A Method for the Quantitative Recovery of Protein in Dilute Solution in the Presence of Detergents and Lipids

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Received August 22, 1983

A rapid method based on a defined methanol-chloroform-water mixture for the quantitative precipitation of soluble as well as hydrophobic proteins from dilute solutions (e.g., column chromatography effluents) has been developed. The effectiveness of this method is not affected by the presence of detergents, lipids, salt, buffers, and β-mercaptoethanol.

KEY WORDS: protein precipitation; TX-100; lipids; β-mercaptoethanol; sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

The quantitative (e.g., by Lowry's assay) or the qualitative (e.g., by SDS–PAGE) determination of dilute samples of protein containing salt, detergents like TX-100, phospholipids, or β-mercaptoethanol is difficult because of the interference of these substances with the analysis methods. Several procedures which circumvent these problems by initial removal of the detergent (1–3) or β-mercaptoethanol (4) prior to the analysis step have been published. However, these methods are suitable only if the sample contains enough protein to be analyzed without further concentration. Dilute protein samples, however, have to be concentrated. Concentration together with the removal of interfering substances could be attempted by acid precipitation (5–7) and/or precipitation by acetone or methanol (8). However, it is often difficult to find optimal precipitation conditions especially when working with hydrophobic (membrane) proteins in the presence of detergents and/or phospholipids.

In this paper we report a method for the quantitative precipitation of soluble as well as hydrophobic membrane proteins which is not sensitive to interference by the presence of salt, detergents, β-mercaptoethanol, or phospholipids. Samples ready for protein assay or SDS–gel electrophoresis can be prepared in less than 20 min.

MATERIALS AND METHODS

Bovine serum albumin (BSA), sodium dodecyl sulfate (SDS), TX-100, and asolectin were purchased from Sigma; methanol and chloroform from Merck, Darmstadt, FRG; and rabbit serum from Calbiochem. Membrane proteins from spinach envelopes were prepared according to Douce et al. (9). Protein was analyzed by the method of Lowry (10) and SDS–gel electrophoresis was performed as described by Laemmli (11), using 12.5% acrylamide and 0.1% N,N'-methylenebisacrylamide.

Procedure. An aliquot (0.4 ml) of methanol is added to 0.1 ml of protein sample and the samples are vortexed and centrifuged (10 s at 9000g) for total collection of the sample. Then chloroform (0.1 ml) is added and the samples are vortexed and centrifuged again (10 s at 9000g). For samples containing a high concentration of phospholipids (e.g., liposomes) 0.2 ml of chloroform is used. For phase separation 0.3 ml of water is added, and the sam-

1 Abbreviations used: SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; BSA, bovine serum albumin; TX-100, Triton X-100; β-ME, β-mercaptoethanol.
samples are vortexed vigorously and centrifuged for 1 min at 9000g. The upper phase is care-
fully removed and discarded. A further 0.3 ml methanol is added to the rest of the lower
chloroform phase and the interphase with the precipitated protein. The samples are mixed
and centrifuged again for 2 min at 9000g to pellet the protein. The supernatant is removed
and the protein pellet is dried under a stream of air. The dried pellet can be stored now until
use. For protein determination the pellets are solubilized by the addition of 50–100 μl of
SDS (5%) and assayed according to the Lowry procedure (10). For SDS–gel electrophoresis
the pellets are dissolved in 50 μl electrophoresis sample buffer (11) containing 5% SDS.

The above procedure can be reliably adapted to a larger sample volume. Large
samples (1 ml) are precipitated with 4 ml methanol, 1 ml chloroform, and 3 ml water.
After centrifugation and removal of the upper phase the lower phase and the interphase are
transferred to 1.5-ml tubes and the subsequent procedures are as described above.

RESULTS AND DISCUSSION

The above procedure for the quantitative protein precipitation from solutions contain-
ing salt, β-mercaptoethanol, or detergents starts with the addition of 4 parts methanol
and 1 part chloroform to 1 part of sample. A phase separation is achieved by the addition
of 3 parts water whereby the protein is precipitated at the chloroform–methanol–water
interphase. The addition of an excess of meth-
anol and the subsequent centrifugation results
in a protein pellet which is totally free of inter-
fering substances. Table 1 shows the effi-
ciency of this method for the precipitation of
soluble proteins, e.g., for BSA or for the serum
proteins from rabbit, as well as for hydro-
phobic membrane proteins (e.g., the proteins
of the chloroplast envelope membrane or
membrane proteins from rat liver). The pres-
ence of the anionic detergent SDS (up to 5%)
does not appear to affect the recovery of pro-
tein obtained by this method. The same result
is obtained in the presence of the nonionic

| TABLE 1 |
RECOVERY OF PROTEIN PRECIPITATED IN THE PRESENCE OF OTHER SUBSTANCES

<table>
<thead>
<tr>
<th>Amount of protein recovered</th>
<th>Soluble proteins</th>
<th>Membrane proteins from</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>BSA</td>
</tr>
<tr>
<td>Control, not precipitated (μg)</td>
<td>40</td>
<td>120</td>
</tr>
<tr>
<td>Protein precipitated (yield, %) in samples containing</td>
<td></td>
<td></td>
</tr>
<tr>
<td>—</td>
<td>96 ± 1.2</td>
<td>97 ± 2.3</td>
</tr>
<tr>
<td>5% SDS</td>
<td>92 ± 2.5</td>
<td>92 ± 1.8</td>
</tr>
<tr>
<td>1% TX-100</td>
<td>100 ± 2.3</td>
<td>92 ± 4.6</td>
</tr>
<tr>
<td>2% TX-100</td>
<td>98 ± 1.8</td>
<td>96 ± 3.5</td>
</tr>
<tr>
<td>1% TX-100 plus 20 mg/ml asolectin</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>1% TX-100 plus 0.5 M KCl</td>
<td>95 ± 2.1</td>
<td>100 ± 3.8</td>
</tr>
<tr>
<td>1% TX-100 plus 1% β-ME</td>
<td>95 ± 4.0</td>
<td>98 ± 2.2</td>
</tr>
</tbody>
</table>

<sup>Note</sup>. The volume was always 100 μl containing the given amounts of protein.
<sup>a</sup> Membrane proteins were first solubilized in 5% TX-100 and then diluted to 1% TX-100. For protein determination
of the not precipitated membrane proteins solubilized in TX-100, SDS was present during the Lowry procedure as
described in (12).
<sup>b</sup> Membrane protein was solubilized in 5% TX-100 and afterward adjusted to the stated conditions.
detergent TX-100, which is a common reagent in membrane biochemistry. Quantitative recovery of protein is also obtained when the samples in addition to TX-100 also contain β-mercaptoethanol (up to 1%) or salts like potassium chloride. These substances are quantitatively removed during the precipitation procedure, so that this simple method is applicable for total recovery of both soluble and hydrophobic membrane proteins. As pointed out already, this method avoids heating or acid conditions for the precipitation of protein. Therefore this procedure is also useful for the removal of protein from extracts which are to be analyzed for various substrates labile to extreme pH or heat.

Quantitative recovery of protein can be obtained in even more dilute protein solutions, and 2–3 μg protein can be quantitatively precipitated so that the method seems to be limited only by the sensitivity of the protein determination method applied subsequently. So far, little is known about the molecular weight cutoffs for this procedure, but even with carbonic anhydrase ($M_r$ 30,000), trypsinogen ($M_r$ 24,000) and β-lactoglobulin ($M_r$ 18,400) the protein recovery is comparable to that outlined in Table 1 (results not shown). The method is also effective for the removal of lipids from membranes with a high lipid/protein ratio. Due to the high lipid content, a distortion of the SDS-gel pattern of those membranes, for example, the outer envelope membrane of spinach chloroplasts with a lipid/protein ratio of 3 (13), is observed (Fig. 1, lane 2). Removal of the lipids by the above method leads to an improved protein pattern without any apparent quantitative loss of protein (Fig. 1, lane 3).

ACKNOWLEDGMENT

This research was supported by the Deutsche Forschungsgemeinschaft. (Fl 126/2-2)

REFERENCES