

## Effects of Lectins Affinity Chromatography on Glycoproteins Enrichment Using AP-MALDI Ion Trap Mass Spectrometry

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### Introduction

Glycosylation is a common co- and post-translational modification of proteins, which may have profound effects on protein structure and function. In glycoproteins, different carbohydrates can be attached to different position on the protein back bone at a given time. In addition, different substituents such as acetyl, lactyl, formyl and sulfate can modify a single carbohydrate thus making a protein structure more complex. For example, sialic acid, which is the outermost molecule in most of the glycoconjugates, occurs in nature in more than 50 different varieties. To analyze minute changes which occur in glycoproteins, we need to concentrate the specific glycopeptides by using lectin affinity chromatography. The specific binding of lectins to carbohydrate structures is used to identify and characterized these structures. Thus, lectins can be used for the selective isolation, purification and concentration of glycoconjugates. After enrichment of glycoproteins, these can be analyzed and identified well-suited atmospheric pressure matrix-assisted laser desorption/ionization (AP-MALDI) and the identity of the glycoprotein can be confirm by MS/MS structural identification analysis.

### Experimental

Experiments were carried out on a Thermo Finnigan (San Jose, CA, USA) LCQ Deca XP ion trap mass spectrometer integrated with an AP-MALDI ion source (MassTech Inc., Columbia, MD, USA). Operating conditions for all full MS and MS/MS experiments were as follows: automatic gain control was off, ion injection time was 220 ms, the temperature of the LCQ input capillary was held at 280 °C. The LCQ was operated in high mass range mode (up to 4000 Da), and was calibrated using the AP/MALDI source and a standard proteins.

Ovalbumin, glycoporphin and fetuin were digested either after reduction and alkylation or directly in a volatile buffer containing 100 mM ammonium bicarbonate. The digestion was carried out by using 10 µl trypsin beads with 1:100 v/v enzyme to substrate ratio. Digestion was carried out at +37 °C, with overnight shaking. After digestion, an aliquot of digested peptides and glycopeptides was applied to 10 µl immobilized lectin columns (TopTip). After several washes with binding buffer and water, the glycopeptides were eluted from the column by using a 300 mM monosaccharides solution (e.g. WGA-bound glycopeptides were eluted with N-acetylneuraminic acid and ConA-bound glycopeptides were eluted with N-acetylglucosamine or mannose). The samples were then further purified by using C-18 or carbon + C-18 micropipette tips (Nutip). After affinity purification, the samples were then placed onto the MALDI target plate with 1.5 mM 4-HCCA matrix solution for analysis.

### Results

This study shows that different lectins and/or different immobilization techniques provide varying results with respect to the identification of different glycopeptides from fetuin, glycoporphin and ovalbumin. Only three spectra of Fetuin are presented from the entire study as an example. In Figure 1, we demonstrate AP-MALDI spectra of Fetuin trypsin digest (A) different Lectins (B) affinity chromatography and MS/MS data for different glycopeptide fragments (C).

### Discussion

After the partial purification and enrichment of glycopeptides with different lectins using micro pipette tips containing different immobilized lectins, glycopeptides were analyzed using AP-MALDI ion trap mass spectrometry. The mass fragment peaks obtained after lectin purification of glycopeptides were then

