Post-translational modifications on human cell expressed (HCX™) human recombinant proteins

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hcx™ Human Cell Expressed

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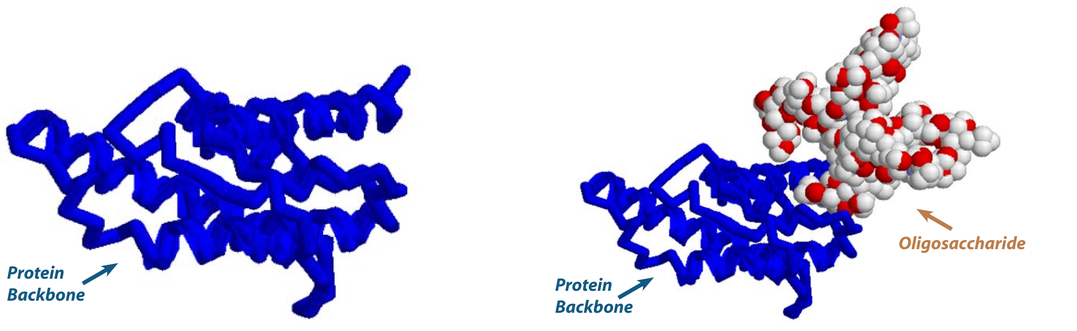
INTRODUCTION

Most proteins undergo post-translational modification (PTM), which can alter their physical and chemical properties (e.g., MW, pI, folding, stability, activity, antigenicity, and function). The presence or absence of PTMs may be significant to both the activity and longevity of the protein in a biological system.

Recombinant proteins are dependent on the machinery of the cell line in which they are made to determine their PTMs. Hence the PTMs of human proteins made recombinantly in a human cell line may differ significantly from the same protein made in NS0, CHO, *E. coli* or any other non-human cell line. For example *E. coli* does not possess the type of cellular machinery used for glycosylation in higher organisms, hence human proteins produced in an *E. coli* cell line are non-glycosylated. Consequently, the function of this protein may vary significantly from the glycosylated version.

Various methods were used to study the differences in PTM, in particular glycosylation, between recombinant human proteins expressed in modified human 293 cells as opposed to non-human cells. These methods determine not only the differences in glycosylation but may also give some insight into the possible differences in function of the protein.

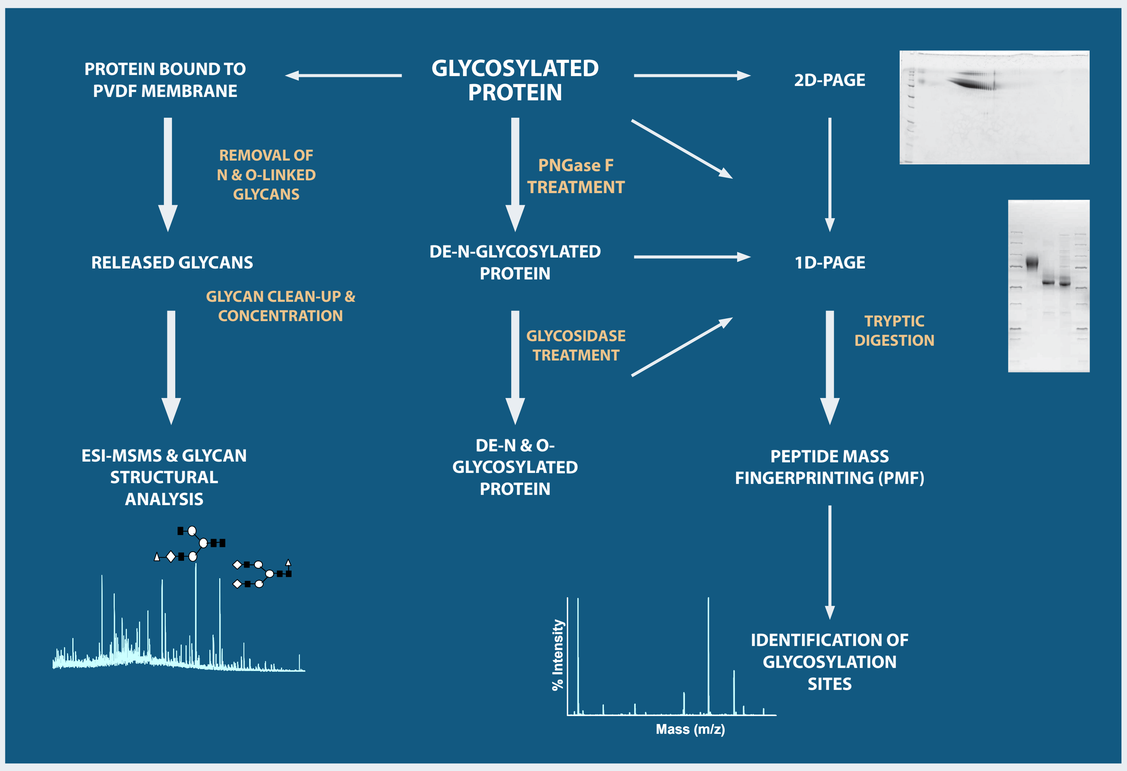
One-dimensional electrophoresis combined with enzymatic deglycosylation was used to determine the relative mass of the glycosylated versus the deglycosylated protein. Enzymatic and chemical de-glycosylation methods combined with MALDI-MS and LC-MS were used to determine the glycosylation sites as well as the N- and O-linked oligosaccharide structures present on the protein.



Human protein expressed in *E. coli* cell line

Human protein expressed in modified human 293 cell line

MATERIALS AND METHODS



CONCLUSION

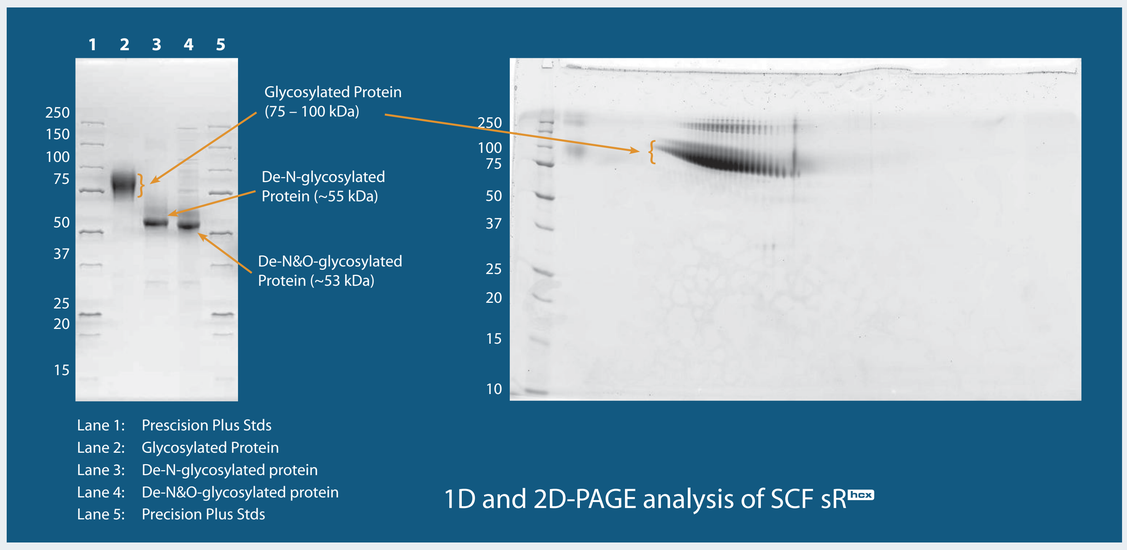
- Human cell expressed proteins differ from other recombinant proteins produced in non-human cell lines, such as NS0, *E. coli* and CHO, in the types of post-translational modifications that are present on the protein backbone.
- PTMs can alter the physical and chemical properties of the protein, and this may have a significant effect on the protein's activity, longevity, and immunogenicity in a biological system.
- *E. coli* does not possess the type of cellular machinery used for glycosylation, and hence human proteins produced in an *E. coli* cell line will be non-glycosylated.
- Proteomic and glycoproteomic techniques can be used to determine the relative quantity, structure, and position of glycosylation, which help enhance our knowledge of how a particular protein may react in a biological system.

RESULTS AND DISCUSSION

1D-PAGE

Using enzymes which remove the oligosaccharides while leaving the protein backbone intact provides valuable information into the amount of glycosylation that occurs on a particular protein. This can be expressed as a percentage of the total mass of the protein as it appears on a one-dimensional gel.

In the example shown here the glycoprotein appears as a fuzzy band with a molecular weight between 75-100 kDa. After removal of the N-linked oligosaccharides the protein resolves into a tighter band at 55 kDa, and subsequently drops to 53 kDa with the additional removal of the O-linked oligosaccharides. This indicates that approximately 25-45% of the glycoprotein mass is due to the N-linked oligosaccharides and 2% is due to the O-linked oligosaccharides.

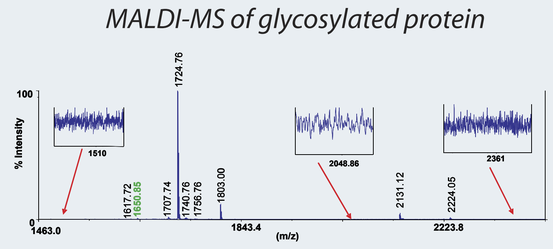


1D and 2D-PAGE analysis of SCF sR[hcx]

PEPTIDE MASS FINGERPRINTING (PMF) ANALYSIS

MS methods such as MALDI-MS typically are not able to detect glycopeptides due to their very large mass. By removing the oligosaccharides not only does the chance of seeing the peptides increase, but also critical information can be obtained as to the sites of glycosylation. Removing the N-linked oligosaccharides using enzymatic methods changes the asparagine amino acid to an aspartic acid. This changes the mass of the peptide, hence enabling the determination of whether the site is non-, partially or completely glycosylated. Extra peptides visible after the removal of O-linked oligosaccharides, also enables the prediction of peptides where O-linked glycosylation may occur.

MALDI-MS of glycosylated protein



MALDI-MS of de-glycosylated protein

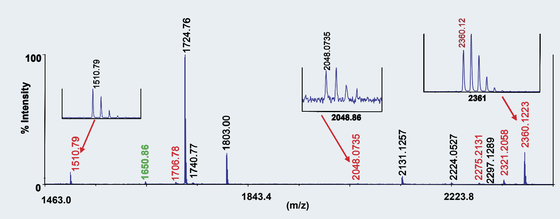


Table 1. *PMF analysis from 1D gel*

Treatment	Tryptic Coverage	N-linked Sites	O-linked Sites
none	31%	1 site seen not N-glycosylated	
PNGase F	47%	4 de-N-glycosylated sites seen	
PNGase F, Sialadase ß(1-4)Galactosidase ß-N-Acetylglucosaminidase O-Glycanase	39%	4 de-N-glycosylated sites seen	2 peptides not previously seen which contain 14 possible O-linked sites.

MS OLIGOSACCHARIDE STRUCTURAL ANALYSIS

Using the above methods to remove the oligosaccharides also enables the structure of the oligosaccharides to be determined using LC-MS/MS. This allows determination of the oligosaccharide epitopes, which contribute to the function of the protein. Our studies have shown major differences in the glycosylation between human protein produced recombinantly from modified human 293 cells as opposed to CHO or NS0 cell lines.