Glycosylation-specific separation of glycoproteins for glycomics studies

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Introduction

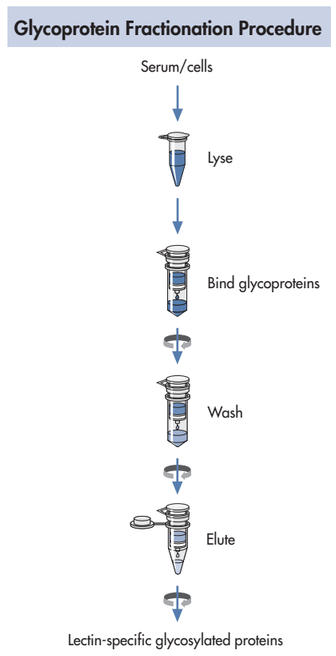
One of the most common post-translational protein modifications is the glycosylation of serine, threonine, and asparagine residues with mono- or oligosaccharides. Several diseases (e.g., rheumatoid arthritis) may be caused by a defect in protein glycosylation. Glycosylation of proteins plays a vital role in a wide range of cellular processes, such as:

- Cell adhesion and signaling
- Stabilization of protein structure and function
- Protein trafficking and sorting
- Oncogenesis

We set out to develop a system for analysis of glycoproteins that delivered:

- Highly specific separation of glycoproteins according to their glycan moieties
- Reduction in complexity of proteomics samples
- Standardized and reproducible separation
- Fast and easy-to-use protocols

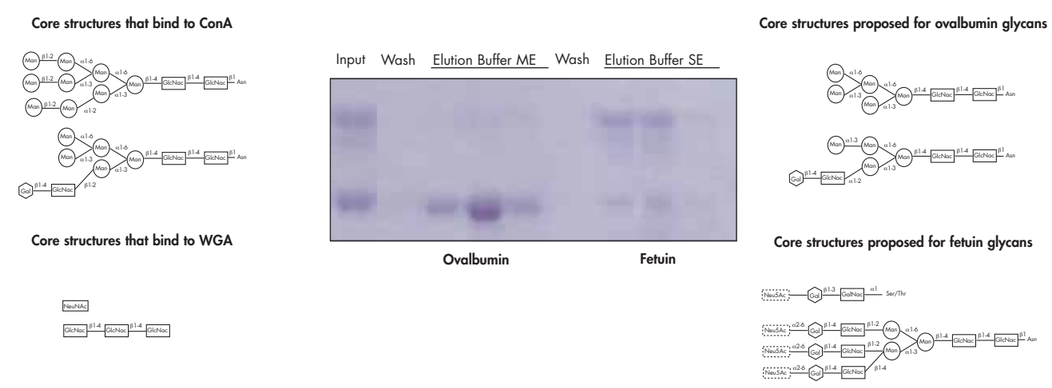
The result is the Qproteome range of glycoprotein kits presented here.



Efficient fractionation according to glycan moiety structure

A mixture of the two model proteins ovalbumin (which binds to ConA lectin) and fetuin (which binds to WGA lectin) was separated using the Qproteome Total Glycoprotein Kit. Proteins were applied to Total Glycoprotein spin columns and eluted using elution buffers specific for ConA-binding proteins (Buffer ME) and proteins that bind to WGA (Buffer SE). There was good agreement between the structures known to bind the respective lectins and those proposed to be present in the respective proteins (references 1–2).

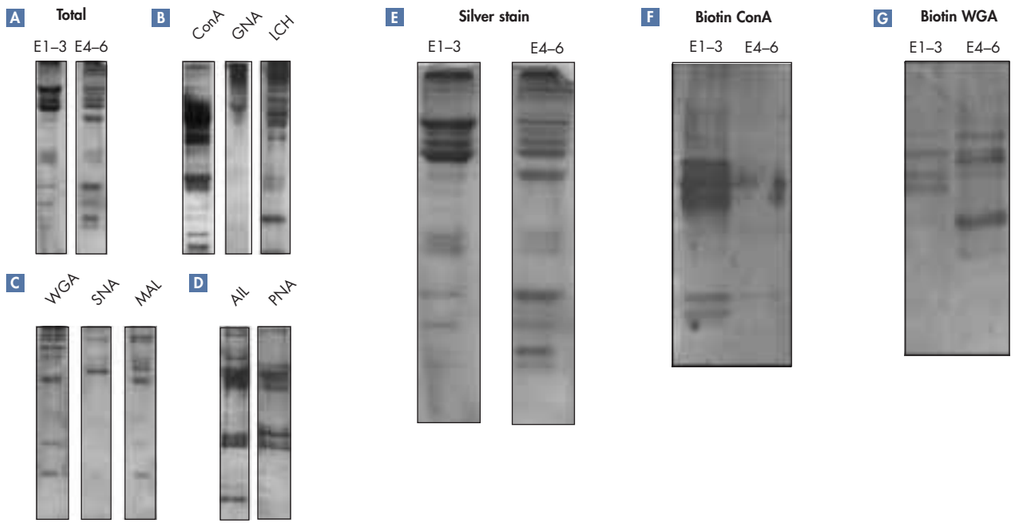
Efficient Separation of a Protein Mixture on the Basis of Glycan Moiety Makeup



100 µg of ovalbumin and 100 µg fetuin (Input) were mixed and applied to a Total Glycoprotein spin column. Protein was eluted in a two-step procedure using buffers specific for ConA-binding proteins (Buffer ME) and WGA-binding proteins (Buffer SE). After elution, aliquots from each fraction were separated by SDS-PAGE and visualized by Coomassie® staining.

A strategy for characterization of glycoproteins

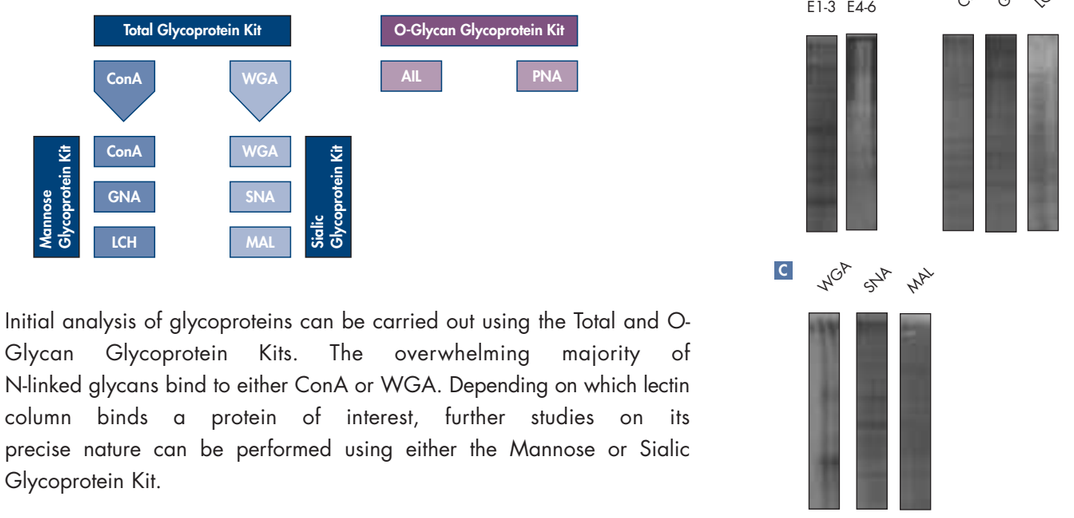
Highly Specific Glycoprotein Fractionation Using Lectin Spin Columns



A Pooled elution fractions 1–3 and 4–6 from Total Lectin Spin Columns in the Total Glycoprotein Kit. B Eluted glycoproteins from ConA, GNA, and LCH Spin Columns in the Mannose Glycoprotein Kit. C Eluted glycoproteins from WGA, SNA, and MAL Spin Columns in the Sialic Glycoprotein Kit. D Eluted glycoproteins from AIL and PNA Spin Columns in the O-Glycan Glycoprotein Kit. Glycoproteins were fractionated from serum using the different lectin spin columns in glycoprotein fractionation kits and analyzed by SDS-PAGE followed by silver staining E Silver stain of pooled elution fractions 1–3 and 4–6 from Total Lectin Spin Columns in the Total Glycoprotein Kit. Pooled fractions were transferred to a nitrocellulose membrane in a western blotting procedure and probed with F biotinylated ConA lectin or G biotinylated WGA lectin. Proteins were subsequently visualized using a streptavidin–HRP conjugate in a colorimetric reaction.

A strategy for characterization of glycoproteins

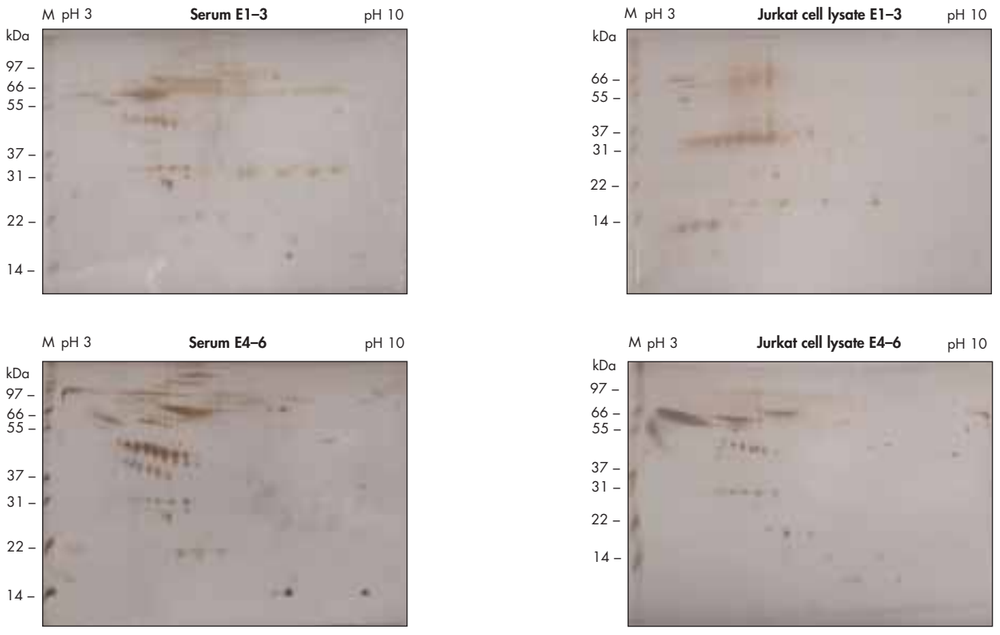
Comprehensive and Precise Analysis of Glycan Moieties



Initial analysis of glycoproteins can be carried out using the Total and O-Glycan Glycoprotein Kits. The overwhelming majority of N-linked glycans bind to either ConA or WGA. Depending on which lectin column binds a protein of interest, further studies on its precise nature can be performed using either the Mannose or Sialic Glycoprotein Kit.

Glycoproteins were fractionated from Jurkat cell lysates (1 x 10^7 cells) using the different lectin spin columns in glycoprotein fractionation kits and analyzed by SDS-PAGE followed by silver staining. A Elution steps 1–3 and 4–6 from Total Lectin Spin Columns in the Total Glycoprotein Kit. B Eluted glycoproteins from ConA, GNA, and LCH Spin Columns in the Mannose Glycoprotein Kit. C Eluted glycoproteins from WGA, SNA, and MAL Spin Columns in the Sialic Glycoprotein Kit.

2D-PAGE analysis of glycoprotein elution fractions



2D-PAGE analysis of A human serum and B Jurkat cell lysate Total Glycoprotein column elution fractions.

Summary

Glycoproteins fulfill a wide range of functions in the cell and new functions are constantly being discovered. Therefore, their analysis and characterization is currently of great interest. Using Qproteome glycoprotein kits we were able to achieve:

- Efficient separation of glycosylated proteins from serum and cell lysates
- Specific separation of glycosylated proteins on the basis of the chemical makeup of their glycan moieties
- Reduction in complexity of proteomics samples for detailed analyses of the glycome

References

1) Nilsson, B., Norden, N.E., and Svensson, S. (1979) Structural studies on the carbohydrate portion of fetuin. J. Biol. Chem. **254**, 4545.

2) Lattova, E., Perreault, H., and Krokhin, O. (2004) Matrix-assisted laser desorption/ionization tandem mass spectrometry and post-source decay fragmentation study of phenylhydrazones of N-linked oligosaccharides from ovalbumin. J. Am. Soc. Mass Spectrom. **15**, 725.

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