High-Throughput Quantification of Glycoprotein Sialylation

by

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Abstract

Sialic acid can improve qualities of therapeutic glycoproteins, such as circulatory half-life, biological activity, and solubility. In production of therapeutic glycoproteins, a high-throughput method (HTM) is required for process monitoring and optimization to ensure consistent and optimal sialic acid content. The HTM is also required for cell clone screening in cell line development. Current methods for quantifying sialic acid, however, require chromatographic separation that is time consuming and cannot rapidly analyze many samples in parallel.

Here we develop a novel HTM for quantifying glycoprotein sialylation. Using chemical reduction, enzymatic release of sialic acid, and chemical derivatization of the sialic acid, the HTM can accurately, rapidly (15 min), and specifically analyze many samples in parallel. It requires only 45 µL of sample and has a quantitation limit of 2 µM sialic acid. We validated the HTM for monitoring sialylation of recombinant interferon-gamma (IFN-γ) produced in Chinese Hamster Ovary (CHO) cell culture. The HTM was accurate in monitoring sialylation of IFN-γ in batch cultures of CHO cells. It was also used for monitoring sialylation of proteins produced in CHO-IgG4 cell cultures.

Furthermore, we used the HTM to study the effects of feeding ManNAc, Cu^{2+}, and Mn^{2+} on sialylation of glycoproteins produced in CHO-IFN-γ cell cultures. We found that feeding these chemicals increased sialylation from 20 to 36 mg sialic acid/g protein on the fifth day of batch CHO cell cultures. Moreover, the increase due to ManNAc and Cu^{2+} was higher when they were added together than separately. This enhancement might be caused by increases in several steps prior to sialylation, such as galactosylation and glycan transfer by oligosaccharyltransferase, due to feeding Cu^{2+}. In contrast, feeding ManNAc and Mn^{2+} in combination decreased sialylation when both were supplemented at concentrations more than 0.4 mM and 5 µM, respectively. Furthermore, a quadratic least square model predicts that the feeding 2 mM ManNAc and 100 µM Cu^{2+} will increase the sialylation to 41 ± 4 mg sialic acid/g protein, which is close to the experimental value of 35 ± 5 mg sialic acid/g protein.

We also used the HTM to study intraclonal variability in glycoprotein sialylation. A batch culture of CHO-IFN-γ cells was serially diluted, and 24 subclones were expanded. We found that there was significant variability in sialic acid content and productivity in these subclones. The sialic acid content varied from 1 to 70 mg sialic acid/g protein, and the total protein concentration varied from 41 to 214 mg/L. In addition, the sialic acid content was negatively
correlated with productivity ($r = -0.6, P = 0.003$). This correlation agrees with previous study showing that shorter golgi residence time caused lower sialylation.

Overall, these studies developed a novel HTM and demonstrated its versatility for various applications in bioprocesses. In monitoring sialylation, the HTM can provide sialic acid content in 15 minutes, while conventional methods require more than one day, during which sialic acid content can decrease significantly. Similarly, the HTM can finish the analysis of the feeding and intraclonal variability studies within one day, while conventional methods require at least 48 and 24 days, respectively. Thus, the HTM is an important analytical tool for producing therapeutic proteins with consistent and optimum sialylation.

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