CHEMISTRY OF MAILLARD REACTIONS: RECENT STUDIES ON THE BROWNING REACTION MECHANISM AND THE DEVELOPMENT OF ANTIOXIDANTS AND MUTAGENS

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I. INTRODUCTION

The interaction between amino and carbonyl compounds resulting in complex changes in biological and food systems, called the "amino–carbonyl reaction," has exerted a strong influence on human existence throughout the ages as evidenced in, among other phenomena, the formation of humus on the earth's surface and the browning and flavor changes associated with the processing and cooking of foods. Although unaware of this reaction prior to its discovery, in 1912, by Louis-Camille Maillard, its importance was nonetheless felt, most often in the enhancement of food flavor by heating treatment (i.e., cooking) and sometimes in inferior food quality (e.g., browning).

As mentioned by Maillard (1912b), Ling (1908) first noted the enhancing effects of heating at 120–140°C on the flavor and browning of malt. Maillard's discovery of the amino–carbonyl reaction was made in the course of his studies on the synthesis of peptides under physiological conditions (Maillard, 1911), and he succeeded in synthesizing glycine peptides by heating a glycerol system where aldose instead of alcohol was used in the amino acid reaction. As a result, Maillard found marked development of color and flavoring (Maillard, 1912a; cf. Kawamura, 1983).

Since that time these reactions, often called Maillard reactions, continue to be a significant focus of attention in the chemistry of foods. During the past 75 years, numerous works have been published on the Maillard reactions. As summarized and reviewed by Hodge (1953), the interest of researchers was at first limited to the browning and flavoring of foods. Later, studies on the effects of the reactions on nutritional and physiological properties, as well as changes in the physicochemical properties of proteins and antioxidative activity, began to increase in number. Recent studies of these reactions have widened to include problems of food safety (e.g., mutagen formation), the chemistry of proteins in vivo, and diabetic and geriatric studies.

Why are the amino–carbonyl reactions so important in food and biological systems and the subject of growing scientific interest?

Regarding food quality, let us suppose a synthetic and nutritionally complete food is prepared by mixing polysaccharides (e.g., starch), pure proteins, lipids, vitamins, and minerals. Such a food containing no low-molecular-weight compounds may be adequate for feeding experimental animals but utterly tasteless to humans. The kind of food we wish to have in our daily diet usually contains large amounts of low-molecular-weight compounds (such as amino acids, sugars, and fatty acids). Not only do these components make food taste good but, as a result of amino–carbonyl reactions, they are also the source of appetizing smells and colors when the food is properly cooked, processed, or stored. Throughout the long history of food culture, humans have acquired various techniques for selecting and combining materials and processing conditions to control these reactions.
Fermented Oriental foods, notably soy sauce and *miso* (bean paste)—which are composed of amino acids, peptides, proteins, and sugars whose complex interactions during processing result in fine flavors and colors—are good examples.

It is interesting to note that research on the Maillard reaction in Japan has been conducted, for the most part, with emphasis on the coloring and flavoring which occur in soy protein foods, a major source of protein in the Japanese diet; the Maillard reaction is thus very desirable in making food more appetizing. In America and Europe, however, research has focused on the undesirable effects of coloring as a result of the Maillard reaction in dairy products, a major source of protein in the Western diet.

The following chemical aspects also underscore the importance of the amino-carbonyl reactions in food and biological systems. Despite the great abundance and complexity of possible biological compositions, the main constituents of all biological systems, as well as those of foods (which are, after all, derived from biological materials and consumed with or without some kind of processing), are only of three types: proteins, polysaccharides, and lipids. The functional groups of their structural units—amino acids, monosaccharides, fatty acids, and alcohols—are limited to the following four groups: $-\text{COOH}$, $-\text{OH}$, $-\text{NH}_2$, and $-\text{CHO}$. These groups are directly responsible for the formation of polymeric biological constituents by one-step reversible condensation reactions mediated by enzymes. This reversibility is apparent in the enzymatic polymerization of amino acids, which goes as far as to form, for example, proteins as the final product. However, the combination of $-\text{CHO}$ and $-\text{NH}_2$ is quite different (see Fig. 1). Although the first step of the nonenzymatic reaction between these groups is reversible (formation and decomposition of glycosyl-amino products), its products undergo Amadori rearrangement to form ketosyl-amino products, which undergo complex irreversible reactions involving dehydration, rearrangement, scission, and so on to yield decomposed and polymerized products including flavor constituents and melanoidins. The physicochemical and physiological functions of proteins are naturally affected by these reactions. This irreversibility and complexity involving the two functional groups $-\text{CHO}$ and $-\text{NH}_2$ are unique features of the initial stage of the Maillard reaction and distinct from the combinations formed by the other functional groups.

Given the importance and complexity of the reaction, numerous articles have appeared over the years; more than 500 directly related to the Maillard reaction are listed in *Chemical Abstracts* for the past 10 years, and more can be found that deal indirectly with the reaction in food systems.

In response to this growing interest, the First International Symposium on the Maillard Reaction was held in 1979 in Sweden under the auspices of the International Union of Food Science and Technology (IUFST) and other organizations. The proceedings of the first (1979, Sweden), second (1982, United States), and third (1985, Japan) symposia have been published [Eriksson, ed. (1981), Waller
Functional groups
- COOH, -OH, -NH₂, -CHO(-CO) : (-SH, -PO₃H, etc.)

Reactions between functional groups

(Enzymatic)

\[ \text{RCOOH} + \text{R'}-\text{NH}_2 \rightleftharpoons \text{RCONHR'} \] Peptide, Protein

\[ \text{RCOOH} + \text{R'}-\text{OH} \rightleftharpoons \text{RCOOR'} \] Fat

\[ \text{RCHO} + \text{R'-OH} \rightleftharpoons \text{R-OR'} \] Glycoside

(R-OH)

(Nonenzymatic)

\[ \text{RCHO} + \text{R'}-\text{NH}_2 \rightleftharpoons \text{RCH=NR'} \] Amadori product

Scission products

\[ \text{Maillard reaction products} \]

**FIG. 1.** Reactions between main functional groups in food and biological constituents.

and Feather, eds. (1983), and Fujimaki et al., eds. (1986), respectively). Many reviews have also appeared, two of them, concerning mainly the chemistry of nonenzymatic browning, in this series (Reynolds, 1963, 1965). The most recent review, also in this series, treats the Maillard reaction as related to the development of flavor (Danehy, 1985).

As can be seen in Table I, the Maillard reaction covers a broad spectrum reaching into a number of various fields. In this article, however, no attempt is made to cover the advances in all of these diverse albeit related fields. Only chemical studies on quality changes in foods effected by the amino-carbonyl reactions, particularly the browning reaction mechanism and the formation of antioxidants and mutagens, are reviewed. Nutritional aspects and physicochemical quality changes involving proteins are not discussed.

Flavor formation is, of course, an important part of the chemistry of the amino-carbonyl reaction. Since there are several articles which list the vast number of volatile flavor compounds generated by the Maillard reaction (Hurrell, 1982; Maga, 1982; Shibamoto, 1983a; Fors, 1983) and relatively little work has been done on the study of the mechanism of flavor formation by Maillard reactions in foods since the reviews by Hodge (1967) and Hodge et al. (1972), discussion of the chemistry of flavor formation is excluded from this article.
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TABLE I
MAILLARD REACTION IN FOOD AND BIOLOGICAL SYSTEMS

<table>
<thead>
<tr>
<th>Reactant</th>
<th>Browning reaction mechanism, isolation and identification of intermediate products, structure and properties of melanoidin</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH, temperature, moisture content, heavy metal ions, oxygen, light, sulfite and other constituents</td>
<td>Flavor development, physicochemical quality changes, antioxidant, control of browning</td>
</tr>
<tr>
<td>Chemistry</td>
<td>Nutritional aspects Loss of amino acid (lysine, arginine etc.), loss of nutritive value, antinutritive properties, metal ion chelation</td>
</tr>
<tr>
<td>Food technological aspects</td>
<td>Toxicology Mutagen formation, Antimutagenesis</td>
</tr>
<tr>
<td>In vivo</td>
<td>Diabetic diseases, aging, etc.</td>
</tr>
</tbody>
</table>

II. BROWNING REACTION MECHANISM

A. INTRODUCTION

As is well known, the rate of coloration, the color produced, and the product properties of the browning reaction (the most characteristic consequences of the Maillard reaction) are strongly dependent on the nature of the reactants and the reaction conditions, especially pH and temperature. For example, the browning rates of aldoses in general are higher than those of ketoses, those of pentoses are higher than those of hexoses, and two- and three-carbon sugar analogs brown very rapidly. Basic amino acids generally brown more easily than acidic amino acids in the following order: lysine > β-alanine > α-alanine > glutamic acid. Alkaline pH and higher temperatures greatly enhance the reactions and result in changes in the product distribution.

Despite the number of chemical studies, these phenomena have not yet been explained in full due to the high reactivities of the reactants and products, the
interwining reaction routes, and the diversity of products. At present, attempts to establish an inclusive, general reaction mechanism would be largely futile, and separate explanations must be given for each individual reaction. However, the reviews by Hodge (1953, 1967) on the early stages of the formation of the precursors to the various browned matter present an extremely well-organized review of the subject and include numerous data. These works serve their purpose well as far as the processes of the initial stages are concerned. However, essentially no new findings have been added in the more than 30 years since the first review was written despite rapid developments in the research techniques necessary to isolate and identify the reaction products. Based on Hodge's generally accepted scheme (Fig. 2), an attempt is made here to describe step-by-step the new findings on each stage of the browning reaction.

Hodge divided his scheme of browning into three stages. (1) An initial stage (reactions A and B) involving the formation of glycosyl- amino products followed by an Amadori rearrangement. (2) An intermediate stage (reactions C, D, and E) involving dehydration and fragmentation of sugars, amino acid degradation, and others. (3) A final stage (reactions F and G) involving aldol condensation, polymerization, and the formation of heterocyclic nitrogen compounds and colored products.

According to the accepted view, the key step is the Amadori rearrangement (reaction B), which irreversibly produces ketosyl compounds that enolize and lead to the complex reactions in the intermediate stage. Until recently it was believed that this part of the reaction sequence was common to all Maillard reactions and that different individual reactions occurred in the later stages. Each stage will be described in the following sections.

B. REACTIONS DURING THE INITIAL STAGE

The scheme proposed by Hodge for the initial stage of the Maillard reaction is shown in Fig. 3. The first step of the reaction is the simple condensation between the carbonyl group (as the aldehyde form of the reducing sugar) and the free amino group of amino acid, protein, or amine, to give an N-substituted glycosylamino compound followed by the reversible formation of the Schiff base derivatives (Fig. 3a). This condensation reaction is initiated by an attack of a nucleophilic amino nitrogen, with an unshared electron pair, on the carbonyl carbon. The reaction usually requires an acidic catalyst. Protonation of the carbonyl group should enhance its reactivity to the nucleophilic reagent, while protonation of the nitrogen of the amino group inhibits the attack on the carbonyl carbon. The favorable combination of the reactants is shown in Fig. 4. In this step, the maximum rate occurs when the product of the concentrations \([>\text{C}==\text{O}] \quad [\text{RNH}_2]\) is maximum. These concentrations vary in the opposite direction with pH,
so the rate of condensation reaches maximum at a weakly acidic pH in the reaction involving aldose and amine.

The remaining steps for formation of the Schiff base usually occur faster than the first combination step. The increase in the nucleophilic strength of the amine increases the rate of the carbonyl–amine reaction, but has almost no effect on the equilibrium (Feeney et al., 1975).

With aromatic aldehydes, the equilibrium is shifted in favor of Schiff base formation, but aliphatic aldehydes, which possess a hydrogen atom on the carbon adjacent to the carbonyl group, do not generally yield Schiff bases. In the case of sugar, the Schiff base may be an intermediate which rapidly cyclizes to the \( N\)-
substituted glycosylamine. Up to this step, the reaction is reversible because the glycosylamine can be hydrolyzed, in aqueous solution, into the parent compounds. The formation of the *N*-substituted glycosylamine is usually very fast and the product is unstable; especially in an aqueous system, it is susceptible to reverse hydrolysis as well as to additional irreversible reactions (even at room temperature). The glycosylamines derived from amines show a certain stability, while those from amino acids are difficult to isolate because they are immediately converted into the Amadori products, *N*-substituted 1-amino-1-deoxy-2-ketoses. However, the isolation of the *N*-glycosylamino derivatives of amino acids is possible using an amino acid ester or metal salt.

This Amadori rearrangement of the *N*-substituted glycosylamines, the most

\[
C=O + \text{NH}_2-R \rightarrow C=O + \text{NH}_2-R
\]

\[
\text{C}=\text{N}-\text{R} \quad \text{(1)}
\]

\[
\text{C}=\text{N}-\text{R} \quad \text{(2)}
\]

**FIG. 4.** Condensation of carbonyl compounds with amino compounds.
important in the Maillard reaction, is believed to be the only reaction in the second step of the initial stage. The Amadori reaction is catalyzed by weak acids, where the protonation of the Schiff base and the subsequent protonic shift constitute the critical step; the amino acids serve as their own acid catalysts so the reaction is rapid even in the absence of added acid.

It should be noted here that the reactions in the initial stage of the Maillard reaction are all favored by acidic conditions and are apparently not compatible with the observed fact that neutral or alkaline conditions promote the browning reactions. A group of reactions involving the early scission of sugar molecules, which was recently reported by this author’s group, may serve to at least complement the existing, widely recognized scheme. This is described in the following section.

C. SUGAR FRAGMENTATION DURING THE INITIAL STAGE AND A NEW BROWNING REACTION MECHANISM

1. Development of Novel Free Radicals during the Initial Stage

It is generally agreed that the Amadori compounds in the Maillard reaction mixture could exist as enaminol structures. The reducing power of the reaction mixture, probably attributable to such reductones, increases with browning (Kirigaya et al., 1968), and the browning products have been shown to possess antioxidative activity. A representative reductone, ascorbic acid, has been shown to have a free radical structure as the intermediate of its oxidation process by electron spin resonance (ESR) spectrometry, and it also acts as a kind of antioxidant (Bielski, 1982). On the other hand, the presence of fairly stable free radicals was observed in the amino-carbonyl reaction of ninhydrin with amino acids (Yuferov et al., 1970) as well as in the highly alkaline solutions of some reducing sugars (Lagercrantz, 1964). These facts suggest that the Maillard reactions may involve a free radical process or produce some kind of free radical product. However, no studies have indicated the presence of free radical in the Maillard reaction except for that of Mitsuda et al. (1965). They showed the presence of a stable free radical in the melanoidin from glucose and glycine heated for a long time by detection of a broad singlet ESR spectrum that may exist in the unsaturated structure of the polymerized product as is found in humus (Tollin et al., 1963; Steelink and Tollin, 1962).

In 1973, Namiki et al. found a novel free radical in a very early stage of the sugar–amino acid reaction mixtures. The free radical showed an ESR spectrum with a clear hyperfine structure and was apparently different from that observed in the melanoidin (Namiki and Hayashi, 1975, 1981, 1983; Namiki et al. 1977).

Figure 5 shows the results of reactions of D-glucose with ω- or β-alanine. The important characteristics are as follows. (1) The ESR signal was detected soon after the reaction mixture was heated, and the relative intensity of signal in-
creased rapidly very early in the reaction when the mixture was not yet significantly colored. (2) After reaching a maximum peak, the intensity of the ESR signal decreased with heating time and was accompanied by a gradual increase in browning, a disappearance of the hyperfine structure, and a gradual increase of the broad singlet in the ESR spectra. (3) The intensity and speed of the ESR signal with browning was stronger and faster for β-alanine than for α-alanine. (4) The hyperfine structure of the ESR signal for these two systems apparently differs in splitting number.

The development of similar ESR spectra was observed for almost all reaction mixtures of sugar–amino compounds, indicating that the formation of a novel free radical product is a normal process of the Maillard reaction and occurs at an early stage. The results of the reactions of various sugars with α- and β-alanine are summarized in Table II. The sugars and their related carbonyl compounds all gave essentially the same types of ESR hyperfine structure, i.e., the hyperfine structures split into 19 lines in the sugars with α-alanine systems, while those with β-alanine systems split into 25 lines (except in the case of three-carbon sugars—the spectra of three-carbon sugars were more complex and resembled each other).
## Table II
### ESR Spectral Data on Free Radicals and Browning in the Reaction of Sugar and Other Carbonyl Compounds with α- and β-Alanine*<sup>a,b</sup>

<table>
<thead>
<tr>
<th>ESR Spectra</th>
<th>Splitting line number</th>
<th>Intensity&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Browning&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Alanine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d-Glucose</td>
<td>19</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>d-Fructose</td>
<td>19</td>
<td>±</td>
<td>+</td>
</tr>
<tr>
<td>d-Arabinose</td>
<td>19</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>d-Xylose</td>
<td>19</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>d-Ribose</td>
<td>19</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Glycolaldehyde</td>
<td>19</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>β-Alanine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d-Glucose</td>
<td>25</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>d-Fructose</td>
<td>25</td>
<td>±</td>
<td>++</td>
</tr>
<tr>
<td>d-Arabinose</td>
<td>25</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>d-Xylose</td>
<td>25</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>d-Ribose</td>
<td>25</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Glyceraldehyde</td>
<td>∼35</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Dihydroxyacetone</td>
<td>∼35</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Glycolaldehyde</td>
<td>25</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>3-Deoxyglucosone</td>
<td>25</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>5-Hydroxymethylfurfural</td>
<td>25</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Furfural</td>
<td></td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Glyoxal</td>
<td>25</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Crotonaldehyde</td>
<td></td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Propionaldehyde</td>
<td></td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

* Namiki and Hayashi (1983).

<sup>a</sup> Aqueous solutions (each 3 M) were heated in boiling water bath.  
<sup>b</sup> ESR Spectral Data on Free Radicals and Browning in the Reaction of Sugar and Other Carbonyl Compounds with α- and β-Alanine.  
<sup>c</sup> ++++, High; ++, moderate; +, low; ±, insignificant.

Carbonyl compounds that are highly reactive in the formation of a free radical are also effective in browning, while carbonyl compounds, such as furfural and crotonaldehyde, show high activity only for browning. This suggests that the presence of an enediol or a potential enediol grouping in the carbonyl compounds as a reducing sugar is necessary for the radical formation. Glycolaldehyde showed extremely high activity in both free radical formation and browning.

With the exception of certain compounds such as aniline, cysteine, and ethylamine, radical formation was observed only in the case of the primary amino compounds.

The radical formed even under weakly acidic conditions and, like browning, increased markedly with pH, although the radical product was rather stable under acidic conditions. Interestingly, the radical was produced without necessarily
removing oxygen from the system. It appeared to be fairly stable within the reaction mixture, but it disappeared rapidly when air was bubbled through the system (Namiki and Hayashi, 1975).

2. Structure of Novel Free Radical Products

Based on many ESR experiments on the various amino compound–sugar systems, Hayashi et al. (1977) found that, as shown by α- and β-alanine, the difference in the hyperfine structure depends upon the difference in the number of protons on the α carbon of the amino compound and not upon the carbon number of the common sugar. They also found that all spectra have in common splittings that arise from interactions of the free radical with two equivalent nitrogen atoms and four equivalent protons in addition to an even number of equivalent protons with different splitting constants. These assignments led to the assumption that the radical products are N,N'-disubstituted pyrazine cation radical derivatives, as shown in Fig. 6.

This is strongly supported by the fact that the hyperfine structure, as well as g value of the ESR spectrum of the reaction mixture of ethylamine with D-glucose, was in good agreement with those of the ESR spectrum of the radical from synthesized N,N'-diethylpyrazine (Curphey and Prasad, 1972). Despite a vast number of pyrazine derivatives in the Maillard reaction products, the presence of such N,N'-disubstituted pyrazinium derivatives has rarely been demonstrated. This is probably due to their high instability, which makes them difficult to track.

Milic and others (Milic et al., 1980; Milic and Piletic, 1984) also conducted ESR studies on this kind of free radical developing at an early stage of the Maillard reaction in model systems of 2-, 3-, and 4-aminobutylic acids with D-glucose. The results of their ESR spectral analyses are consistent with the previously mentioned assignments of the free radical products. Their studies also included the investigation of the kinetics of the formation of the free radicals.

3. Formation Process of the Free Radical Products

The novel pyrazine derivatives have no substituents on the ring carbons, so the plausible formation mechanisms are limited to the following two pathways: (a) formation of a two-carbon enaminol compound by sugar fragmentation and subsequent dimerization, or (b) bimolecular condensation of the enaminol of the Amadori products followed by elimination of the substituted sugar residues.

Investigations of these possible pathways (Hayashi and Namiki, 1981; Namiki and Hayashi, 1983) indicated (1) that the free radical developed rapidly prior to (or simultaneously with) the formation of the Amadori product and then began to decrease while the Amadori product continued to increase and 3-deoxyosone was produced thereafter, and (2) that the glycosyl amino compound alone resulted in marked free radical formation while no free radical was formed from the
Amadori product alone or with amine or sugar. In addition, as mentioned previously, glycolaldehyde showed especially high activity in both free radical formation and browning. These results indicate that the novel free radical is produced before the Amadori rearrangement and possibly via the pathway (a). This is quite interesting since the occurrence of changes such as fragmentation prior to Amadori rearrangement had never been considered in the Maillard reaction mechanism and required substantiation.

4. Sugar Fragmentation at an Early Stage of the Maillard Reaction

The proposed scheme of radical formation involves sugar fragmentation to give a very reactive enamino compound for browning. Sugar fragmentation and the role of fragmentary low-molecular-weight carbonyl compounds in browning have been noted by Hodge (1953):

The fission products of the sugars very conceivable in their potential for browning. Fragments which retain the α-hydroxycarbonyl grouping will undergo browning alone in aqueous solutions;
and, in the presence of amino compounds, the browning is greatly accelerated. The most highly reactive compounds are glycolaldehyde, glyceraldehyde, pyruvaldehyde, dihydroxyacetone, acetoin, and diacetyl. . . [Ep. 935]

He proposed the formation of pyruvaldehyde and other carbonyl compounds from 1-deoxyglucosone, the product (through 2,3-enolization) of the Amadori compound, and also postulated that cleavage would occur at the C-2/C-3 position of the sugar to give two-carbon carbonyl products by a reverse aldol mechanism (Hodge, 1953). However, the formation of such carbonyl compounds at an early stage of the Maillard reaction was not chemically demonstrated, and their role in browning, especially concerning two-carbon products at a very early stage of the Maillard reaction, was not clarified.

Hayashi and Namiki (1980) demonstrated the production of a two-carbon fragmentary product at an early stage of the Maillard reaction by isolating and identifying glyoxal and its diimine derivatives in glucose–alkylamine mixtures by thin-layer chromatography (TLC), gas–liquid chromatography (GLC), mass spectroscopy (MS), and nuclear magnetic resonance (NMR) analyses. Quantitative measurements indicated that the production of the two-carbon sugar fragmentation product increased after the production of the glycosyl amino compound, and that this was followed by free radical formation and subsequent browning. Formation of the four-carbon product, the residual of hexose fragmentation, was also demonstrated to occur almost in parallel with the two-carbon product formation (Namiki and Hayashi, 1983).

It should be noted here that glyoxal was known to be an artifact on the TLC from glyoxal diimine in the reaction mixture, and neither compound is the direct precursor of the pyrazine radical because glyoxal showed only weak activity in radical formation with amines and glyoxal diimine itself is inactive. However, when ascorbic acid was added at an early stage (prior to free radical formation and browning) to the glucose–alkylamine reaction mixture, an intense ESR spectrum of the pyrazine radical appeared instantaneously, together with marked browning (Hayashi et al., 1985a). The same fact was also observed when ascorbic acid was added to the glyoxal dialkylimine solution. The initial two-carbon fragmentary product of sugar, the precursor of the free radical formation and browning, is assumed to be glycolaldehyde monoimine or its enamino. The \(N,N'\)-disubstituted pyrazinium salt was shown to be highly unstable in aqueous systems and produced instantaneous browning. Possible pathways for the formation of the novel free radical, browning, and the production of glyoxal diimine derivatives were proposed as shown in Fig. 6 (Namiki and Hayashi, 1983; Hayashi et al., 1985a).

It should be noted that the evidence indicating the formation of the fragmentary products at an early stage of the Maillard reaction was obtained mainly in glucose–alkylamine systems. Systems employing alkylamines usually react fast-
er and more intensely to give the free radical product and to produce browning than do ordinary amino acids; this may be due to the tendency of amine-catalyzed reverse aldol condensation reactions to give higher amounts of intermediate fragmentary products, which results in more intense browning as well as in a higher yield of glyoxaladiimino, especially in ethanol reaction systems (Hayashi et al. 1985a). The formation of glyoxal diimine derivatives was also observed in the reactions of glucose and amino acids, e.g., glycine, lysine, arginine, and β-alanine, although in amounts smaller than amine derivatives (Hayashi et al., 1985a).

As early as 1962 Kitaoka and Onodera reported sugar fragmentation into one- and two-carbon products in the reaction of 1,2-diamino–sugar derivatives and suggested its occurrence at an early stage browning of the Maillard reaction. However, they started from diaminated sugar derivatives formed by further amine addition of Amadori products and differs from the sugar fragmentation described previously.

5. Formation of Three-Carbon and Other Sugar Fragmentary Products

Strictly speaking, the discussion which follows may be better placed under the intermediate rather than the initial stage of the Maillard reaction. However, while the evidence is far from complete, there exists a good possibility that the formation of the C₃ compound occurs in the early stage.

As mentioned previously, Hodge (1967) proposed the formation of fragmentary products such as methylglyoxal and diacetyl from 1-deoxyglucosone, which is formed from the Amadori product through 2,3-enolization and deamination, although no clear evidence has been presented on this process.

Recently, Hayase and Kato (1986) investigated the formation of low-molecular-weight products of the glucose–n-butylamine reaction system by GLC analyses. The results showed that at pH 4.0 the ether extractable products are mainly heterocyclic compounds which are assumed to be formed from the Amadori product through 1,2-enolization. On the other hand, large amounts of low-molecular-weight fragmentation products, such as N-butylformamide and N-butylacetamide were produced very rapidly and abundantly at pH 11.4 while no heterocyclic compounds were produced. The reaction at pH 6.5 already showed a tendency to increase the fragmentation products. Hayase and Kato proposed that these low-molecular-weight amides are formed from glyoxal and diacetyl, respectively, and that diacetyl is formed through the scission of the C-2/C-3 and C-4/C-5 bonds of glucose after 2,3-enolization of the Amadori product. Production of N-butylformamide was observed as the first and most abundant product in the GLC, especially at a pH above 6.5. If, indeed, this indicates the formation of glyoxal or other two-carbon products at an early stage of the Maillard reaction, it is very interesting in light of the results obtained by Namiki and Hayashi (1983).
Diacetyl was detected as a main product in the headspace gas analysis of the reaction at pH 6.7. Formation of the acetamide derivative did not appear to occur via diacetyl, but from the C$_6$ compound or methylglyoxal. In any case, the results are interesting because diacetyl and glyoxal are known to be active intermediates in browning and, moreover, active cross-linking agents in the polymerization of proteins by the Maillard reaction (Cho et al., 1986).

Meanwhile, Hayashi and others (Hayashi et al., 1986a; Hayashi and Namiki, 1986b) directly observed the formation of C$_3$ compound in the sugar–amine reaction by isolation and identification of the diimine derivative of methylglyoxal. This was noted at a very early stage of the reaction, closely following the formation of two-carbon product and together with the Amadori product. Heating of glucose alone at pH 9.3 did not produce a detectable amount of methylglyoxal, indicating that the fragmentation occurred by the Maillard reaction. In this case, the reaction of the Amadori product with n-butylamine rapidly produced the C$_3$ compound in a manner similar to that of the glucose–n-butylamine system. A clear difference was observed between the reaction of glucose with n-butylamine and t-butylamine, especially in the production of the C$_3$ compound, which was significantly suppressed and delayed in the latter case, probably due to the bulky structure of t-butylamine. These results are difficult to explain from the scheme proposed by Hodge, in which the C$_3$ is formed by the scission of 1-deoxyosone, and suggest the presence of a new pathway of sugar fragmentation to give C$_3$ imine by a reverse aldol-type reaction of the additionally aminated Amadori product. C$_3$ carbonyl compound formation in the n-butylamine system may occur mainly via a newly proposed pathway, while that in the t-butylamine system may possibly occur according to the scheme of Hodge.

6. Role of Sugar Fragmentation Products in Browning Reaction

It has been demonstrated previously that sugar fragmentation producing the C$_2$ and C$_3$ carbonyl compounds or their imine derivatives occurs at an early stage of the Maillard reaction. In order to evaluate the role of sugar fragmentation in browning, it is necessary to quantify the yields as well as the browning abilities of the main fragmentation products. However, little research has been done on these aspects of the Maillard reaction, perhaps because until recently sugar fragmentation at an early stage of the reaction was not taken into account. Moreover, it is often difficult to measure accurately the amounts of active intermediates in the complicated processes of the reaction, especially glycoaldehyde in alkaline solution.

Recently, Hayashi and Namiki (1986a) quantified C$_2$ and C$_3$ carbonyl products in a glucose–β-alanine reaction mixture by the o-phenylenediamine method using GLC and showed that the production of these products was greatly influenced by pH: production was negligible at acidic pH, observable at neutral pH,
and increased greatly at alkaline pH (although the yields were very low compared to that of the Amadori product). The production of these fragmentation products increased rapidly very early in the reaction, and then decreased when browning and Amadori product formation started to decrease rapidly.

The fragmentation products of C₂ and C₃ carbonyl compounds are assumed to be glycolaldehyde, glyoxal, glyceraldehyde, methylglyoxal, etc., or their imine derivatives. These are well known to be very active compounds in Maillard browning, but no quantitative evaluation has been done on their browning ability. Hayashi and Namiki (1986a) evaluated their browning activity and compared it with that of xylose and glucose at 80 and 95°C respectively. As shown in Table III, low-molecular-weight carbonyl compounds showed extremely high brown-
ing ability, i.e., the relative values of glycolaldehyde and glyceraldehyde are about 2000 times greater than that of glucose. The high values of the fragmentation products, despite their low yields in the reaction, correspond to the browning observed at the early stage of the Maillard reaction. In other words, the browning observed at the early stages of the Maillard reaction with pH at and above neutral may be caused mainly by the fragmentation pathways.

D. REACTIONS DURING THE INTERMEDIATE STAGE

What is called the "intermediate stage" in Hodge's scheme (1967) of the Maillard reaction involves a series of reactions which starts with the Amadori rearrangement products (i.e., 1-amino-1-deoxy-2-ketose derivatives produced from glycosyl amino compounds in the initial stage) to produce melanoidins. Compared to the relative simplicity of the first stage, the facts accumulated by the numerous studies on the second stage are chaotic and await systematization via some universally recognized dominant pathway(s) of melanoidin formation. Obviously, this situation has arisen because the high reactivities of almost every product in this stage result in complex paths, all of which, in turn, lead to a complex mass of colored polymers called melanoidins. We know very little of the chemistry of melanoidins despite the advanced analytical techniques currently available.

A number of studies on the products of this stage, especially the precursors of pigments, overlap to a large extent with studies on the production of flavors and antioxidants as well with those on protein modification as a result of the reactions. In this review, the emphasis is mainly on the formation of colored products.

1. Amadori Rearrangement Products

Amadori products, the key intermediate in Maillard reactions, are obtained from glycosylamino products (Gottschalk, 1952) and sugar–amino compound model systems (Abrams et al., 1955; Finot and Mauron, 1969), and the Amadori products from aromatic amines and sugars are especially easy to isolate in a pure and stable state (Baltes and Franke, 1978). Amadori products are also isolated from treated foods, e.g., freeze-dried fruits (Anet and Reynolds, 1957), liver extracts (Heyns and Paulsen, 1959), and soy sauce (Hashiba, 1978).

Recently, due to their very important effects on the physical, nutritive, and physiological properties of proteins, a number of studies have been done on Amadori product formation by sugar–protein reactions in food and biological systems. Major studies include Adrian (1974), Bayns et al. (1986), Erbersdobler (1986), Mauron (1981), Mester et al. (1981), and Monnier and Cerami (1983). However, these will not be discussed in this review.

Numerous studies on the features of Amadori rearrangement and product
isolation have been thoroughly reviewed elsewhere (e.g., Reynolds, 1963, 1965) and will not be discussed here.

Recent analytical techniques have produced more detailed information on the nature of Amadori products. High-performance liquid chromatography (HPLC) has become a popular tool for analyzing Amadori products (Moll and Gross, 1981; Moll et al., 1982) because of its rapidity and simplicity. Structural analyses by high-resolution NMR have shown that Amadori rearrangement products exist in the β-pyranose form at pH greater than 7 and favor the β-furanose form at pH 3 (Altena et al., 1981). The equilibrium of \(N-(1\text{-deoxy-}d\text{-fructos-1-yl})-l\)-amino acids in \(D_2O\) was shown by \(^1H\)- and \(^13C\)-NMR to be \(\sim 64\% \) β-pyranose, \(15\% \) α-furanose, \(\sim 15\% \) β-furanose, and \(\sim 6\% \) α-pyranose forms (Röper et al., 1983).

Amadori products are fairly stable and are weak in browning activity even in the presence of amino compounds. The main processes giving colored and flavor products from Amadori products are as follows (Fig. 7) (Hodge, 1967): formation of labile enolized intermediates, 1,2-enolization followed by elimination of the hydroxy group at C-3 and deamination at C-1 yielding 3-deoxyhexosone, the reactive dicarbonyl product itself, and later furfural as well as the reactive carbonyl compound.

On the other hand, 2,3-enolization of the Amadori products followed by elimination of the amino group from C-1 gives the 1-deoxydicarbonyl intermediate, which further reacts to produce reactive fission carbonyl products such as methylglyoxal, diacetyl, and others (Hodge, 1967). In most of the studies on the Maillard reaction, browning and flavor are considered to be caused by additional reactions of these active intermediates with amino compounds.

However, several cases have been reported in which the Amadori products yielded colored products without undergoing conversion to deoxyosones or other compounds. Hashiba (1976, 1978) reported oxidative coloration of Amadori products, focusing on the coloration of soy sauce. Although Kato (1963) reported oxidative degradation of \(N\)-glucosides, model experiments show that oxygen does not interfere in the Maillard reactions.

In actual foods—miso, sake, soy sauce, wine, etc.—the browning effected by oxygen is of practical importance. Hashiba et al. (1981) confirmed that Maillard reactions, rather than polyphenol oxidation, are responsible for this kind of browning and isolated several Amadori products as key intermediates. These products produce intense red color on contact with oxygen, especially in the presence of Fe ions. Hashiba (1986) observed that the addition of \(Fe^{2+}\) ions increased the absorption at 550 nm of glucose–glycine melanoidin as well as of the Amadori product from that system, and that addition of EDTA had the opposite effect. Apparently, the \(Fe^{2+}\) ion is involved not only as a catalyst but in the coloration itself. Pigment isolated from the reaction of a fructose–glycine Amadori product with \(Fe^{2+}\) and air (or \(H_2O_2\)) was identified as 2-hy-
FIG. 7. Reactions at the intermediate stage (Hodge, 1967); enolization and degradation of Amadori compounds.
droxymethyl-3,5-dihydroxy-1-(4-pyridone)acetic acid, (Fig. 8, 1) and was assumed to be formed by cyclization of the Amadori product, resulting in the pigment by 1:1 chelation with the Fe$^{2+}$ ion. The Fe$^{3+}$ ion also produced a colored product with this pigment. Maltol behaved similarly toward Fe ions, but 4-hydroxy-2,5-dimethyl-1,3(2H)-furanone chelated only with the Fe$^{2+}$ ion. Hashiba concluded that the oxidation of Amadori products is involved in the coloration of soy sauce and that the presence of similar structures may be responsible for the coloration and metal chelating activity of melanoidin.

Many nitrogen-containing heterocyclic products have been isolated from sugar–ammonia caramel products, some of which are pyridine and pyrazine derivatives presumed to originate from Amadori products. For example, Tsuchida et al. (1973, 1975) isolated a number of polyhydroxy alkylated pyridines, pyrazines, and pyroles from weakly acidic sugar–ammonia systems with or without sulfite by GLC and ion-exchange chromatography (Fig. 8, 2–8). The formation of the β-hydroxypyridines (4 and 5) by the reaction of furfural and amino compounds had already been shown by Aso (1939). Deoxyfructosazine [(2-α-arabino-tetrahydroxybutyl)-5-(α-erythro-trihydroxybutyl)pyrazine] (Kuhn et al., 1961) and its 6-isomer (Fig. 8, 2 and 3) were present in this reaction mixture in approximately a 1:1 ratio. These pyrazine derivatives were considered to be condensation products of Amadori products with 3-deoxyosone and ammonia. They also isolated and identified many new polyhydroxy heterocyclic compounds (Tsuchida et al., 1986). With caramelization in the presence of sulfite, the variety of products decreased, and the main product was deoxyfructosazine. Furan derivatives with cyclized side chains have also been isolated (Tsuchida et al., 1986). Many nitrogen-containing heterocyclic products from cyclization, including sugar fragments (e.g., methylglyoxal), are found in the products of caramel formation under alkali conditions (Tsuchida et al., 1976).

2. Enolization and Degradation of Amadori Products

In the intermediate stage of Hodge’s scheme, 1,2-enolization is considered to be favored under acidic conditions, and 3-deoxyosones (Anet, 1960; Kato, 1960), furfural, and hydroxymethylfurfural (Anet, 1962, 1964) are the main products which have been isolated and identified from acidic sugar–amino acid reaction mixtures as well as in actual food systems. These intermediate products are also known to be produced by acidic decomposition of sugars and caramelization (Feather and Harris, 1973). On the other hand, 1-deoxyosones, the main 2,3-enolization Amadori products, have not yet been isolated from food systems and are presumably produced only by the Maillard reaction (Ledl et al., 1986a).

Detailed examination of these processes was carried out by Feather and coworkers (1969; 1970; 1981) using isotope incorporation (D$_2$O, T$_3$O) and NMR.
FIG. 8. Some active intermediates and colored precursors of melanoidin. 1, Hashiba (1986); 2–8, Tsuchida et al., (1975); 9, Severin and Seilmeir (1968) and Feather (1981); 10a–c and 11a–c, Ledl and Severin (1981); 12, Nursten and O’Reilly (1983); 13, Obretenov and Arginov (1986).
The study of the decomposition of sugars in 3 N HCl using furfural as the marker showed that deuterium is not incorporated into the furfural ring, suggesting that the formation of the furan ring is fairly straightforward from 1,2-enolization without intervening keto–enol equilibria in the process. In contrast, examination of the decomposition of the Amadori products using aromatic N-substituents of different basicities and under different acidities of the medium (with acetic acid or HCl) showed (1) that the Amadori products produce hydroxymethylfurfural in higher yields than do the sugars and (2) that deuterium is incorporated into the furan ring (especially with acetic acid). This suggests the ease of 1,2-enolization and the presence of intervening keto–enol equilibria in the case of Amadori products. It was shown also that 1,2-enolization occurs more easily when the nitrogen atom of the Amadori compound is nearly completely protonized in acidic media.

Feather et al. also studied 2,3-enolization using 4-hydroxy-5-methyl-3(2H)-furanone (9) as the marker; this has been isolated from cooked beef flavor and is produced by the reaction of amines with xylose (Severin and Seilmeier, 1968) and with ribose (Peer et al., 1968). It has been confirmed that this compound is derived from 1-deoxyosone by 2,3-enolization of the Amadori product according to evidence of incorporation of the 14C-1 atom of the labeled Amadori product into its methyl group and by NMR studies (Hicks and Feather, 1975). In heating Amadori products formed from N-substituted aromatic amines with different basicities, Feather (1981) also showed that furfural production in a strongly acidic medium increased with the basicity of substituted amines, and a slight amount of methylfuranone was detected with only weakly basic Amadori products. On the other hand, no furfural and only methylfuranone production was observed at pH 7.0 in all Amadori derivatives. These results indicate that the formation of furfural is favored by strong acidity because the nitrogen atom protonized completely and only weakly basic Amadori products produced furfural in acidic media, while under less acidic or neutral conditions, the amino group is only partially protonized and tends to result in 2,3-enolization followed by the formation of methylfuranone.

3. Colored Intermediates and Precursors of Melanoidin

Given this background, we will now consider how the pigments are formed from their precursors. Concerning the additional reactions of the products of 1,2-enolization, it became known early on that the reactions of furfural with amino compounds produce pyrrole and pyridine derivatives (Aso, 1939, 1940). Several other products of the same type also became known (Pachmeyer et al., 1986). Exhaustive studies of colored intermediates and melanoidin precursors by Severin, Ledle, and others continue to be conducted. Their achievements include the isolation of colored furanone derivatives as an intermediate (Fig. 8, 10a–c) from
pentose–methylamine (or glycine) model systems (Krönig and Severin, 1972). These intermediates are considered to be the condensation products of furfural or the respective pyrrole derivatives with a furanone derivative (9) isolated earlier from the glucose–methylamine–acetic acid system (Severin and Seilmeier, 1968), and a number of their analogs have been synthesized (Ledl and Severin, 1978). Another colored condensate (11a–c) of furfural with 10a–c was obtained by increasing the content of primary amine (Ledl and Severin, 1981). The same products have been obtained by HPLC fractionation of petroleum ether extract of a xylose–glycine reaction mixture by Nursten and O’Reilly (1983, 1986b); they have also isolated a colored product assumed to be a condensate of furfural with methylfuranone at the 4-hydroxy position of the latter (Fig. 8; 12).

Colored products from furfural, e.g., N-furilidene-β-furyl-α,β-dehydroalanine methyl ester (13), have been obtained by Obretenov and Arginov (1986) by reaction with glycine methyl ester in ether.

Ledle et al. (1983) also isolated colored products having furanone and β-pyranone moieties (Fig. 9a, 16 and 18). Since these were formed by the reaction of 3-deoxyosone and furfural, they were considered to be the condensate of furfural and β-pyranone (14), the product of its ring opening and ring closure to furanone, the product of further addition of furfural, etc. The analogous colored pyrrolinone derivatives (19) were also obtained by the reaction of furfural and methylamine (Ledl et al., 1986) (Fig. 9a).

As for the colored products from the intermediates of the reaction path from 1-deoxyosone produced by 2,3-enolization, Ledl and Fritsch (1984) isolated a pyrrolinone-type reductone (Fig. 9b, 25) as a key intermediate in melanoidin formation, which they considered to have formed from 1-deoxyosone of monosaccharide via acetylformin (24) and condensation with primary amine as glycine ester (Fig. 9b). The actual contents of 25 were 60 and 100 ppm in the glucose–glycine reaction mixture (after 3 hr at 80°C and 15 min at 130°C, respectively), and 40 ppm in colored malt. They also obtained a β-pyranone intermediate (14) from disaccharide, e.g., lactose, believed to originate from 1,5-deoxy intermediates. The presence of two reaction paths of disaccharides, one via the 1,5-deoxy intermediates and the other via 1,6-deoxy intermediates, has been surmised (Ledl et al., 1986).

The formation of pyrroles and pyridinols in Maillard reactions, e.g., from a glucose–glycine system (Nyhammer et al., 1983) or a fructose–alanine system (Shaw and Berry, 1977), is known. This has been attributed to a hypothetical path from the Amadori products via formation of osones, Strecker degradation with (for example) glycine to form deoxynaminols, and ring closure (Olsson et al., 1981a). (Fig. 10a). However, Nyhammer et al. (1983) presented contradictory results in their study using [13C]glucose. They demonstrated that compounds 28 and 29 form more easily from 3-deoxyosone (26) than from glucose and is favored by a lower reaction pH. On the other hand, the formation of
FIG. 9. Formation pathways of intermediates and colored precursors of melanoidin (Leidl et al., 1986a). a, from 3-deoxyosone; b, from 1-deoxyosone.
FIG. 10. Proposed pathways of the formation of 2-acetylpyrroles and 3-pyridinols from D-glucose and glycine. a, Olsson et al. (1981a); b, Nyhammer et al. (1983).

Compounds 30 and 31 is hindered by low pH and did not form from 3-deoxyyosone. The reaction of D-[1-13C]glucose with glycine yielded 13CHO-28, 5-13C-30, 2-13C-29, and 6-13C-31. A conceivable alternate origin of 30 and 31 as 1-deoxyyosone (27) was, however, not supported by the 13C study. The results indicated overlooked reaction route(s) and that 2-deoxy sugars may be a possible
origin of compound 31, but no explanation was given concerning the origin of 30 (Fig. 10b).

4. Reactions of Sugar Fragmentary Products

As mentioned previously, Hayase and Kato (1986) examined low-molecular-weight products in an ether extract of a glucose–butylamine (or glucose–glycine) Maillard reaction mixture. They found considerable amounts of N-butylformamide and N-butylacetamidine as well as 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one and pyrrole derivatives in the neutral and alkaline reaction mixtures; the products from the acidic reaction mixture were mainly N-butyl-2-formyl-5-(hydroxymethyl)pyrrole and contained hydroxymethylfuran. The assumed origins of the products were Amadori products for hydroxymethylfuran (through 1,2-enolization), 1-deoxyosone or diacetyl for the acetamide derivatives (through 2,3-enolization), and 3-deoxyosone for the formamide derivative. However, the results for the formamide derivatives seem to suggest some processes other than 1,2-enolization because of the very small quantities obtained from the acidic reaction mixture as opposed to the rapid formation of large amounts in neutral and alkaline reaction mixtures.

Ledl (1982) obtained a pyrrole derivative as the main product of the reaction of furfural with hydroxyacetone (a simplified sugar model) and methylamine. Among complex products of a similar reaction using piperidinum acetate, a cyclic dihydroxypentenone, a colored furan derivative, an amino reductone, and a reductic acid were isolated.

The carbonyl compounds, which are fragmented products of sugars (hexoses, pentoses) such as methylglyoxal, diacetyl, and others, are obtained directly from sugar by caramelization, from Amadori products through 2,3-enolization and 1-deoxyosone formation (Hodge, 1967), and from additional amination of Amadori products (Hayashi and Namiki, 1986a). Many studies have been done on the browning reactions of these products with amino compounds, mainly on the Strecker degradation of amino acids by these products leading to flavor formation. Recent results of Hayashi et al. (1986a) have been described previously. Takagi and Morita (1986) also examined the browning activities of low-molecular-weight carbonyl compounds with lysine, which revealed the order glycolaldehyde > glyceraldehyde > dihydroxyacetone > methylglyoxal >> glyoxal.

Piloty and Baltes (1979a,b) reported that the reactivity of amino acids with diacetyl at pH 4 and 100°C is greater for basic or hydroxylated amino acids (e.g., arginine, histidine, lysine, and threonine) than for others; they also isolated and identified oxazolines, pyrazines, and pyrroles from the reaction mixtures.

There are a number of reports on the reactions of active carbonyl compounds from sugar fragmentation, especially diacetyl and methylglyoxal, with amino
acids (e.g., Maga, 1982; Piloty and Baltes, 1979b); they focus mainly on the formation of the flavor components as aldehydes and heterocyclic compounds by Strecker degradation.

To summarize, the main products of the intermediate stage of Maillard reactions are heterocyclic compounds produced from osones (by 1,2- and 1,3-enolization in Hodge’s scheme), furfurals, and carbonyl fragmentation products produced by mutual reactions and, especially, by reactions with amino compounds. Many of these products have been obtained as flavors, precursors to flavors, and pyrolysis products from sugars. Although some of them are colored and may be regarded as intermediates of melanoidin formation, we know very little about the melanoidin formation itself. Does it occur as the straightforward polymerization of a single or mixed species? What role do these products actually play in melanoidin formation? In seeking the answers to these questions, it must be kept in mind that no matter how small the quantity of a product isolated from the reaction mixture, it may be important in melanoidin formation. In other words, the more reactive the product, the more difficult it is to isolate and identify.

E. NONSUGAR MAILLARD BROWNING REACTION

Certain nonsugar carbonyl compounds of foods (e.g., products of ascorbic acid, polyphenols, and unsaturated fatty acids) are known also to take part in the Maillard reaction with amino compounds and are involved in browning and other reactions.

Reactions involving oxidized products of polyphenols and unsaturated fatty acids mainly involve proteins, so they are not included in the following discussion. For recent studies on reactions involving these compounds the reader is referred to Suyama and Adachi (1986) for fatty acid products and to Igarashi and Yasui (1985) regarding polyphenols. Coloration attributed with ascorbic acid oxidized products is the main subject of discussion in this section.

1. Ascorbic Acid and Oxidized Products

Ascorbic acid (AsA), besides being a nutritionally important food component, is widely used as a meat coloring agent, as an antioxidant, as a baking additive, etc. (Counsel and Horning, 1980; Bauernfeind, 1982). While these uses originate mainly from its nature as a reductone with an enediol structure, the high reactivity of AsA and its decomposition and oxidation products often cause problems in food processing, for example, by discoloration and other effects, mainly as a result of reaction with amino compounds.

a. Nonoxidative Decomposition of AsA. The mode of decomposition of AsA is mainly oxidative, but nonoxidative AsA decomposition also occurs. AsA
is most stable at pH 6, when anaerobically decomposed at pH 2, 4, or 6. Furfural, by decomposition of the undissociated form of AsA, is the main product of the reaction under strongly acidic conditions (Herbert et al., 1933; Huelin et al., 1971). Since 3-deoxypentosulose (3-DP) was also identified as the another carbonyl decomposition product, 3-DP or its enol form may be an intermediate to furfural, although details are not known (Kurata and Sakurai, 1967a). The formation of reductic acid, 2,5-dihydro-2-furoic acid, and others are also reported, but their origins are not known (Coggiola, 1963). Although furfural and 3-DP are highly reactive products, the role of nonoxidative decomposition of AsA in browning reaction is considered relatively small (Kurata et al., 1967).

b. Oxidative Decomposition of AsA. The most important factors in the oxidative decomposition of AsA are pH and the presence of metal ions; decomposition is rapid in an alkaline medium and is much slower at a pH less than 7 in the absence of metal catalysts, e.g., Cu ions (Seib and Tolbert, 1982). Oxidation starts with a one-electron withdrawal from an AsA anion that produces the ascorbate anion radical, which disproportionates to dehydroascorbic acid (DHA) and AsA (Yamazaki et al., 1959; Bielski, 1982). DHA, which exists in a hydrated form in aqueous solutions, produces 2,3-diketo-L-gulonic acid (DKG) by ring opening, which is very slow at pH 2.4 and proceeds faster with increasing pH (Terada and Ohmura, 1966). The decomposition of inherently unstable DKG produces 2-furoic acid and 3-hydroxy-2-pyrone through decarboxylation and dehydration (Kurata and Sakurai, 1967b). The enol form of DKG also decomposes to 3-keto-4-deoxypentosulose (KDP) by dehydration and decarboxylation (Kurata and Fujimaki, 1976). A highly unstable DKG 3-lactone having a 3,4-enediol structure has recently been isolated and found to be very susceptible to browning; this compound is considered to be an important intermediate in the oxidative browning of AsA (Ohtsuka et al., 1986).

Besides the browning of AsA by the interaction of various decomposed carbonyl compounds, the reaction of carbonyl products (notably α-dicarbonyl compounds) with amino compounds leads to the active formation of pigments and flavors. The activity and variety of the reactions of DHA with amino compounds, e.g., Strecker degradation with α-amino acids, makes DHA an interesting subject of investigation.

2. Dehydroascorbic Acid–Amino Acid Reactions

a. Formation of Red Pigment. Initially, the reaction of DHA with α-amino acids in aqueous media rapidly produces a conspicuous red pigment, which is then followed by browning (Koppanyi et al., 1945; Kurata et al., 1973a,b). This pigment was found to be related to the coloration that occurs during the processing and storage of dried vegetables and other foods and is also considered to be an intermediate of the browning reaction (Nakabayashi and Shibata, 1967;
Ranganna and Setty, 1968). Since the reactions of DHA with most α-amino acids give the same kind of red pigment and liberate carbon dioxide and aldehydes, these reactions occur through typical Strecker degradations. The lactone ring in DHA seems to be a requisite for pigment formation because DKG does not form the pigment (Kurata et al., 1973a). Kurata et al. (1973b) successfully obtained this unstable pigment in a pure crystalline form by oxidation of L-scorbamic acid (SCA), a DHA–amino acid reaction product, and elucidated its structure as 2,2′-nitrilodi-2(2)-deoxy-L-ascorbic acid ammonium salt (NDA), which is formed by the condensation of SCA with oxidized SCA (dehydro-SCA) (Fig. 11). The hypothesis that the red pigments were pyrazine or dihydropyrazine derivatives was thus proved wrong (Lecocq, 1951). The murexide-type structure of red pigment was confirmed by detailed NMR and ESR studies and the calculation of π-electron densities or spin densities of the chromophore system using Hückel’s molecular orbital method (Kurata et al., 1986).

b. Formation of Free Radical Species by DHA–Amino Acid Reactions. Namiki et al. (1974) and Yano et al. (1974) found strong ESR signals with the formation of NDA in the reactions of DHA and α-amino acids in water or in an ethanol mixture. Analysis of hyperfine structures of the signals showed two components, A and C, in the signal of the aqueous reaction mixture and two components, A and B, in the signal of the ethanolic reaction mixture. Surprisingly, thin-layer chromatography yielded a blue spot indicating the radical species A (R—A) and a yellow spot indicating component C (R—C) close to that of NDA. The blue spot of R—A changed to the red spot of NDA with oxidation in air (Yano et al., 1976a). Increasing the pH of reaction mixture of DHA with amino acid to a neutral or weakly alkaline pH dramatically increased R—A, suggesting the presence of a precursor (Yano et al., 1978b). Hayashi and Namiki (1979) and Hayashi et al. (1981) succeeded in isolating this product and identified it as tris(2-deoxy-2-~-ascorbyl)amine, having a 3-fold symmetric structure around one nitrogen atom originating from the amino acid. Tsuji et al. (1980) examined the processes using electrochemical methods and confirmed that the dianion of the product produces R—A by eliminating one electron; the product additional one-electron oxidation produced a labile oxidized form which liberates a deoxy-AsA molecule and produces the red pigment NDA. R—C was found to be a one-electron reduction product of NDA (Yano et al., 1976a; Kurata and Fujimaki, 1974). Production of these radical species was enhanced by the addition of AsA. These products do not appear to play important roles in browning reactions (Hayashi et al., 1983b).

c. Browning Reactions. Hayashi et al. (1983a) also found considerable amounts of a yellow pigment on the developed chromatogram of a DHA–amino acid reaction mixture; analysis showed the presence of two nitrogen atoms,
FIG. 11. A possible pathway for formation of colored products, free radical products, and browning in the reaction of dehydroascorbic acid (DHA) and amino acid (Kurata et al., 1973b; Hayashi et al., 1983b).
cyclic DHA, hydrated DHA, and AsA moieties. The reactions of SCA with NDA or DHA produced large amounts of this pigment, which was thus assumed to be a condensation product of SCA with NDA. Heating the yellow pigment or its mixture with DHA in an aqueous solution produced pronounced browning, which suggests a browning reaction scheme involving the DHA–amino acid system via NDA and yellow pigment. One-electron reduction of this pigment produced a stable free radical species identified with the formerly observed radical component B.

From these experimental results, Hayashi et al. (1983b) proposed a whole scheme for the formation processes of the colored products, the free radical products, and browning in the reaction of DHA–amino acid system as shown in Fig. 11. The pathway involves complicated mutual redox reactions between starting materials, intermediates, and the products. This reaction system was also shown to produce a purple pigment (Hayashi et al., 1984) and new antioxidative products (Namiki et al., 1982a,b).

d. DHA–Alkylamine Reaction Systems. Paralleling the DHA–amino acid reactions, the reactions of DHA with alkylamines produce red pigments and free radical species. Since the Strecker degradation cannot take place in these cases, the presumed products are red pigments having a substituted nitrogen atom and corresponding free radicals. Yano et al. (1976b, 1978a) and Hayashi et al. (1978) isolated and deduced the structures of several products from DHA–methylamine and other systems.

An interesting finding is related to the DHA–alkylamine reaction of DHA with casein, serum albumin, and egg albumin under freeze-dried conditions at room temperature or 60°C (Namiki et al., 1986; Hayashi et al., 1986b). It was shown that NDA, the same red pigment as produced from DHA and α-amino acid, was also formed by this reaction. Studies including protein analyses and experiments using hexylamine and other model compounds confirmed that DHA reacts with the ε-amino group of lysine in proteins, resulting in deamination to give NDA and, at the same time, the formation of a carbonyl group, which leads to the polymerization of protein. The reactions of DHA with proteins in the presence of water produced rapid browning; no NDA was obtained, but deamination of this type is considered to play an important role in browning.

F. CHEMISTRY OF MELANOIDINS

In the final stage of the Maillard reaction, colored intermediates and other reactive precursor products (such as enamino products, low-molecular-weight sugar analogs, and unsaturated carbonyl products) proceed to condense and polymerize to form brown polymers, or melanoidins, under acceleration by an amine catalyst. As mentioned previously, the formation of intermediates differs
greatly by reactant and reaction conditions, and a variety of intermediate products are known to form even under fixed reactant and reaction conditions; so the browned products naturally differ in chemical structures and properties at least in degree of polymerization, depending on preparation conditions. It is wishful thinking to assume a single structure for a product of a Maillard reaction; even the most advanced separation and analytical methods currently available cannot be expected to clarify their structures completely. Nevertheless, continuous efforts are being made to determine the structures of representative melanoidin products, e.g., those prepared from glucose–glycine heated for a long time and then dialyzed. HPLC and GLC for separation, infrared (IR), NMR, MS, and ESR spectrometry for structural analysis, and refractive index (RI) and other advanced techniques are now available to accomplish what ionophoresis and elemental analysis did in the past, but the accumulated data are limited and, so far, only partial structures of the melanoidin products have been determined.

A general formula, "sugar + amino acid $- 2 \sim 3 \text{H}_2\text{O}," has been proposed for melanoids based on the consideration that the main pathway of melanoidin formation involves the reactions of amino compounds with deoxyosones, furfural, and other fragmentation products which accompany the dehydration reaction (Wolform et al., 1953; Kato and Tsuchida, 1981).

However, the evolution of carbon dioxide during melanoidin formation has been demonstrated, and it was noted that decarboxylation by Strecker degradation of $\alpha$-amino acids is responsible for this evolution. This was supported by studies using $^{14}\text{C}$-labeled amino acid (Stadtman et al., 1952; Wolform et al., 1953). Sugars can also be a source of carbon dioxide (Stadtman et al., 1952).

Liberation of aldehydes by Strecker degradation is also conceivable, as reported by Feather and Hung (1985, 1986) on melanoids derived from D-glucose-1- and -6-$^{14}\text{C}$, glycine-1- and -2-$^{14}\text{C}$, or methionine-1- and -[1-$^{14}\text{C}$]methyl. The fastest loss of carbon dioxide occurs by Strecker degradation, but loss of $^{14}\text{C}$-2 in glycine was also found to occur. The remaining numbers of carbon atoms were glucose-6-C > glucose-1-C > glycine-1-C > glycine-2-C after a 16-hr reaction, suggesting liberation of carbon dioxide and aldehydes. $^{13}\text{C}$-NMR results also support liberation of carbon dioxide and aldehyde. However, the remaining amounts of $^{14}\text{C}$ during the reaction (at 8, 16, and 24 hr) were not always constant, except in the glucose–methionine reaction system, suggesting the involvement of dehydration and rearrangement reactions after polymer formation. $^{13}\text{C}$ analyses of melanoids from glucose and L-alanine-enriched $^{13}\text{C}$-1 or $^{13}\text{C}$-2 indicated that both $^{13}\text{C}$-labeled carbons of L-alanine are incorporated into the polymer and C-1 and C-2 of L-alanine remain as a carboxyl carbon and a substituted amino carbon, so the source of the liberated carbon dioxide may not be limited to the carboxyl group of the $\alpha$-amino acid.

The study of xylose–glycine reactions by Benzing-Purdie and Ratcliff (1986), utilizing cross-polarized–magnetic angle spinning (CP-MAS) $^{13}\text{C}$- and $^{15}\text{N}$-
NMR, partly simulated soil humus formation at relatively low temperatures (22, 68°C). As the reaction progressed, $^{13}$C-NMR absorption corresponding to the aromatic, heteroaromatic, and heteroaliphatic C=C, C=O, and C=N bonds (120 ~ 160 ppm) increased while the absorption corresponding to the ester (or amide) C=O group decreased. $^{15}$N CP-MAS NMR also showed a decrease of amide nitrogen and an increase of tertiary nitrogen atoms of pyrroles and indoles in melanoidin. In general, the sugar species and amino acid species did not have a significant affect on the spectra, but the use of ammonia or urea as the nitrogen component produced some differences (Benzing-Purdie et al., 1983).

Nursten and O'Reilly (1986a) studied the petroleum ether and ethyl acetate-soluble fractions, which amounted to 0.1 wt% of the materials, of xylose–glycine melanoidin by HPLC. Over 100 fractions absorbing at 450 nm were found; these were divided into four classes by the peak characteristics. The largest number of fractions was found to absorb at 260 nm. A significant number of the fractions fluoresced by irradiation at 254 or 360 nm, and the authors considered them to be intermediates of the browning reaction. Even though some of the fractions were identified with furan derivatives as the colored intermediates by Ledl (1982; see Section III,D), his data serve mainly to highlight the extreme complexity of the melanoidin structure.

Formation of stable free radical species in melanoidin was first reported by Mitsuda et al. (1965) and was based on broad singlet ESR signals. Namiki and Hayashi (1973) found a new free radical species producing hyperfine ESR signals in the initial stage of melanoidin formation, as mentioned in Section II,C. Following this work, Wu et al. (1986) examined the ESR of melanoidin fractions. Melanoidins prepared from glucose with glycine, glutamic acid, and lysine (at pH 9.0, 120°C, 15 ~ 240 min) showed hyperfine ESR signals in their 90% ethanol-insoluble fractions. Glucose–glycine and glucose–glutamic acid melanoidins gave broad singlets and almost no hyperfine structure, but the glucose–lysine melanoidin retained some part of the signal with 33-line hyperfine structures (Namiki and Hayachi, 1983) after a 4-hr reaction. The main low-molecular-weight fraction of this melanoidin showed 9-line hyperfine structures by Sephadex G-50 gel chromatography.

The structure of melanoidin was considered to consist mainly of a repeating aromatized moiety because of its dark brown color. However, $^{13}$C-NMR studies by Kato and Tsuchida (1981) on pyrolysate and oxidized products of melanoidin from glucose–glycine, in addition to those by Olsson et al. (1981) on melanoidin from glucose–glycine, indicate that the spectrum of melanoidin is fairly simple, similar to that of Amadori products, and almost devoid of aromatic and olefinic carbon atoms. Kato and Tsuchida (1981) proposed that the structure of melanoidin has a repeating unit involving conjugated carbon double bonds and tertiary nitrogen (Fig. 12).

Recent studies by Kato et al. (1986), using GLC analysis of the ether-soluble
fractions of colorless ozonolysate or alkaline hydrogen peroxide decomposition products of melanoidin from glucose-glycine, suggested MeCO<, HOCH₂CH<, HOCH₂COH<, EtCHOHCH₂<, EtCHOHCOH<, Me₂COHCH<, and Me₂COHCOH< as groups forming partial structures of melanoidin. The water-soluble fraction of the ozonolysate contained 5.7% glycine, and incorporation of glycine in melanoidin was suggested by ¹³C-NMR of melanoidin from C-2-labeled glycine. ¹³CNMR of melanoidin from C-1-labeled glucose showed the possibility of glucose C-1 incorporation as methyl, saturated and unsaturated carbons, and aliphatic and aromatic carbons. The presence of a saturated aliphatic carbon chain stable against ozonolysis, in addition to aliphatic and aromatic unsaturated C=C bonds and C=O bonds in melanoidin, has been suggested by comparison of ¹³C CP-MAS NMR and ¹³C-NMR spectra before and after ozonolysis. These results support the chemical structure of melanoidin proposed previously (Kato and Tsuchida, 1981). The proposed scheme of melanoidin supports the presence of reductone (enaminol) structures, which have been considered to play
important roles in antioxidative activity as well as in the metal chelating activity of melanoidin (see Section III). It was also recently known that melanoidin inactivates some mutagens, perhaps because of its reducing activity and inclusion power (see Section IV).

So far, the application of modern analytical methods has succeeded only in accumulating data on the partial structures of melanoidins. Further investigations must continue to seek characteristic partial structures as well as variations in the reaction products using related starting materials and reaction conditions.

III. ANTIOXIDANTS AND THE MAILLARD REACTION

A. INTRODUCTION

Fatty acids (especially polyunsaturated fatty acids) in foods are very susceptible to oxidation by oxygen in air, which results in the formation of hydroperoxides by a free radical chain reaction. The hydroperoxides are subjected to further reactions giving various secondary products, such as volatile carbonyl compounds, polymerized products, and some active oxygen radical species. These processes result in the development of rancid flavor and other deteriorative changes in food quality that limit the storage life of many food products and materials. The lipid peroxidation processes are very complicated and involve numerous factors, which requires the use of various techniques suited to the characteristics of each food to prevent lipid peroxidation. The most effective methods are tight packaging with removal of oxygen gas and the use of antioxidants. Recent developments in packaging materials and oxygen-removal techniques have shown remarkable progress and contributed greatly to protecting food against microbial as well as oxidative deterioration. In many cases, however, it is desirable for the food to possess antioxidative properties, which necessitates effective antioxidants. The most widely utilized antioxidants are phenolic compounds, such as butylated hydroxyanisol (BHA) and butylated hydroxytoluene (BHT), but these synthetic compounds are currently in disfavor because of the potential hazards associated with their use (Ito et al., 1983a,b). The use of natural antioxidants, such as tocopherols, is increasing, although they are not as effective BHA and BHT.

On the other hand, much attention has recently focused on dietary antioxidants, such as vitamins E and C, as preventive agents against biological damage caused by active oxygen free radical species, especially damage to DNA and other important cell constituents which may possibly induce mutagenesis, carcinogenesis, and aging.

Given these circumstances, past and recent findings that the stability of foods increases when Maillard reactions occur within the food system are encouraging and, currently, the subject of much attention.
There are numerous examples of this kind of stabilization. Findley et al. (1946) reported increased antioxidative stability of powdered milk by treatment at 88–93°C for 20 sec before drying. This stabilization was considered to be due to the Maillard reaction that occurred during the treatment (Patton, 1955). The antioxidative action of the Maillard reaction products (MRPs) themselves (i.e., that of the MRP of glucose–glycine or glutamic acid system upon margarine oxidation) was first demonstrated by Franke and Iwainsky (1954) and Iwainsky and Franke (1956). Griffith and Johnson (1957) showed that using glucose instead of sucrose in cookie dough enhances the browning and antioxidative stability. Yamaguchi et al. (1964) observed increased stability with the addition of glucose and amino acids (especially valine, glycine, and lysine) to cookie dough in addition to the stabilizing effect of acetone extract on cookies baked after the addition of glucose and lysine. Improvement of antioxidative stability by heat treatment was observed by Anderson and co-workers (1963) in cereals such as wheat, corn, and oats.

These findings demonstrate the effectiveness of MRPs in the prevention of lipid peroxidation in foods. The Maillard reaction is very common in food processing and storage, especially in protein-rich foods subjected to heat treatment, so we have made use of this kind of process-induced antioxidants without fully understanding what is involved.

B. REACTION CONDITIONS FOR INDUCTION OF ANTIOXIDATIVE ACTIVITY

In MRPs from reducing sugars and amino compounds, the presence of an amino compound is required to induce antioxidative activity. This is evident from the fact that neither sugars nor their pyrolytic products (caramel) alone show activity, while there are many studies on the antioxidative activity of the compounds of secondary and tertiary linear or cyclic amines and amino acids or peptides, e.g., Marcuse (1962), Harris and Olcott (1966), Matsushita and Ibuki (1965), and Karel et al. (1966). However, the antioxidative activity of amino compounds reacting without sugar is far weaker than that induced by the Maillard reaction with sugars.

d-Glucose is used as the reactant carbonyl compound in most model Maillard reaction systems because it is the most common and abundant reducing sugar present in food and biological systems. However, as mentioned previously, pentoses are generally more susceptible to browning than hexoses, and, parallel to browning, xylose induces more potent antioxidative activity than does glucose in sugar–amino acid systems (Kirigaya et al., 1968; Lingnert and Eriksson, 1980a). Low-molecular-weight sugars and some dicarbonyl compounds, such as methylglyoxal, glyceraldehyde (Hayashi et al., 1986a), and dehydroascorbic acid (Namiki et al., 1982b), are known to be far more reactive in inducing the Maillard browning than are hexoses, such as glucose. The MRPs from dihydroxy-
yacetone with methionine and leucine (Itoh et al. 1975) and from methylglyoxal with leucine and isoleucine (Kawashima et al., 1977) prepared in corn oil at 175°C exhibit more potent antioxidative activity than those from xylose or glucose. Dehydroascorbic acid with tryptophan heated in ethanol for 30 min resulted in intense browning along with the induction of potent antioxidative activity. These two activities were negligible when glucose was used instead of dehydroascorbic acid (Namiki et al., 1982b).

Among the amino acids, on the other hand, MRPs from arginine, histidine, β-alanine, and cysteine with glucose showed stronger activities than MRPs from other amino acids (Kirisgaya et al., 1969). Tomita (1971a,b) studied the antioxidative activities of MRPs from several amino acids with glucose and demonstrated the superiority of MRPs from tryptophan over the others. Lingnert and Eriksson (1980a,b) examined the antioxidative activities of MRPs from glucose, fructose, or xylose with arginine, cysteine, glutamic acid, histidine, lysine, or valine using polarographic and gas chromatographic techniques. They observed potent activities in the MRPs from histidine and lysine with any sugar and in those from arginine with xylose, while MRPs from glutamic acid with each sugar showed no activity. They concluded that MRPs from basic amino acids produce higher activities than those from glutamic acid, valine, and cysteine. The arginine-xylose system produced especially strong activity.

Other reaction conditions (including pH, reaction temperature and time, molar ratio of the reactants and their concentrations, water activity, atmosphere, and the reaction medium) also greatly affect the induction of antioxidative activity. In general, higher media pH and higher amino compound/sugar molar ratios enhance the production of undialyzable melanoidins, the inclusion of nitrogen in MRPs, and the induction of antioxidative activity (Kirisgaya et al., 1969). Essentially the same tendencies in the effects of pH, molar ratio, and reactant concentration on the induction of antioxidative activity were reported for MRPs from glucose with various amino acids (Tomita, 1971a) and for MRPs from histidine-glucose (Lingnert and Eriksson, 1980a). Tomita (1971b) also noted the effect of buffer solution as a medium, and phosphate buffer more effectively enhanced the antioxidative activity from tryptophan-glucose than veronal or borate buffer. The optimum conditions are 0.1 M phosphate buffer and an initial pH of 9.0.

Maillard browning is shown to be most effective at intermediate water activity ($a_w$), e.g., 0.44–0.53 for whey powder (Saltmarch et al., 1981) and 0.60–0.70 for milk powder (Loncin, 1968). However, Eichner (1981) indicated that the lower water activity in the glucose-lysine-avicel freeze-dried system favors the production of antioxidative browning intermediates, which were assumed to be hydroperoxide-reducing Amadori products. That is, in the low-$a_w$ system, the formation of visible browning is suppressed while colorless reducing intermediates are still being formed.

Nonparallelism between browning and antioxidative activity during the heat-
ing process has, at times, been observed. Antioxidative activity in the glucose-histidine system does not necessarily parallel the formation of undialyzable brown products; it is known that the antioxidative activity increases with the browning in the first stage but decreases with further heating after reaching a maximum (Lingnert and Eriksson, 1981). Park and Kim (1983) studied antioxidative activity in ethanol extracts of the glucose-glycine reaction mixture. The activity was found at an early stage of browning in products which were almost colorless but showed intense fluorescence; the activity remained unchanged with prolonged heating.

These results suggest that the relationship between heating and antioxidative activity is not a simple one, and the manifestation of the activity appears to be a complicated process (although melanoidins themselves are known to be antioxidative).

C. STRUCTURE–ANTIOXIDATIVE ACTIVITY RELATIONSHIP

The antioxidative activity observed in Maillard reaction mixtures is assumed to be present mainly in melanoidins, but since our knowledge of their structural aspects is limited to some of their partial structures, a structure–activity investigation of melanoidins is, at present, beyond reach. From what we know about the MRPs, the first possible candidates for active principles come from the reductone group. The key intermediate of the early stage of the Maillard reaction is the Amadori rearrangement product, which is a kind of amino reductone. Moreover, an increase in the consumption of Tillman’s reagent as browning progresses has been reported (Kirigaya et al., 1968), which suggests the presence of reductone or amino reductone structures in melanoidin. Isolation and identification of amino reductones as an important intermediate product to melanoidin formation has been made (Ledl et al., 1986a,b).

The reductones are commonly considered to be an effective antioxidant due to their reducing activity and metal chelating ability; the experimental results, however, sometimes indicate an enhancement of lipid oxidation but not necessarily of the antioxidant (Yamaguchi, 1969). This contradiction was shown to be dependent upon the experimental conditions, especially on the moisture content of the system, in an experiment on ascorbic acid, a typical reductone, and a hydrogen donor (Ueda et al., 1986); whereas ascorbic acid is an antioxidative in a nonaqueous or low-moisture-content system, it is inactive (or may even act as an oxidation promotor) in an oil–water system.

Compared to the enediol-like reductones, amino reductones are more effective and more likely to be stable. Evans et al. (1958) showed the strong antioxidative activity of amino reductones prepared from secondary amines with aldohexose, and Obata et al. (1971) and Itoh et al. (1975) indicated that amino reductones from triose reductone with glycine, methionine, and valine have superior antiox-
idative activities than the original triose reductone, ascorbic acid, and reductic acid. Eichner (1981) suggested that reductone-like products, probably 1,2-enaminols formed by the Amadori rearrangement, may be responsible for the antioxidative activity of MRPs, which is assumed to be caused by the reduction of hydroperoxides to inactive products. Thus, the contribution of the enaminol structure in MRPs to antioxidative activity seems highly probable, but does not appear to depend on its reducing activity, as indicated by the results of the ozonolysis of melanoidins described shortly. In any case, the effect of reductone in MRPs remains inconclusive.

The metal chelating ability of reductone is assumed to contribute to the antioxidative activity, since Cu, Fe, and other heavy metal ions play an important role in promoting lipid peroxidation. The metal chelate does not always act as an inhibitor, but it sometimes promotes oxidation as exemplified by the copper–ascorbate system, possibly due to the production of some active oxygen species in the presence of oxygen (Martell, 1982).

Melanoidins are known to have high metal binding activity (Johnson et al., 1983), which is also assumed to be due to the reductone moiety. Yamaguchi and Fujimaki (1974b) observed that the antioxidative activity of tocopherol decreased in the presence of a small amount of copper ion, while that of melanoidin was not significantly affected. This suggests that the MRPs have some metal inactivating activity which may contribute synergistically to the antioxidative activity of melanoidins.

Several studies have been conducted to elucidate the chemical structure, by fractionation and chemical modification, of melanoidin as it relates to antioxidative activity. Yamaguchi et al. (1981) examined the activities of Sephadex gel chromatographic fractions of MRP from xylose–glycine (1:1). The first chromatography, G-15, gave two reducing peaks, and only the higher molecular weight fractions showed antioxidative activity. Further fractionations (G-50, G-100), and paper and thin-layer chromatography gave a markedly antioxidative fraction, with a molecular weight of around 4500 that gave a single spot by paper chromatography, but was not a purified substance. The antioxidative activity, however, was found widely distributed in other fractions by gel chromatography.

Lingnert and Eriksson (1983) attempted to characterize the antioxidative products of MRP from histidine and glucose. Upon dialysis through membranes with a nominal molecular weight cutoff of 1000, antioxidative compounds were concentrated in the retentate. Further purification using isoelectric precipitation and preparative electrophoresis gave somewhat more active fractions but did not yield a special fraction having intense activity, while some correlations were found between ESR signal intensity and the antioxidative activity of the fractions.

These results, even though interesting in themselves, are not sufficient to draw any conclusion about the nature of the antioxidative activity of melanoidins. However, a recent study of ozonolysis of antioxidative melanoidins (Yamaguchi,
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1986) suggests a direction for future investigation: ozonolysis discolored the melanoidin and decreased its reducing capacity to a constant level but, interestingly, had no significant effect on the antioxidative activity. Sephadex G-25 fractionation of the ozonolyzed product gave a degraded, low-molecular-weight, nearly colorless fraction having higher antioxidative activity. IR examination showed a disappearance of C=C bonds and an increase of C=O groups, which agrees with the results of Kim et al. (1985). These ozonolysis products also showed synergistic activity with tocopherol. Although it is not yet clear if the initial activity in the melanoidin remained intact or the activity was an artifact of ozonolysis, this finding suggests that unsaturated melanoidins may not be essential for manifestation of the activity.

To elucidate chemical structure of the antioxidative principle of amino-carbonyl reaction products, Namiki et al. (1982b) investigated the reaction mixture of dehydroascorbic acid with tryptophan. This system demonstrated far stronger antioxidative activity than glucose-glycine; the reaction was conducted in refluxing ethanol to prevent production of unseparable high-molecular-weight melanoidin. From active HPLC fractions of butanol extracted from the reaction mixture, one of main active principles was isolated in crystalline form and identified as being a new equimolar condensation compound consisting of tryptophan and dehydroascorbic acid. (Namiki et al., 1982a).

D. APPLICATION

Apart from the chemical nature of the active principles, there is no doubt about the effectiveness of MRPs in practical food processing. Yamaguchi et al. (1964, 1967a,b) found that the antioxidative stability of lard in cookies was increased greatly by adding glucose and certain amino acids (especially valine, glycine, and lysine) to the dough. An observation has also been made that the addition of a histidine and glucose mixture to cookie dough before baking showed more effective antioxidation than the addition of MRPs prepared from the same mixture (Lingnert, 1980; Lingnert and Eriksson, 1981) and that the increase in activity paralleled browning. Regarding the oxidative stability of sausage during frozen storage, the development of rancid flavor was found to be effectively retarded by the addition of MRPs from glucose and histidine, but no effect was obtained by the addition of a histidine-glucose mixture (Lingnert and Lundgren, 1980). The inconsistency in these two cases may be due to insufficient heating in the latter. Enhancement of antioxidative activity of spray-dried whole milk was also observed by preheating with the addition of histidine and glucose, although it was accompanied by a decrease in the lysine content and some coloration (Lingnert and Hall, 1986).

The search for economical and effective MRP materials for practical food processing includes a study by Obretenov et al. (1986), who tested, using lard as the substrate, the antioxidative effectiveness of the reaction products of acid-
hydrolyzed bovine blood or sunflower groat with hydrolyzed starch and found that the former product is more effective, perhaps due to the higher content of sulfur-containing, chain-branched, basic amino acids. The best results were obtained when a 2:1 amino material–carbohydrate mixture was heated for 20 hr at 100°C.

Utilization of MRPs for food preservation may sometimes cause problems because of their inherent color. The decoloration of MRPs by ozonolysis without a significant decrease in the antioxidative activity (Yamaguchi, 1986) may provide a simple solution. While ozonolysis by itself is impractical for use in food processing from the standpoint of food safety, it may be possible to develop an acceptable method of biological oxidation based on the process.

Synergistic effect with other antioxidants, especially those of natural origin, is very important for preventing undesirable coloration with melanoidin. Yamaguchi and Fujimaki (1974a,b) demonstrated that MRP from xylose with ammonia and purified by gel chromatography showed marked synergistic effect in a model system with β-, γ-, and δ-tocopherol, but not with α-tocopherol. In margarine, the effect was stronger with the addition of citric acid and, in combination with 0.005–0.01% tocopherol and 0.0025% melanoidin [below its coloring level (0.005%)], inhibits autoxidation very effectively.

In any event, antioxidation is a very important factor in food processing and preservation, and the fact that it can be induced in food by intercomponent reactions is not only valuable information for food production and storage but also a very interesting problem in basic food chemistry. Although considerable information has been obtained with regard to conditions for inducing the activity, little is known about the chemical properties of the active principles involved, except that a complex MRP structure involving nitrogen appears to be responsible for the antioxidative activity. In order to fully utilize the antioxidative activity of MRPs, much more information on controlling the Maillard reaction in food processing, as well as on the chemical properties of melanoidins, is needed.

IV. MUTAGEN FORMATION

The development of simple and reproducible mutagenicity tests (Kada et al., 1972¹; Ames et al., 1975²) resulted in the detection of numerous mutagenic

¹The Rec-assay system is a method used to detect DNA-damaging substances by comparing the growth of DNA recombination-proficient (H17 rec+) and -deficient (M45 rec−) strain of Bacillus subtilis on a medium on which a paper disk containing test sample has been placed (Kada et al., 1972).

²The Ames test is a microbial assay method using mutants of Salmonella typhimurium LT2 to detect gene mutagens by counting revertant colonies having a histidine requirement to prototrophy after treatment with mutagens. The TA98 strain, specific for frameshift-type mutagens, and the TA100 strain, for base-pair substitution-type mutagens, are the most commonly used strains. The S9 mix, rat liver cell homogenates, is used to metabolically activate the test sample. (Ames et al., 1975).
substances in various foods and in the environment. Although mutagens are not always carcinogenic, nearly all known carcinogens are mutagenic (McCann et al., 1975; Sugimura et al., 1976). Since epidemiological research has revealed that the major causes of human cancer occur via the oral route (Wynder and Gori, 1977; Weisburger et al., 1980; Doll and Peto, 1981; Ames, 1984), studies on the presence and the formation of mutagens in food are of extreme importance.

A. PYROLYSATE MUTAGENS

Besides the mutagens which occur naturally in foods (such as bracken and cycad) and those due to contamination by chemicals or mycotoxin-producing molds, mutagens produced by chemical reactions of food constituents during storage, processing, and cooking are of equal importance. N-nitrosoamines are the best known of this kind (Gough, 1978), but recent findings of mutagenic pyrolytic products found in broiled fish and meat, for example, have been the cause of general alarm. The study of these pyrolytic products was initiated by the discovery of mutagens other than benzo[a]pyrene, which was formerly believed to be the major mutagen in smoke condensate of broiled fish (Masuda et al., 1966; Sugimura et al., 1977a) and in cigarette smoke (Kier et al., 1974; Hutton and Hackney, 1975; Sugimura et al., 1976; Mizusaki et al., 1977). Matsumoto et al. (1977), in a study on tobacco smoke, baked various amino acids at 300-800°C and found, by the Ames test (using TA98 with S9 mixture), that the tar produced from tryptophan was essentially highly mutagenic and, in a study of indol derivatives, revealed the importance of 3-position side chains for the manifestation of pyrolytic mutagenicity. The mutagenicity of smoke extracts from broiled sardine and beef was shown by the Ames method (TA98, S9 mix) (Nagao et al., 1977a), as was that of the scorched part of grilled beef (Commoner et al., 1978). The strong mutagenicity of pyrolysates of proteins (e.g., lysozyme) and tryptophan and the nonmutagenicity of pyrolysates of DNA, RNA, starch, and vegetables were noted by Nagao et al. (1977b). Strongly mutagenic products Trp P-1 and P-2 were isolated from pyrolysate of tryptophan at temperatures over 300°C (Sugimura et al., 1977b) as well as Glu P-1 and P-2 (Yamamoto et al., 1977) (Table IV). Tryptophan-containing especially, proteins and, peptides produced stronger mutagenic pyrolysates than did proteins not containing tryptophan, and the main mutagens in tobacco smoke were found to be the pyrolysates of tobacco proteins (Matsumoto et al., 1978). The main mutagens in soybean protein pyrolysate were identified as α-carboline and methyl-α-carboline (Yoshida et al., 1978).

These studies investigated mainly pyrolytic tarry products (of tryptophan or a protein alone) obtained in smoke at high temperatures of over 300°C, conditions which are not encountered in day-to-day cooking. However, in time it was discovered that mutagenicity also resulted in ordinary cooking conditions, e.g., broiled, semifried fish and grilled hamburger, with the formation of extraor-
<table>
<thead>
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<th>Chemical name</th>
<th>Abbreviation</th>
<th>Structure</th>
<th>Source of isolation</th>
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<td>Trp-P-1</td>
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<td>Tryptophan pyrolysate</td>
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</tr>
<tr>
<td>3,4-Cyclopentenopyrido-[3,2-α]imidazole</td>
<td>Lys-P-1</td>
<td><img src="image5" alt="Structure" /></td>
<td>Lysine pyrolysate</td>
</tr>
<tr>
<td>4-Amino-6-methyl-1H-2,5,10,10b-tetraazafluoranthene</td>
<td>Orn-P-1</td>
<td><img src="image6" alt="Structure" /></td>
<td>Ornithine pyrolysate</td>
</tr>
<tr>
<td>2-Amino-5-phenylpyridine</td>
<td>Phe-P-1</td>
<td><img src="image7" alt="Structure" /></td>
<td>Phenylalanine pyrolysate</td>
</tr>
<tr>
<td>2-Amino-9H-pyrido[2,3-b]indole</td>
<td>AaC</td>
<td><img src="image8" alt="Structure" /></td>
<td>Soybean globulin pyrolysate</td>
</tr>
<tr>
<td>2-Amino-3-methyl-9H-pyrido[2,3-b]indole</td>
<td>MeAaC</td>
<td><img src="image9" alt="Structure" /></td>
<td>Soybean globulin pyrolysate</td>
</tr>
<tr>
<td>2-Amino-3-methylimidazo[4,5-f]quinoline</td>
<td>IQ</td>
<td><img src="image10" alt="Structure" /></td>
<td>Broiled sardine</td>
</tr>
<tr>
<td>2-Amino-3,4-dimethylimidazo[4,5-f]quinoline</td>
<td>MeIQ</td>
<td><img src="image11" alt="Structure" /></td>
<td>Broiled sardine</td>
</tr>
<tr>
<td>2-Amino-3,8-dimethylimidazo[4,5-f]quinoxaline</td>
<td>MeIQx</td>
<td><img src="image12" alt="Structure" /></td>
<td>Fried beef</td>
</tr>
</tbody>
</table>

\( \text{a} \) Nagao et al. (1983).
ordinarily high mutagenic products 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) and 2-amino-3,4-dimethylimidazo[4,5-f]quinoline (MeIQ) from broiled fish (Kasai et al., 1980a,b) and 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx) from broiled beef; these mutagens were isolated and identified as such by Kasai et al. (1981a,b). The results of various studies on the formation of mutagens fluctuated, and mutagenicity varied greatly depending on cooking conditions such as temperature, time, and moisture content (Spingarn and Weinburger, 1979; Pariza et al., 1979; Dolara et al., 1979); in the beginning it was not known that the Maillard reactions are involved in mutagen formation.

Significantly, Yoshida and Okamoto (1980a,b) reported the development of mutagenicity when glucose was added to mixed amino acids before pyrolysis at 150°C, while no activity was noted for mixtures of glucose with albumin or adenine, or in the case of albumin or adenine each by itself. Noteworthy was the remarkably strong mutagenicity in pyrolysates of the mixtures of creatine with amino acids (especially cystine, threonine, phenylalanine, and methionine), glucose, or fatty acids, even though pyrolysis of creatin and the other materials by themselves did not produce mutagenicity (Yoshida and Okamoto, 1980c; Yoshida and Fukuhara, 1982).

Jägerstad et al. (1983) found stronger mutagenicity in pyrolysates of beef with high sugar content than with low sugar content and reported that the addition of sugars increases the mutagenicity of pyrolysates; thus, they pointed out the importance of the presence of both sugar and amino compounds. It was as a result of these reports that the relationship between Maillard reactions and mutagenicity came to receive considerable attention (Barnes et al., 1983).

Heating creatine-glucose-glycine or alanine systems in diethylene a glycol-water system at 130°C for 2 hr induced mutagenicity (Ames test, TA98 with S9 mix), which led to a proposal regarding the route of formation of the imidazoquinoline mutagens in fried beef (Jägerstad et al., 1983) (Fig. 13). There is some question concerning the formation of the imidazoquinoline mutagens, especially in pyrolysates of the model system. Formation of IQ was first discovered in broiled fish in Japan. The discovery was widely reported, becoming a source of concern throughout the nation. Since it was found in broiled fish, it was believed that IQ would also be found in grilled beef, a major part of the diet in the West. This turned out not to be the case, however, since fried or grilled beef indicated only slight amounts of IQ; rather, the main mutagen in beef is MeIQx (see Table V). This is another case where dietary customs gave rise to different findings. Jägerstad et al. (1984) isolated MeIQx (but not IQ) from a heated mixture of creatine–glucose–glycine using HPLC. Negishi et al. (1984) isolated another new mutagen from same reaction mixture. Recently, Nyhammer et al. (1986) determined, by HPLC, the presence of MeIQx and a small amount of IQ in a heated mixture of creatine–fructose–glycine, as well as the presence of 4,8-DiMeIQx and a slight amount of MeIQx in a mixture of creatine–fructose–DL–
alanine heated at 140°C for 2 hr. IQ was also isolated from a heated mixture of creatine and proline (Yoshida et al., 1984). Jägerstad et al. (1983) also observed that the addition of 2-methylpyridine in the creatine-glucose-glycine system enhanced the induction of mutagenicity.

Although the formation mechanism is yet to be clarified, these results clearly indicate involvement of the Maillard reactions in the formation of imidazoquinoline mutagens found in broiled fish and beef. The essential point of the formation seems to be the creatine–sugar–amino acid combination, and creatine is believed to be the key component that provides the imidazole ring to the IQ analogs, which are considered to be the active site of the mutagens.

Effective adsorption with "blue cotton" (cotton bearing copper-phthalocyanine) for IQ analogs in food and environmental systems (Hayatsu et al., 1983) and HPLC analysis (Wakabayashi et al., 1986) have been developed, enabling detection of very small amount of IQ and MeIQ. Employing HPLC, Wakabayashi et al. (1986) reported contents of MeIQx in broiled beef (0.5 ng/g), broiled chicken (2.1 ng/g) and fried ground beef (0.3 ng/g), as well as IQ in bacteriological-grade beef extract (41.6 ng/g).

IQ and MeIQ have been shown to be carcinogenic in mice (Ohgake...
### TABLE V
EXAMPLES OF QUANTITATIVE ANALYSES FOR MUTAGENS IN COOKED FOODS

<table>
<thead>
<tr>
<th>Food material</th>
<th>Method of cooking</th>
<th>Methods of identification and quantification</th>
<th>Mutagens in cooked material (ng/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sun-dried sardine</td>
<td>Naked flame</td>
<td>GC/MS with MID</td>
<td>Trp P-1 13.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Trp P-2 13.1</td>
</tr>
<tr>
<td>Sun-dried sardine</td>
<td>Naked flame</td>
<td>GC/MS with MID by addition of CD&lt;sub&gt;2&lt;/sub&gt;-substituted MeIQ</td>
<td>IQ 158</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>MeIQ 72</td>
</tr>
<tr>
<td>Beef</td>
<td>Electric hot plate</td>
<td>GC/MS with MID</td>
<td>IQ 0.59</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>MeIQx 2.4</td>
</tr>
<tr>
<td>Beef</td>
<td>Naked flame</td>
<td>Identified by fluorescence and MF, quantified by GC/MS</td>
<td>Trp P-1 53&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Chicken</td>
<td>Naked flame</td>
<td>Fluorescence</td>
<td>A&lt;sub&gt;a&lt;/sub&gt;C 180</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>MeA&lt;sub&gt;a&lt;/sub&gt;C 15</td>
</tr>
<tr>
<td>Sun-dried cuttlefish</td>
<td>Naked flame</td>
<td>Identified by UV, quantified by MF</td>
<td>Glu P-2 280&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Nagao et al. (1983).
<sup>b</sup> ng/g of raw beef.
<sup>c</sup> ng/g of sun-dried material.

1984, 1985), and IQ to be carcinogenic in rat (Takayama et al., 1984), but their carcinogenicity was revealed not to be as strong as first supposed from their extremely strong mutagenicity as determined by the Ames test (with TA98 and S9 mix). The amounts of these mutagens in ordinary food as shown in some examples are known to be very small, and it is difficult to evaluate whether or not they constitute significant carcinogenicity with respect to human cancer. It should also be noted that mutagen formation in Maillard reactions has been observed only in water-poor model systems at high temperatures (above 130°C), and IQ and other mutagens are not formed in water-rich cooking conditions.

### B. MUTAGENS OF AQUEOUS MAILLARD REACTIONS

The toxicity of melanoidins, which are produced in aqueous systems by browning reactions at lower temperatures (below 100°C) has been discussed (Lang and Baessler, 1971; Lee et al., 1981). Are they mutagenic? Caramel prepared from sugars alone or with ammonia and used in various beverages and liquors was first investigated for mutagenicity by Aeschbacher et al. (1981) using the Ames test, and no mutagenicity was observed in food caramel obtained
by heating pure sugar. No mutagenicity was noted in hydroxymethylfurfural (HMF) or in a plant protein hydrolysate. Recently, Scheutwinkel and von der Hude (1985) also reported no mutagenicity of commercially available caramel colors in Ames tests using four strains with or without S9 mix.

Omura and his group (Shinohara et al., 1980, 1983; Omura et al., 1983) examined the mutagenicity of browned aqueous mixtures of glucose with amino acids at 100°C using the Ames test and Rec-assay. The results showed varying mutagenicity depending mainly upon the kind of amino acid, the reaction conditions (especially pH), and the test systems used. As shown in Table VI, for the reaction systems using glucose–amino acid, the Ames test gave mutagenicity data that divided the amino acids into five groups which behaved differently according to the test bacterial strain (TA98 or TA100) and to the presence or absence of S9 mix. Among the amino acids, tryptophan, glutamic acid, and aspartic acid showed no mutagenicity despite the strong mutagenicity observed in pyrolysis of the first two. Mutagenicity was shown for lysine only with TA100 and without S9; for arginine, with TA100 and TA98 with S9, and for cysteine, with TA100 without S9. Their results also showed dependence of mutagenicity on sugar species, which in turn depend on the amino acid used.

The reaction between glucose and glutamic acid, arginine, or cysteine showed weak mutagenicity in Rec-assay and tests on pupal oocytes of silkworms (Yamashita et al., 1981).

Powrie et al. (1981) demonstrated that the Maillard reaction in the mixtures of glucose with various amino acids (especially lysine and arginine) without S9 mix induced significant increases in the chromosomal aberration test using Chinese hamster ovary (CHO) cells; mitotic recombination and mutation was induced in Saccharomyces cerevisiae. The activities depended heavily on initial pH of the Maillard reaction and were stronger at pH 10 than at pH 7.

<table>
<thead>
<tr>
<th>Group</th>
<th>Kinds of browned solutions with Glc</th>
<th>TA100</th>
<th>TA98</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Lys, HCl, Leu, Ser, Thr, Met, Gln, Pro</td>
<td>+ Decrease</td>
<td>-</td>
</tr>
<tr>
<td>B</td>
<td>Arg, Gly, Ala, Val, Ile</td>
<td>+ Decrease</td>
<td>- Increase</td>
</tr>
<tr>
<td>C</td>
<td>CySH</td>
<td>+ Increase</td>
<td>+ Increase</td>
</tr>
<tr>
<td>D</td>
<td>Phe</td>
<td>+ Increase</td>
<td>-</td>
</tr>
<tr>
<td>E</td>
<td>Trp, (Cys),2, Tyr, Asp, Asn, Glu</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* Shinohara et al. (1983).

+ , Positive mutagenic activity; -, no detectable mutagenic activity.
These results indicate the complexity of determining mutagenicity in Maillard reaction mixtures, with different test methods often giving contradictory results.

Concerning the mutagenic principle of Maillard reaction mixtures, Omura et al. (1983) isolated 5-hydroxymethylfurfural (HMF) from a glucose–phenylalanine system, HMF and a pyrrole derivative from a glucose–lysine system, and a thiazolidine derivative from a glucose–cysteine system. This was the first time that HMF, well known as an important intermediate of browning and flavor formation, was shown to be mutagenic.

According to Shinohara’s detailed study on the formation of furan derivatives (Shinohara et al., 1986), larger amounts of HMF are produced by lysine and arginine reactions with arabinose, xylose, or glucose (all at pH 7.0) than in reactions of phenylalanine, glutamic acid, lysine, and arginine with fructose at pH 2.0. In these cases, the amounts of HMF formed did not parallel the degree of browning; the highest degree of browning occurred in alkaline media with lysine and glycine without the formation of HMF. Some organic acids, e.g., oxalic acid and tartaric acid, promoted the formation of furan derivatives. Although the mutagenicity of HMF, shown by TA100 with S9 mix, is much weaker than that of IQ and its analogs, it is a cause for some concern because it is easily produced by Maillard reactions. Its carcinogenicity, however, is not known.

Shibamoto and co-workers examined the mutagenicity of Maillard reaction model systems of maltol-NH₂ (Shibamoto, 1980), L-rhamnose-NH₂-H₂S (Toda et al., 1981), and cysteamine glucose (Mihara and Shibamoto, 1980) and detected mutagenicity by Ames test in the fractions containing pyrazine, pyrrole, and thiazolidine derivatives; the mutagenic principles were not identified (Shibamoto, 1982).

There are some other mutagenic products in foods which may be related to the Maillard reaction. Nagao et al. (1979) investigated the mutagenicity of coffee and claimed methylglyoxal (~10 μg/ml in regular coffee) to be its mutagenic component (TA100 without S9 mix) and warned of the potential hazards of coffee (Kasai et al., 1982). Aeschbacher et al. (1980a,b), on the other hand, refuted this claim, stating that coffee showed only weak mutagenic activity toward TA100 in the Ames test which, moreover, disappeared with the addition of S9 mix. It should be kept in mind that coffee possesses bactericidal activity, a fact which casts some doubt on results obtained using the Ames test.

Methylglyoxal was proposed to be an important fragmentation product of the Amadori product in the Maillard reaction by Hodge (1967); this was recently confirmed by Hayashi and Namiki (1986a,b). Methylglyoxal is present in many foods, e.g., caramel (Hodge et al., 1963; Severin et al., 1984), bread (Wiseblatt and Kohn, 1960), and broiled potato (Kajita and Senda, 1972). Recently, Hayashi and Shibamoto (1985) noted a much higher content of methylglyoxal than previously found in coffee and root beer using a newly developed analytical method (Hayashi et al., 1985b).
Methylglyoxal is known to be a very reactive compound and is assumed to be an important active carcinogenic carbonyl compound \textit{in vivo} (Szent-Györgyi, 1980), but whether methylglyoxal in food is related to human cancer remains to be elucidated.

C. NITROSO MUTAGENS

\textit{N}-nitrosoamines, the most hazardous of the carcinogens produced by the interaction of food components, are produced mainly from secondary amines and nitrous acid. Since the Maillard reaction produces a number of secondary amines including the Amadori products, the possibility of mutagen formation cannot be ignored. At the same time, it is also possible that the reductive products of the reaction exhibit desmutagenic action (Kada et al., 1982) on the formation of \textit{N}-nitroso compounds, as has been evidenced for ascorbic acid, a representative reductone (Mirvish et al., 1972; Mirvish, 1981); reductone-like properties of Maillard products have been noted previously.

Several reports concerning the Maillard reaction–nitrous acid complex system have been published (Coughlin et al., 1979). Heyns et al. (1974, 1979) described the formation of nitrosated Amadori compounds, and Russel (1983) showed formation of positive dose–response mutagenicity of a nitrosated fructose–tryptophan mixture for both TA98 and TA100 strains without S9 mix in the Ames test. Shibamoto has reviewed Maillard nitrite reactions (1983a) and focused on the mutagenicity of nitroso derivatives of thiazolines, which are important meat flavor components (Fujimaki et al., 1969) produced by the Maillard reaction (Sakaguchi and Shibamoto, 1979; Mihara and Shibamoto, 1980; Sekizawa and Shibamoto, 1981).

Although the mutagenicity of soy sauce was once the subject of some public concern, it was later disproved (MacDonald and Dueck, 1976; Nagahara et al., 1986). However, mutagenicity was found to develop when nitrite was added to soy sauce (Lin et al., 1979), a phenomenon which was not considered to be caused by nitrosamines (Shibamoto, 1983b).

Wakabayashi et al. (1983) also investigated the production of mutagens in the nitrite–soy sauce system and isolated \textbeta-carboline derivatives and Tyramine as precursors of the induced mutagen principle in the mixture (Ochiai et al., 1984). The carbolines, considered to be a product of the reaction of tryptophan with methylglyoxal, furfural, or hydroxymethylfurfural, probably produce nitroso mutagens by reaction with nitrite.

Kinae et al. (1986) demonstrated the induction of significant mutagenicity upon addition of nitrite to a very slightly mutagenic reaction product of tryptophan with carbohydrates and other compounds, especially carbonyl compounds such as triose reductone, diacetyl, and furfural. Mutagenicity has been attributed to the formation of \textbeta-carboline compounds by condensation and
cyclization followed by N-nitrosation by nitrite, although the final products have not yet been identified.

At the time these findings were reported, they became cause for general alarm throughout Japan concerning the safety of soy sauce and other foods. However, mutagenicity under practical conditions was, for the most part, disproved because of the enormous difference in nitrite concentration levels found in soy sauce and the experimental model systems, as well as because of evidence of the presence of reaction inhibitors in soy sauce (Nagahara et al., 1986; Shibamoto 1983b).

Yen and Lee (1986) investigated mutagenicity of a browned casein-glucose system with or without nitrite by the Ames test (TA 100, 102, and 104 with S9 mix). The ether extracts showed mutagenicity in the nitrite-treated casein-glucose mixtures, especially in the browned casein-glucose mixture, but no activity was observed in the ether extracts of casein-glucose, browned casein-glucose, or nitrite-casein systems, nor was any activity observed in the water extract of samples of any test strain. Amino acid analysis showed a marked decrease in lysine content, especially in nitrite-treated systems.

D. ANTIMUTAGENESIS

The formation of these mutagens in foods naturally presents serious problems regarding safety. However, it must also be noted that, because studies on food mutagenicity are still in an early stage, in many cases it is not well understood whether or not mutagens are actually formed and/or function in actual food processing and living systems as they do in model systems. Inactivation of mutagens in food by other components has also been demonstrated. e.g., the inactivation of Trp P-1 by vegetable juices, especially cabbage, broccoli, and burdock (Kada et al., 1978; Morita et al., 1978; Inoue et al., 1981) and also by BHA and others (Barnes et al., 1983). The enzyme peroxidase in horseradish (Yamada et al., 1979) and saliva (Nishioka et al., 1981) has also been found to inactivate mutagens. Unlike peroxidase or other enzymes, the mechanism of inactivation in the case of burdock appears to be adsorption by some polymer (Morita et al., 1984, 1985). The elimination of mutagenicity has been observed with the addition of nitrite (Yoshida and Matsumoto, 1978) (Tsuda et al., 1980) and chlorine (Tsuda et al., 1983), both of which appear to inactivate the active site by some kind of simple chemical reaction. The term desmutagen, proposed by Kada et al. (1982) for substances possessing such action, represents this concept well. Chan et al. (1982) reported the desmutagenic effects of a lysine-fructose reaction mixture and caramelized sucrose on pyrolysate mutagens, although the effective components have not yet been characterized. Kim et al. (1986) examined the effect of the addition of fractionated glucose-glycine melanoidin (molecular weights <1000, 1000–5000, and >5000) on the muta-
genicity of Trp P-1, Trp P-2, Glu P-1, Glu P-2, IQ, etc. and confirmed desmutagenic action that grew stronger with increasing molecular weight and paralleled the reducing power and antioxidative activity. Sodium borohydride reduction lowered desmutagenic activity, and the inactivation of the pyrolysates by humic acid has also been reported (Sato et al., 1986, 1987). The desmutagenic effect of melanoidin, as well as that of humic acid, may be the result of the adsorptive activity of mutagens, as has been observed in the case of a desmutagenic lignanlike fraction of burdock (Morita et al., 1984). Desmutagenic activity against pyrolysate mutagens by low-molecular-weight carbonyl compounds (such as diacetyl and glyceraldehyde, which are assumed to be fragmentation products of the Maillard reaction) has also been reported (Kim et al., 1986); this is thought to be due to an amino–carbonyl reaction between these carbonyl compounds and an amino group of the pyrolysate mutagens.

While these substances possess desmutagenic activity, compounds also exist which, although not mutagenic themselves, appear to promote mutagenicity. β-Carbol ine compounds from soybean protein pyrolysate are an example of this type (Nagao et al., 1977c).

Although it may not be directly involved in the mutagenicity of Maillard reaction products, some Amadori products have been found to induce site-specific DNA breaks on some phages (Komano et al., 1986; Kashimura et al., 1986). The effect depends on the Cu²⁺ ion and is inhibited by radical scavengers, suggesting the involvement of active oxygen radicals.

The involvement of the Maillard reaction and its products in the formation and elimination of mutagens is gradually being elucidated by studies which are being conducted mainly in Japan. The mutagenicity of pyrolysates once caused grave concern but, given the evidence of their low levels in actual food, their inactivation by other food components, and their own weak carcinogenicity, it now seems that their actual involvement in carcinogenesis is less serious than first thought. The actual role of pyrolysate mutagens in carcinogenesis should be carefully examined taking into account both the overall process of food preparation and intake involving the desmutagenic actions.

V. TRENDS IN CONTINUING RESEARCH

As mentioned at the beginning, among the interactions of food and biological constituents, Maillard reactions are unique, important, and involve many problems in numerous fields of food science and technology. The present view covers only recent developments concerning the browning reaction mechanism and the antioxidative and mutagenic activities of Maillard products, dealing mainly with problems concerning low-molecular-weight compounds. In addition to browning and other organoleptic aspects, Maillard reactions involving proteins are, of
course, very important for food quality, especially from the nutritive and physiological viewpoints. However, these topics are treated in a number of other studies and have not been included in this limited review. This final section deals with relevant problems which require further investigation.

A. BROWNING REACTION MECHANISM

The mechanism for the Maillard reaction proposed by Hodge in 1953 has been accepted widely as the most appropriate description for the production of melanoidin, the browning polymer. The mechanism involves Amadori rearrangement as a key step to give major intermediates for browning polymers. However, the browning is known to be influenced greatly by reactants, pH, and other conditions, so it seems reasonable to assume the existence of different pathways that depend mainly on the pH and the reactant. In this respect, the new browning reaction mechanism described in Section II,C is noteworthy since it is the first which emphasizes the importance of sugar fragmentation occurring prior to Amadori rearrangement. Experimental results showed that the occurrence of such sugar fragmentation is negligible under acidic conditions, and the browning probably proceeds according to Hodge's proposal (although the rate of browning is slow). On the other hand, in systems above a weakly alkaline pH, the contribution of the sugar fragmentation to browning becomes dominant, especially in early browning. Thus, it seems important to elucidate the degree of the contribution of the sugar fragmentation pathways to browning in neutral or slightly acidic food systems, and also to browning in different reactant systems, e.g., lysine-glucose, glutamic acid-glucose, fructose-glycine, etc.

If the Maillard browning reactions proceed by additional reactions of furfural or osones with amino compounds in acidic conditions and by condensation of sugar fragmentation carbonyl compounds or their enaminols in alkaline conditions, the browning products which result will naturally be different in structure and chemical properties. Studies on precursor and colored products regarding these respects are needed.

The formation of C₂ fragmentary products of sugar before Amadori rearrangement has been elucidated by ESR spectral analysis and chromatographical isolation as well as by NMR and MS spectral identification. As for the C₃ fragmentary products, even though their production was recognized at an early stage of the reaction (closely after C₂ formation) it has not yet been clearly elucidated whether the C₃ formed directly from the Amadori product or from 1-deoxyosone according to Hodge's proposal. Moreover, a C₃ product was identified as methylglyoxal diimine derivative, but whether it is a direct fragmentary product or secondary product is not yet clear.

To elucidate the mechanism of a reaction as rapid and complicated as the Maillard reaction at neutral and higher pH, it is necessary to develop new and
dynamic experimental techniques employing various new spectrometries to detect and analyze unstable intermediates and their changes in the reaction. Highly reactive intermediates to browning are sometimes difficult to isolate by the usual techniques and the yield is poor, while some products which are fairly stable and obtained in appreciable amounts sometimes turn out not to be the most important intermediates. The discovery of a free radical product in the early stage of the reaction leading to the elucidation of the presence of a new pathway to browning is an example of the application of such research methods.

Concerning the free radical products early in the Maillard reaction, an interesting question is why such free radicals are fairly stable in the reaction mixture. Perhaps they exist because of a balanced oxidation–reduction system. Another question concerns the kind of products involved, and the answer should be useful in further clarifying the browning mechanism.

B. REACTANTS

Recently, large amounts of isomerized sugar have been used in various foods and drinks. The behavior of fructose in the Maillard reaction is known to be considerably different from that of glucose, e.g., in the browning reaction rate and color tone. There are many studies on the Maillard reaction of fructose, and a reaction mechanism involving the Heyns rearrangement has been proposed. However, many problems concerning the reaction of fructose, especially in browning and the development of antioxidants and mutagens, remain.

As for carbonyl compounds other than reducing sugars in the Maillard reaction, this review discusses mainly oxidized ascorbic acid, but there are many interesting and important problems concerning the reactions of oxidized fatty acids and polyphenols.

On the other hand, differences in Maillard reactions that depend on the amino acids involved are well known, and there are many detailed studies on the formation of flavor products in special amino acid–sugar systems, e.g., proline (Tressl et al., 1986), threonine and serine (Baltes and Bochmann, 1986), and cysteine (Shibamoto et al., 1983a). However, not many studies have been done on development of browning, antioxidants, mutagens, etc., and further investigation is required concerning the products and the mechanism of formation by the Maillard reaction of special amino acid–sugar systems, such as lysine, histidine, arginine, tryptophan, and others. The fact that an ε-amino group is eliminated by DHA and other analogous carbonyl compounds (just as the α-amino group is eliminated by Strecker degradation) is very important regarding the nutritive and physicochemical properties of proteins. The kinds of carbonyl compounds involved and to what extent such deamination occurs in food system need further elucidation.
C. ANTIOXIDANTS

As mentioned in Section III, the antioxidative activity of melanoidin may result from multiple factors involving hydrogen or electron donating activity, metal chelating activity, and synergistic activity; it does not appear to be the result of a single and strongly active factor, but seems to involve many different activities. Thus, to elucidate the mechanism of the antioxidative activity, more detailed information on the structure and properties of melanoidin is required. Investigations to determine the Maillard reaction conditions for controlling antioxidative activity, and other properties such as flavor and color, are required. Antioxidative low-molecular-weight Maillard reaction products as observed in glucose–glycine (Park and Kim, 1983), DHA–tryptophan (Namiki et al., 1982a,b), and triose reductone–amino acid (Obata et al., 1971) systems also require examination.

Ozonolysis of melanoidin gave an almost colorless but still antioxidative melanoidin product. This is a very interesting finding and is important with respect to both the chemical study of melanoidin and the practical use of melanoidin as a food additive. Additional studies on the modification of melanoidin are needed on the formation of antioxidative products contributing to safety, color, flavor, solubility, and other important food quality factors.

D. MUTAGENS

Extremely strong mutagens, such as IQ and MeIQx, have been isolated and identified by the Ames test from broiled semidried fish and beef and demonstrated to be formed by Maillard reactions. It was found that creatine–amino acid–sugar systems are the most effective producers of these mutagens and also that their formation is greatly influenced by reaction conditions such as temperature and moisture content. Details concerning the effects of reaction conditions and the formation mechanism are, however, not yet known. Fortunately, additional studies showed that the carcinogenicity of the pyrolysate mutagens was not as strong as originally thought and that their yields in ordinary foods are very small. To prevent the production of the pyrolysate mutagens, it is necessary to elucidate these points as well as the effects of inactivating factors in food systems.

The mutagens are formed under low-moisture conditions. Concerning this and, moreover, the formation of antioxidants, flavors, and other changes in dried foods, more studies on Maillard reactions in low-moisture systems are needed.

Nitroso amino compounds have been noted as being strong carcinogens. As discussed, nitrite-Maillard reaction systems involve mutagen formation while, at the same time, involving desmutagenesis. The relevant reactions are very com-
plicated, and thorough investigations employing reactant concentrations similar to those found in actual foods are required, as are studies on the effects of the presence of other food constituents.

In conclusion, the extremely complex Maillard reaction can perhaps be compared to a river which constantly flows and affects human life. The reactions and interactions are very complicated, and care must be taken not to isolate and focus too much on one aspect without taking into consideration all of the other relevant aspects. The dirt and debris which may be found on the banks do not present the total picture of the benefits and risks the river provides.

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