Effects of Cell Culture Conditions on Antibody N-linked Glycosylation—What Affects High Mannose 5 Glycoform

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ABSTRACT: The glycosylation profile of therapeutic antibodies is routinely analyzed throughout development to monitor the impact of process parameters and to ensure consistency, efficacy, and safety for clinical and commercial batches of therapeutic products. In this study, unusually high levels of the mannose-5 (Man5) glycoform were observed during the early development of a therapeutic antibody produced from a Chinese hamster ovary (CHO) cell line, model cell line A. Follow up studies indicated that the antibody Man5 level was increased throughout the course of cell culture production as a result of increasing cell culture medium osmolality levels and extending culture duration. With model cell line A, Man5 glycosylation increased more than twofold from 12% to 28% in the fed-batch process through a combination of high basal and feed media osmolality and increased run duration. The osmolality and culture duration effects were also observed for four other CHO antibody producing cell lines by adding NaCl in both basal and feed media and extending the culture duration of the cell culture process. Moreover, reduction of Man5 level from model cell line A was achieved by supplementing MnCl₂ at appropriate concentrations. To further understand the role of glycosyltransferases in Man5 level, N-acetylglucosaminyltransferase I GnT-I mRNA levels at different osmolality conditions were measured. It has been hypothesized that specific enzyme activity in the glycosylation pathway could have been altered in this fed-batch process.

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KEYWORDS: Chinese hamster ovary; culture duration; glycosylation; Man5; manganese chloride; osmolality

Introduction

The product quality of therapeutic monoclonal antibodies (mAbs) should be closely monitored and assessed throughout product development from cell line and process development, preclinical, and clinical material production to commercial manufacturing (Lubiniecki et al., 2011). During these different stages of development, product quality profiles can vary due to process changes, equipment, and facility differences, raw material lot-to-lot variation, and process control deviation. Understanding glycosylation is important because most therapeutic recombinant antibodies on the market or in development are glycoproteins produced in mammalian cells. Chinese hamster ovary (CHO) and mouse myeloma NS0 cell lines are common hosts due to their ability to produce large amounts of antibodies with similar glycosylation profiles to human IgGs (Jefferis, 2005; Raju, 2003). Several elements including cell line characteristics, process control parameters, and cell culture media components could affect glycosylation (Andersen et al., 2000; Butler, 2005) and therefore could impact biological activity, efficacy, stability, immunogenicity, clearance rate, antibody-dependent cellular cytotoxicity (ADCC), and complement-dependent cytotoxicity (CDC) (Burton and Dwek, 2006; Goochee et al., 1991). The impact of various cell culture conditions toward antibody glycosylation should be understood in order to produce monoclonal antibody products with consistent quality attributes.

IgG antibodies produced in mammalian cells generally contain low levels of high mannose glycoforms (Man5-9), typically below 5%, with the most common glycan being the complex glycoforms G0F, G1F, and G2F (van Berkel et al., 2009). These dominant glycoforms of IgGs produced in CHO cells are considered mature glycoforms. To illustrate how high mannose, hybrid, and complex glycoforms are formed, a simplified model of the N-link glycan pathway is summarized in Figure 1. As glycoproteins pass through the endoplasmic reticulum (ER), the reducing ends of high mannose oligosaccharides are trimmed by multiple enzymes including Mannosidase-1 to form Man5. The Man5 glycans are then further processed in the Golgi apparatus for the
removal of α-1,3-mannose and the addition of N-acetylglucosamine (GlcNAc) to form a hybrid glycan. The hybrid glycan is then further processed to complex glycans. Variability in the degree of completion in the multiple enzymatic steps involved results in a heterogeneous mixture of mostly complex glycans (G0F, G1F, and G2F), with a small amount of high mannose glycans (Kornfeld and Kornfeld, 1985; Kornfeld et al., 1978).

In general, human IgG has low amount of the Man5 glycoform (Walsh and Jefferis, 2006), and high level of high mannose glycoforms in the therapeutic antibody could be a concern due to uncertainty about its impact on clearance, immunogenicity, and efficacy. Antibodies bearing the Man5 glycoform produced by the glycosylation mutant CHO cell line Lec1 were reported to have deficiency in complement activation, reduced affinity to FcγRI, and reduced in vivo half-life in a mouse study (Wright and Morrison, 1998; Wright et al., 2000). In another mouse model, the pharmacokinetic profile of antibodies with high mannose glycoforms did not show an early phase reduced half-life but exhibited increased beta-phase clearance (Kanda et al., 2007). Another recent study concluded that Man5 and higher mannose glycoforms exhibited comparable clearance in humans to antibodies bearing complex glycans due to high mannose (Man6-9) conversion to Man5. The result suggested that the presence of a mannosidase in human serum was responsible for glycan cleavage, not differential antibody clearance (Chen et al., 2009). A complete understanding of the clearance of antibodies bearing Man5 or other high mannose glycoforms still remains to be resolved as demonstrated by the conflicting reports.

Antibodies with high mannose have become of interest as therapeutics because of potential enhancement of biological activity. A previous study demonstrated the feasibility of producing high mannose antibody by adding glycosylation inhibitor to the cell culture process. As a result, high mannose antibodies that were generated with kifunensine treatment showed higher ADCC activity and greater affinity to FcγRIIIA (Zhou et al., 2008). Binding to the FcγIII receptor is dependent on the fucose content of the Fc glycans where a reduction in fucose can increase effector function. Fucose-deficient IgGs have shown a significant enhancement of ADCC up to 100-fold (Mori et al., 2007; Shields et al., 2002). As afucosyl antibodies have become recognized as having potentially higher therapeutic potency due to enhanced ADCC function, high mannose antibodies, which also lack fucose, could also have therapeutic benefit.

In a cell culture process, the factors that can most affect glycosylation include choice of cell lines, process conditions, and media composition. Cell culture process parameters such as temperature, pH, and dissolved oxygen (DO) have also been shown to have significant effects (Ahn et al., 2008; Borys et al., 1993). Culture temperatures below 32°C have resulted in a reduction of branched structures and degree of sialylation. Variations in pH have resulted in more aglycosylated proteins. Other impacts of cell culture parameters have been identified over the last three decades as bioprocess knowledge accumulates. However, most case studies were based on specific cell line(s), and no general trends on how process control impacts antibody glycosylation can be concluded (Calhoun, 2008). Media composition is another important factor that can significantly alter
processing of oligosaccharides (Gawlitzek et al., 2000; Schmelzer and Miller, 2002; Zanghi et al., 1999). Legacy products that still use serum or hydrolysate-containing media can be subject to lot-to-lot variability which could impact glycosylation profiles. Trace amounts of metals can modify glycosylation properties when added to cell culture media formulations (Hendrickson and Imperiali, 1995; Kaufman et al., 1994). Addition of copper (II) chloride has shown to increase the sialic acid content (Ryll, 1999). Manganese addition to the rHuEPO production process has shown to increase galactosylation and reduce lower sialylated fractions (Crowell et al., 2007).

This study focused on investigating the impact of culture variables on the level of Man5 in antibodies made in CHO cells. Multiple cell culture process conditions and medium components were tested to evaluate their impact on glycosylation profile. By altering the NaCl concentrations of the basal and feed media to increase osmolality and by extending the run duration, general trends in Man5 glycosylation were observed with multiple cell lines. To test whether GlcNAc transferase I (GnT-I) transcription changes during the course of a cell culture production, mRNA levels were measured among the different osmolality conditions. Although the impact of osmolality is still not fully understood, the increase of ammonia in the culture condition suggests an impact on enzyme activity in the glycosylation pathway. This work provides further insight on the role of osmolality, run duration, and manganese on Man5 glycosylation of recombinant antibody in CHO cell culture.

Materials and Methods

Cell Lines and Cell Culture

Several in-house CHO antibody producing cell lines derived from a dihydrofolate reductase-deficient (DHFR–) CHO DUX-B11 host (Urlaub and Chasin, 1980) were used as model cell lines in this study. The plasmid construction, gene transfection, and cell line selection methods were described previously (Guo et al., 2010). Cell line A, which produces humanized IgG1 with unusually high levels of Man5 compared to other in-house cell lines, was used as a primary model cell line. Antibody producing CHO cell lines B, C, D, and E were also selected to demonstrate applicability of various cell culture conditions on Man5 levels. The cell lines were maintained in shake flasks after thaw, adapted for growth in a serum-free medium and cultivated in the presence of methotrexate to maintain selection pressure for a minimum of 2 weeks. At production, shake flask cultures were seeded at 1.0 × 10^6 cells/mL in 125 mL flasks with 40 mL working volume under conditions of 37°C, 5% CO2, 150 rpm rotation, 85% humidity in an incubator. The cells were grown initially at 37°C then shifted to lower temperature of 35°C from 48 h post inoculation through the production duration which was up to 22 days in fed-batch conditions.

Cell Culture Medium Preparation

Proprietary, in-house cell culture basal and batch feed media were used during these production studies. The original osmolalities are 300 and 750 mOsm/kg for basal and feed media, respectively. Osmolalities of both basal and feed media were adjusted during media preparation by adding NaCl (Gibco Invitrogen, Grand Island, NY) to meet desired specifications. Upon preparation, osmolality was verified with a micro osmometer (Advanced Instruments, Norwood, MA). To make media with different levels of osmolality, one batch of basal medium was split into four solutions and adjusted to have osmolalities of 300, 330, 360, and 400 mOsm/kg. Similarly, one batch of feed medium was split into two solutions to have 750 and 1,250 mOsm/kg.

Production Conditions

The first experiment was designed to evaluate the impact of basal and feed osmolality. Suspension cultures were grown in respective shake flasks containing corresponding basal media with osmolalities of 300, 330, 360, and 400 mOsm/kg. The cultures were fed on days 3, 6, and 9, and each feed was 10% (4 mL) of the initial culture volume. For each basal osmolality condition, the culture was fed with the low (750 mOsm/kg) and high (1,250 mOsm/kg) batch feed formulations.

In another experiment, a high basal medium osmolality condition of 400 mOsm/kg was used to determine the effects of MnCl2 supplementation. A 1 mM stock solution of MnCl2 (Sigma, Atlanta, GA) was prepared and then titrated into individual cell culture shake flasks on day 3 to achieve final concentrations of 0.25, 0.50, and 1.0 μM. These cultures were fed with 10% of the 750 mOsm/kg feed media on days 3, 6, and 9. A control condition using 300 mOsm/kg normal basal medium, 750 mOsm/kg feed medium, and the same feeding schedule was also tested to determine the benefits of MnCl2. A stock solution of MnCl2 was added directly to shake flask on day 3 to achieve a final concentration of 1.0 μM.

Cell, Metabolite, and Osmolality Analysis

Cell culture samples were taken from each shake flask every 2–3 days for the entire run. Viable cell density, viability, and cell size were determined using a Beckman Coulter Vi-Cell Viability Analyzer (Beckman Coulter, Fullerton, CA). Lactate, ammonia, glucose, glutamine, glutamate, and pH were monitored using a Nova Biomedical Bioprofile 400 Analyzer (Nova Biomedical, Waltham, MA). Osmolality was measured using a micro osmometer (Advanced Instruments). Supernatant samples were stored at −80°C.
At the end of experiments, frozen cell-free supernatant samples were thawed and collectively submitted for titer and glycan analysis.

### Protein Titer Analysis

Cell culture supernatant samples at five different time points, day 7, 12, 14, 17, and 22 were collected and purified by protein A column using an automated sampling platform, OASIS. After desalting with a Microcon 30 concentrator and treating with Peptide-N-Glycosidase F, the samples were spun down multiple times at different speed and duration and digested overnight in a water bath at 37°C. Released carbohydrates are recovered in the supernatant, following precipitation of the protein at 95°C, and as pellet following drying in the evaporator. One molar sodium cyanoborohydride in Tetrahydrofuran was added to dissolve the pellets, which were then incubated at a 55°C prior to injection to capillary electrophoresis with fluorescence detection (Beckman P/ACE MDQ series and PA800). The detailed protocol can be found in Ma et al. (Ma and Nashabeh, 1999). The relative amounts of glycans with major and minor carbohydrate moieties such as G0F, G1F, G2F, and Man5 were quantified in percentile proportions.

### Protein Glycosylation Analysis

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### mRNA Analysis

The mRNA level of enzyme GnT-I was measured for both control and high osmolality conditions. Cell pellets were collected and frozen to −80°C on day 3, 7, 12, and 14. Cell pellets at different sampling times were thawed and used for quantitative PCR (qPCR) analysis to measure the endogenous mRNA level of GnT-I. The primer used for amplification of the GnT-I mRNA has the following sequences; GnT-I forward: CGT TGT CAC TTT CCA GTT CAG, GnT-I Reverse: CGT TGT CAC TTT CCA GTT CAG, and GnTI probe: AGC TGT CCA CCT GGC ACC CC. The house keeping gene β-2-microglobulin (B2M) has the following sequences; B2M forward: TTC TCT CAG TGG TCT GCT TGG, B2M reverse: TGG CGT GTG TAG ACT TGC ACT T, and B2M probe: TGC CAT CCA GCC TCC CCC A. The B2M was chosen to normalize the GnT-I levels due to its stable transcription level during the entire culture. All mRNA measurements were normalized to B2M as the endogenous control gene. To perform qPCR, mRNA was isolated by RNaseaTM 96 well kit (Qiagen) or MagMAX™-96 total RNA isolation kit (Ambion, Applied Biosystems, Austin, TX) according to manufacturer protocol. A TAQMAN® analysis was performed using ABI RT-PCR 7900HT (Applied Biosystem) to measure GnT-I mRNA level. The data was analyzed using software accompanied by the ABI RT-PCR instrument to compare relative GnT-I mRNA level to the level on day 3 of the control condition.

### Results

#### Effect of Osmolality and Run Duration on Cell Growth, Metabolism, and Antibody Production

The effect of osmolality and run duration on cell growth and antibody production of cell line A was evaluated by testing combinations of basal and feed media osmolalities. Overall, cell growth was higher with lower media osmolality. Within the tested osmolality ranges, cell growth was more sensitive to the high feed medium osmolality than to the basal media osmolalities. Whereas various basal media combined with 750 mOsm/kg feed reached peak viable cell count (VCC) of 6.5–8.5 × 10⁶ cells/mL, the high osmolality feed cases only reached 5.0–6.5 × 10⁶ cells/mL (Fig. 2a). Trending similarly for both osmolality feed conditions, viability profiles at basal osmolalities of 360 and 400 mOsm/kg were higher than 300 and 330 mOsm/kg. The viabilities at the end of run were 50–63% at higher basal osmolalities whereas 32–48% for the lower basal osmolality conditions (Fig. 2b). At the end of production at day 22, the titer for the 750 mOsm/kg feed conditions reached 4–5 g/L, compared to 2.5–3.5 g/L of 1,250 mOsm/kg feed conditions (Fig. 2c). For cell metabolism, end of run NH₃ levels were lower with 750 mOsm/kg feed cases at 4.5–6.5 mM compared to 1,250 mOsm/kg feed cases at 5.5–10 mM (Fig. 3a). Due to the lack of pH control in shake flasks, the culture pH decreased in the early stage of culture and then drifted up after day 3. This pH shift correlated with lactate metabolism in culture, where lactate was accumulated in the early stage of the run and was then consumed. The lactate profiles were very similar for all the experimental conditions (data not shown). The overall trend of off-line pH profiles were similar between the two feed cases, but drifted higher later in the culture for the high feed osmolality (Fig. 3b). End of run osmolality levels were lower for the 750 mOsm/kg feed cases at 290–360 mOsm/kg compared to the 1,250 mOsm/kg feed cases at 395–490 mOsm/kg (Fig. 3c).

#### Effect of Osmolality and Run Duration on Man5 and Other Glycoforms

Model cell line A showed an increase in the Man5 glycoform during the course of the cell culture. As run duration was extended to 22 days, the glycan analysis results from a total
of five different sampling time points showed continuing increase of Man5 levels from days 7 to 22. Each time-point represents the cumulative formation of Man5 over the course of the run. The increase in Man5 levels correlated with the increase in media osmolality. The baseline medium osmolality at 300 mOsm/kg in basal and 750 mOsm/kg in feed media represents the control condition. This condition has 15% of Man5 on day 22. Other basal conditions of 330, 360, and 400 mOsm/kg with the same feed have higher Man5 levels up to 18% on day 22. With the 1,250 osmolality feed cases, Man5 levels are significantly higher, in the range of 23–27% (Fig. 4a). During the entire culture duration, as the Man5 level increased, the other glycoforms such as G0F and G1F decreased proportionally (Fig. 4b and c).

To further examine the general trend of media osmolality and run duration effects, four other antibody producing CHO cell lines B, C, D, and E were also tested in the 300 and 400 mOsm/kg basal media with the feed medium of 750 mOsm/kg. Although these cell lines had low Man5 levels that are more typically found in mAbs expressed in CHO cells, the general trends of the increase in Man5 level with culture duration and medium osmolality were consistent in all tested cell lines. The control condition of cell line B at 300 mOsm/kg showed 0.36% Man5 on day 7 and 0.84% at the end of run. The observation is consistent with the high osmolality case with higher level of Man5 levels at 0.67% Man5 on day 7 which was increased to 1.85% at the end of run. Other cell lines, C, D, and E followed a
similar trend but with different absolute levels of Man5 (Fig. 5).

The data of cell line A with respect to osmolality and run duration was further analyzed. Statistical analysis showed a significant linear correlation of osmolality with ammonia at each time point. This analysis showed that increasing osmolality correlated with increasing ammonia levels (Fig. 6). Cell line B also showed a similar correlation (data not shown).

Effect of Manganese Chloride on Man5

Manganese (Mn), a metal ion co-factor for a number of glycosyltransferases such as GnT-I, GnT-II, and B4GalT-I, was tested with cell line A to determine the impact on Man5 levels. The original basal medium formulation had very low levels of MnCl₂. An initial MnCl₂ titration study was conducted to determine a testing range without significant toxic effects to cells after supplementation (data not shown). With appropriate concentrations of MnCl₂ added to cultures, VCC, and titer were comparable to culture without the addition of MnCl₂ (Fig. 7a and b). The condition of 400 mOsm/kg in the basal medium that corresponds to high level of Man5 was selected to test the impact of MnCl₂. Manganese chloride was titrated directly to the shake flasks to achieve final concentrations of 0.25, 0.50, and 1.0 μM. A control condition using 300 mOsm/kg was also tested with and without 1.0 μM MnCl₂. Upon supplementation of

Figure 3. Metabolic and control profiles of model cell line A at various basal (300, 330, 360, and 400 mOsm/kg) and feed (750 and 1,250 mOsm/kg) osmolality combinations over a 22-day cell culture process. (a) NH₄⁺; (b) Off-line pH; (c) Osmolality.
1.0 μM MnCl₂ to the high osmolality media condition, Man5 decreased significantly from 25% to ~14% (Fig. 8a). Similarly, the control condition of 300 mOsm/kg basal with 12% Man5 decreased to 5% (Fig. 8b).

**Effect of Osmolality to mRNA Levels**

Cell pellets from the control and high osmolality conditions were collected on day 3, 7, 12, and 14. The mRNA was isolated for TaqMan analysis. All mRNA measurements were normalized to B2M as the endogenous control gene. In addition to normalization to the endogenous control, the data were further normalized to the mRNA level of GnT-I on day 3 of the control condition. Therefore, the data shown were calculated using the following equation:

\[
\frac{\text{mRNA GNT1}_{D(x)} / \text{mRNA B2M}_{D(x)}}{\text{mRNA GNT1}_{D3\text{,Control}} / \text{mRNA B2M}_{D3\text{,Control}}}
\]

The data is illustrated in Figure 9. The relative mRNA levels do not appear to vary significantly over the course of the culture in both conditions. The GnT-I mRNA level between the two conditions also appeared to be similar, suggesting that the transcript level of the GnT-I enzyme was not affected by different osmolality levels.
IgG antibodies produced in mammalian cells generally contain low levels of high mannose glycoforms (Man5-9), typically below 5%, because most glycoproteins are processed to complex glycoforms G0F, G1F, and G2F (van Berkel et al., 2009). Even though oligosaccharide formation takes place inside the cell, we have demonstrated that physicochemical environment changes such as culture duration or cell culture media osmolality can alter glycosylation of antibodies. Model cell line A demonstrated the changes of inhibited growth, lower titer, and a higher proportion of the Man5 glycoform when exposed to higher media osmolality (Figs. 2–4). In addition to model cell line A, other mAb cell lines including B, C, D, and E which have diversity in properties such as growth, metabolism, and specific productivity demonstrate similar effects of high osmolality and run duration on Man5 glycosylation even when using a different lot of media. These cell lines have exhibited same response of increase in Man5 level at higher basal osmolality, and the Man5 level steadily increased during a 21-day culture duration (Fig. 5). Since these mAbs have lower starting levels of Man5, the increase of Man5 levels is relatively modest. Nevertheless, an increase of Man5 levels due to osmolality and run duration has been confirmed with multiple cell lines. While we have shown the impact of osmolality and run duration on Man5 production in CHO cells, an increase of high mannose during the course of a run and with extended duration has also been observed in other hosts such as NS0 cells (Bibila and Robinson, 1995; Hooker et al., 1995; Robinson et al., 1994).

Referring back to the simplified glycosylation model (Fig. 1), it has been hypothesized that glycosyltransferase
enzyme activity in the glycosylation pathway could have been altered in this dynamic fed-batch process. To test this hypothesis, the GnT-I mRNA levels of high and low osmolality conditions were measured to determine whether the increased Man5 level with increased culture osmolality correlated with reduced GnT-I mRNA transcript level. The relative mRNA levels appear to be steady over the course of the culture, and the mRNA level was also similar for both control and high osmolality conditions (Fig. 9). This data suggested that the GnT-I enzyme transcription level was likely unaffected by osmolality, and the increased Man5 level observed for the high osmolality condition could have resulted from decreased enzyme activity of GnT-I, decreased translation of mRNA, decreased stability of GnT-I, or varying activity of other enzymes along the glycosylation pathway.

It has also been reported that increased ammonia levels in the cell culture environment can alter intracellular pH and therefore compromise intracellular glycosyltransferases activity due to the pH sensitivity of glycosyltransferases. Ammonia concentrations were found to be higher in increased osmolality conditions (Figs. 3 and 6). Ammonia is a common metabolic waste product accumulated in CHO cells cultures, driven by a major energy source such as glutamine or asparagine. Elevated ammonium levels have shown to inhibit galactosylation and sialylation of TNFR-IgG N-glycans by increasing the pH of the trans-Golgi compartment (Gawlitzek et al., 2000). Ammonia effects in the Golgi can modulate many enzyme branching mechanisms that give rise to more heterogeneity (Chen and Harcum, 2006). Many mechanisms have been proposed to explain how ammonium toxicity affects glycosylation. We
agree with the proposed mechanism suggesting that ammonium inhibits the glycosylation process by increasing the pH within the ER and golgi compartments, resulting in inhibition of pH-sensitive enzyme activity (Schneider et al., 1996). We expect cascading effects of higher osmolality which can lead to increased ammonia levels in the cell culture environment and compromise intracellular pH sensitive glycosyltransferases, therefore resulting in the increase of high mannose glycoforms.

In addition to media osmolality and run duration effects on Man5 levels, we observed that increasing MnCl2 concentrations reduced Man5 glycoforms for the recombinant antibody produced by model cell line A (Fig. 7a). Adding appropriate MnCl2 in the range of 0.25–1.0 μM into the cell culture media can be an effective approach to reduce Man5 if the level is undesirably high and to maintain typical levels of major glycoforms such GOF, G1F, and G2F. With the supplementation of 1 μM MnCl2, Man5 level was effectively reduced from 12% to 5%, which is the typical levels of glycoform found in IgG1 (Fig. 7b). Many attempts to use oligosaccharide precursors and enzyme metal cofactors have been tried to control glycosylation (Clark et al., 2005; Hills et al., 2001). Several metal ions have been tested to determine impacts on glycosylation. Manganese has been demonstrated to be more effective as a metal cofactor compared to other co-factors such as magnesium (Mg2+) and calcium (Ca2+) (Hendrickson and Imperiali, 1995). In recombinant human erythropoietin (rHuEPO), MnCl2 supplementation increases galactosylation and sialylated fractions. It has increased carbohydrate site-occupancy and narrowed carbohydrate branching to biantennary structures (Crowell et al., 2007). Manganese is involved in oligosaccharide addition within the secretory pathway. The depletion of such metal cation has shown to inhibit the processing of complex N-link oligosaccharide (Kaufman et al., 1994).

Previous studies have proposed mechanism on the role of manganese in converting high mannose to hybrid and complex oligosaccharides. X-ray crystallographic structures of catalytic fragments suggested that Mn2+ ions complexes with UDP-GlcNAc, which binds to the enzyme GnT-I and subsequently to the high mannose. The formation of the hybrid glycoform from high mannose takes place by transferring the first GlcNAc residue onto a mannose branch. A similar mechanism activated by Mn2+ may transfer the second GlcNAc forming complex oligosaccharide G0F, which resulted in two GlcNAc being present at the non-reducing ends of the biantennary structure. Mn2+ ions further play a role as a metal cofactor in the conversion of G0F to G1F and G2F glycoforms. Mn2+ is a required cofactor for the enzyme β-1,4-galactosyltransferase (B4Gal-T1) that transfers galactose to the GlcNAc forming G1F and G2F (Gordon et al., 2006; Unligil et al., 2000; Zhang et al., 2001). We have shown that the addition of appropriate MnCl2 concentrations can decrease Man5 levels, most likely due to manganese ion’s involvement to effectively convert high mannose to complex glycoforms (Fig. 7a and b).

Conclusions
While production of consistent and reproducible mAb glycoform profiles still remains a considerable challenge of biopharmaceutical industry, variations in cell culture processes play a role in mAb glycosylation profile. Potential variables in the cell culture physicochemical environment including culture pH, cell culture media composition, raw materials lot-to-lot variations, equipment, and process control differences are just a few examples that can potentially alter glycosylation variation on therapeutic antibodies, this work has provided additional knowledge on how media osmolality, culture duration and MnCl2 influence mAb glycosylation patterns.

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