Improving Glycosylation Patterns and Consistency Through Media Optimization

Answers by: Dr. Adam Elhofy, Ph.D., CSO.

We recently finished our Ask the Expert discussion on “Ask We recently finished our Ask the Expert discussion, "Media Optimization Can Improve Glycosylation Patterns and Consistency to Impact Protein Efficacy". During this Ask the Expert session, we discussed factors that influence glycosylation, the relationship between media and glycosylation, and the effect of glycosylation on the protein. Additional topics included glycosylation in biosimilars, glycoengineering, other post-translational modifications and protein aggregation.

When producing a biologic therapeutic, protein quality is a key consideration. While manufacturing parameters such as high protein yield, protein solubility and protein stability are important, it is also critical that the biologic exhibits the desired therapeutic activity. Post-translational modifications impact efficacy and pharmacokinetics of the biotherapeutic, where improper glycosylation can result in an ineffective drug. Proteins are commonly post-translationally modified by adding sugar residues in a process called glycosylation. Half of all human proteins are estimated to be glycosylated.

Essential Pharmaceuticals is addressing the challenges associated with glycosylation including increasing consistency of glycosylation profiles with their Cell-Ess universal titer boost and optimizer. Cell-Ess was used as a feed in a Wave bioreactor, resulting in greater than 25% increase in monoclonal antibody titer, which was similar to the titer increases observed in earlier experiments using shake flasks. In addition to increasing titer, Cell-Ess was shown to significantly increase consistency of glycosylation patterns when used as a feed. Further, Cell-Ess was shown to increase higher order glycoforms measured by increase in galactosylation. These new data suggest supplementing with Cell-Ess as a feed increases protein titer while improving or maintaining quality. To learn more about the use of Cell-Ess, please see our previous article, Cool Tool – Novel universal titer boost and enhancer improves CHO cell protein production in small bioreactors.”

This Ask the Expert session was hosted by Dr. Adam Elhofy, Ph.D., CSO. Dr. Elhofy developed the core technology for the Ess line of products and aided in creating patents around novel uses of materials. Dr. Elhofy has over 14 years of scientific research experience in the areas of immunology, neuroscience, and oncology. He was funded by both the National Institutes of Health and the Multiple Sclerosis society as an investigator at Northwestern University Medical School. His doctoral research won him the award of the Top 5 trainee scientists by the American Association of Immunologists. Dr. Elhofy has 15 scientific publications in peer reviewed journals. He has played a variety of roles with start-up biotech companies ranging from Principal Investigator to Director of Corporate Development.

Question:
Have you looked at any other post translational modifications? Lipidation?

Answer:
We have not specifically looked at other post-translational modifications such as lipidation. Cell-Ess can improve the lipid membranes including those of the ER and Golgi. Therefore, theoretically there should be a benefit in using Cell-Ess for lipidated proteins, but we have not tested it yet. Lipidation, similar to glycosylation, is a complicated multistep process where Cell-Ess can most likely play a role, but we look forward to hearing from users who have directly tested what their experiences have been.
**Question:**
What is the relationship between glycosylation and aggregation?

**Answer:**
There are many reasons for aggregation. To simplify it, imagine there is the ability to formulate an equation to determine if and how much proteins will aggregate and at what concentration. The formula would have many inputs. Glycosylation is one of the many inputs that would determine whether a protein will aggregate. In the case for monoclonal antibodies, as touched upon in an earlier question, glycosylation can affect stability and solubility. If a monoclonal antibody is less soluble and less stable, then it will have a higher propensity to aggregate. Furthermore, glycosylation also affects the folding of a protein. In cases where the protein is mis-folded, the protein would also have a higher likelihood of precipitating. While glycosylation can play a very important role in aggregation, it is one of many components. Therefore, the amount, type, and location of the glycosylation will also be relevant. How the aggregation equation is formulated may vary for each protein, but the basic inputs will be common between the equations. Glycosylation and aggregation are related, but to what degree and how much would likely depend on your specific protein.

**Question:**
I have been seeing articles on the use of supplements, peptones, etc. regulating glycosylation. Do you think it is better to start with a CD media or with a media that already contains some supplements? Why would these supplements be having an impact on glycosylation? Healthier cells?

**Answer:**
The optimal way to start would be with a CD media and establish a baseline. Once you have a baseline for your system, then CD supplements can be added individually to determine whether the profile changes. If you are not able to change your profile with the CD supplements, then you can move to the cGMP non-CD supplements such as peptones. If using peptones, you should try to find one with the most defined characteristics so you are able to reproduce your results.

Depending on the supplement, you can have a range of effects on your cells, from providing a broader base of sugar nucleotides to improving the function of the glycolytic enzymes.

**Question:**
What impact, if any does Cell-Ess have on aggregation and downstream purification?

**Answer:**
It is a great question. Cell-Ess does not have any effect on downstream purification. We have not looked at the aggregation issue directly. However, in cases where we have increased titer, we have not seen an increase in aggregation. We do hypothesize there may be a slight reduction in aggregation based on mechanism of action where the Golgi is functioning better. A more properly functioning Golgi leads to correct protein folding and proper post-translational modification, and those factors can be removed from the complex aggregation equation.

**Question:**
What impact does Cell-Ess have on glycosylation patterns? When added can your product affect fucosylation or galactosylation?

**Answer:**
As mentioned, the approach with Cell-Ess is to target the physiological make-up of the ER and Golgi to effectively make them work more efficiently and function appropriately. The hypothesis is if you are able to target the membrane constituents of the ER and Golgi, then there would be greater consistency and higher order glycoforms. With the addition of Cell-Ess in two different media bases, we have seen increased consistency in the glycosylation pattern of monoclonal antibodies, suggesting that the Golgi is functioning more uniformly across groups to increase reproducibility. Further, with the addition of Cell-Ess, we also observed increased galactosylation in two different base media, also suggesting more efficient Golgi. The amount of glycoform G0F was decreased, masking the increase of fucosylation associated with higher order glycoforms, so the net result is a decrease in fucosylation. In other work, we have shown a greater than 20% increase in titer by increasing the amount of protein made per cell, which also points toward a more effective ER and Golgi.

**Question:**
We are trying to match an existing product profile. What tools are available to achieve this goal? What do you think about glycoengineering? Do you think it is better to try and control the glycosylation or engineer it?

**Answer:**
The term glycoengineering has been changing over time. Glycoengineering was traditionally defined as any tool used to modify or change the glycosylation patterns. There are 4 basic levers to pull to control the addition of glycans. They are (1) the substrate – namely the sugar nucleotide mix and quantity, (2) the enzymes – glycotransferases and glycosidases, (3) the physical location where the additions occur – namely the ER and Golgi, and (4) the environment which can affect all of the other levers. The early forms of glycoengineering used media components to shift the glycosylation patterns by adding a variety of sugars in addition to glucose. Once the environmental parameters were shown to affect the glycosylation pattern, a new form of engineering developed in which parameters (such as pH and oxygenation) were monitored to target the profile of choice. As technology has progressed, the specific genetic and protein targets became more clearly identified, and methods to specifically alter them have been developed and utilized. As an example, CRISPR can be used to make a specific genetic modification that would be beneficial for the particular biologic and glycosylation profile you are targeting. The ideal strategy to optimize glycosylation pattern and achieve consistency would be to use a variety of engineering tools rather than utilizing an either/or strategy. In our studies, we have looked at providing a method to improve the physical location where post-translational modifications occur – namely the ER and Golgi. By targeting the physical structure, we have seen both an increase in the higher order glycoforms and increased consistency when Cell-Ess is used.
Question: What impact does glycosylation have on the protein?

Answer: Glycosylation is a critical quality to consider for proteins. The amount and type of glycosylation has a dramatic effect on the physical nature of the protein and its biological function. In addition to amino acid sequence, glycosylation can affect the folding of proteins and their ability to aggregate with each other. The different folding and aggregate structures then have a resultant effect on stability and solubility. The function is also affected in many ways by glycosylation, including the pharmacodynamics and pharmacokinetics of antibodies. Antibodies with lower glycoforms can be cleared faster leading to a lower physiological half-life. The binding of the protein to the target is also affected in how long and how tightly it will bind. Antigenicity can be impacted by glycosylation where certain glycoforms can increase antigenicity. Amount of glycosylation addition and complexity of the glycoform both impact the protein, and there is a wide range of effects glycosylation can have on these various protein characteristics. In many cases, differential glycosylation effects can impact therapeutic efficacy of a protein.

Question: Do you find that glycosylation is an issue mainly in biosimilars? It seems everything I read on this talks about trying to match the profile of an innovator product?

Answer: Conceptually this questions addresses the issue of targeting a particular glycan profile. It is true that there is a target profile for both innovator biologics and biosimilars. For the biosimilars, the target is clear – it is the glycan profile of the innovator. For the innovator, in the case of antibodies, there may be a desire to target a profile that increases antibody-dependent cell-mediated cytotoxicity (ADCC) over complement-dependent cytotoxicity (CDC) or increases half-life of the antibody in circulation, or a certain profile may have been specifically targeted in R&D. In some cases, the optimal candidate was chosen empirically during biological testing and then becomes the target profile. In any scenario, glycan addition drives functionality, and this is true for both the innovator and the biosimilar. In some cases, researchers have determined that the innovator glycan profile is not be the best and have developed therapeutics with improved glycan profiles. These “improved” therapeutics are part of a relatively new class called bio-betters.

Question: What do you think are the media ingredients that influence glycosylation?

Answer: Glycosylation is affected by several factors, including: movement of the protein through the ER and Golgi apparatus, environmental factors, and sugar availability. The movement of protein through ER and Golgi apparatus can be affected by cholesterol and other free fatty acid availability that impact health of ER and Golgi membranes. Environmental factors include cell culture parameters such as pH, CO2, dissolved oxygen. Sugar availability is driven by the media constituents and any feeds that are used. Finally, lot-to-lot variation in commercially available supplements and feeds also introduce variation in glycosylation. It is important to select vendors and materials that have proven lot-to-lot consistency.

The wide variety of factors impacting glycosylation can make controlling the quality profile a complex challenge. Further, these same factors that drive post-translational modification also impact protein titer. In many cases scientists will focus optimization efforts on either titer or glycosylation first, but find that both titer and glycosylation are affected by any one change. In some cases, scientists will have to make a hard choice between quality and yield. It is possible to focus on the physiology of the cell and target the ER and Golgi to increase both titer and consistency.

Question: What factors influence glycosylation?

Answer: You ask an interesting question. We did see the same effect of increased consistency in two different media bases when adding Cell-Ess. Interestingly as you imply there were two different glycosylation patterns because the constituents in the base media were different but the increase in consistency of the glycoform patterns within each group remained the same. We hypothesize that the effect would be similar throughout a variety of different media because the way Cell-Ess works is to improve the function of the Golgi and the ER which is one of the major drivers of glycan addition. By improving the health of those organelles, the consistency will improve.

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