

## Study of Glycoproteins in Human Serum and Human Plasma Using Multi-Lectin Affinity Chromatography Coupled with RPLC-MS/MS

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### Introduction:

Glycosylation is one of the most common post translational modifications and it plays a fundamental role in a diversity of biological processes, such as the immune system, cellular regulation, processing and function of receptors, and targeted clearance of serum proteins. It is proposed that about 50% human plasma proteins are glycosylated. Lectins are the proteins that can recognize and capture specific carbohydrate residues. In this study, a multi-lectin affinity column containing concanavalin A (Con A), wheat germ agglutinin (WGA), and jacalin lectin (JAC) has been prepared and used to isolate glycoproteins from human serum and human plasma samples provided by Human Proteome Organization (HUPO). The glycoproteome of Caucasian American, Asian American, and African American have been compared.

### Method:

The multi-lectin affinity column was prepared from a mixture of equal amount of agarose gel immobilized with Con A, WGA, and JAC lectin. Serum or plasma samples were diluted and loaded on the multi-lectin affinity column. The non-captured components were then removed by a washing step and the flow-through fraction was collected. Specific displacers of the lectins were used to elute proteins captured by the affinity column. The flow-through and elution fractions were compared on SDS-PAGE with staining using Schiff's reagent. In addition, the elution fraction was analyzed by trypsin digestion followed by LC/MS/MS. The glycoproteins are identified using the SEQUEST algorithm. The HUPO serum and plasma samples, and the samples from three ethnic groups were compared.

### Results:

The unbound fraction and captured fraction were compared to indicate the specificity and efficiency of the multi-lectin affinity column using SDS-PAGE with Schiff's reagent stain, which specifically stains glycoproteins, and with LC-MS/MS tests (Table 1.). It was found that the multi-lectin column was highly specific for glycoproteins and extended the dynamic range of the proteome measurement. In order to investigate reproducibility of the enrichment procedure, the complete experiment was repeated, and the capture was found to be reproducible (Figure 1.). In the first captured sample, 50 glycoproteins were identified with 2 or more peptides. While 49 glycoproteins were identified in the second captured sample, and 46 proteins were found to

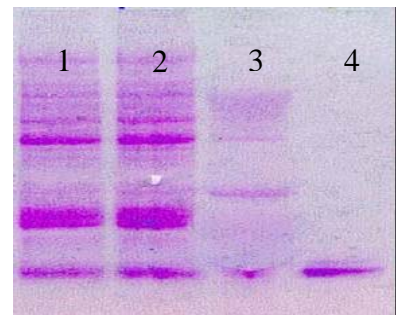


Figure 1, The similar SDS-PAGE profile of two multi-lectin column captured samples (1 and 2), and the less intense staining the flowthrough ( 3 ) indicated the reproducibility and the efficiency of the procedure. Lane 4 is a positive control.

be in common with those found in the first sample. The results obtained with this affinity step also indicated that about 10% of human serum proteins are glycosylated (weight/weight).

Rank	Reference	Hits in EL	Hits in FL	Glycoprotein
1	ALPHA-2-MACROGLOBULIN	86	0	Y
2	SEROTRANSFERRIN	61	14	Y
3	HAPTOGLOBIN-2	60	0	Y
4	HAPTOGLOBIN-RELATED PROTEIN	60	0	Y
5	Alpha-1-antitrypsin	53	7	Y
6	HEMOPEXIN (BETA-1B-GLYCOPROTEIN)	51	0	Y
7	IG ALPHA-1 CHAIN C REGION	42	1	Y
8	COMPLEMENT C3	34	0	Y
9	IG KAPPA CHAIN C REGION	34	0	Y
10	A-FOLIOPROTEIN A-I	24	17	Y
11	SERUM ALBUMIN	22	307	N
12	ALPHA-1-ANTITRYPSIN	19	0	Y
13	ALPHA-2-HS-GLYCOPROTEIN	18	0	Y
14	IG MU CHAIN C REGION	16	0	Y
15	INTER-ALPHA-TRYPsin INHIBITOR HEAVY CHAIN	14	0	Y
16	PREGNANCY ZONE PROTEIN	14	0	Y
17	CERULOPLASMIN	13	0	Y
18	COMPLEMENT FACTOR H	13	0	Y
19	COMPLEMENT C4	12	0	Y
20	HISTIDINE-RICH GLYCOPROTEIN	12	0	Y

Table 1. Top 20 proteins captured by the multi-lectin column. Except for serum albumin, all are glycoproteins. The efficiency was shown by the absence of 15 glycoproteins in the flowthrough fraction and significant enrichment of the other 4 glycoproteins.

Compared with plasma, fibrinogen and plasminogen were the only major glycoproteins absent in the serum glycoproteome. The glycoproteins which had significant up and down regulated in the different HUPO samples are listed in table 2.

ID	Protein name	Regulation difference
ANGT	ANGIOTENSINOGEN	UP in CA
AG2R	TYPE-1A ANGIOTENSIN II	UP in FA
AG22	TYPE-2 ANGIOTENSIN II RECEPTOR	UP in AA
VTNC	VITRONECTIN	DOWN in FA; Up in AA
HRG	HISTIDINE-RICH GLYCOPROTEIN	DOWN in CA

Table 2. The glycoproteins found to have significant up or down regulations in one ethnic group comparing to the other two HUPO samples. CA: Caucasian American; FA: African American; AA: Asian American

### Conclusions:

- Multi-Lectin affinity chromatography can be used to enrich glycoproteins from human serum.
- The enrichment procedure is efficient, specific and reproducible.
- Serum keeps major glycoproteome from plasma.

### References:

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