A New Monolithic ConA Affinity Column for Purification and Analysis of Glycans, Glycopeptides, and Glycoproteins

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INTRODUCTION

Lectin-affinity chromatography has been widely used for the purification and analysis of oligosaccharides, glycopeptides, and glycoproteins. However, most of the lectin-affinity columns currently available are agarose bead-based spin columns, which have to be operated manually and can only be used for a limited number of purification cycles. With the growing interest in glycoproteomic studies, such as biomarker identification, there is an increasing need for a robust HPLC lectin column. Presented here is the development and applications of a new monolithic Concanavalin A (Con A) affinity column. Concanavalin A is a lectin derived from *Canavalia ensiformis* (Jack bean) seeds. At neutral and alkaline pH, Con A exists as a tetramer of four identical subunits with a total molecular weight of approximately 104 kDa. Below pH 5.6, Con A dissociates into active dimers of 52 kDa. Con A is one of the most well characterized and widely used lectins. It binds to α-mannose, and to α-glucose with weaker affinity. Divalent metal ions such as calcium (Ca^{2+}) or magnesium (Mg^{2+}) need to be present to keep Con A active for its binding to carbohydrates. Figure 1 shows the four monomer units, each of which binds a calcium and a transition metal, typically manganese. The high-capacity ProSwift® ConA-1S column can provide fast and efficient purification and analysis for various Con A-binding glycoconjugate samples. The HPLC compatibility of this column allows automatic sample injection, high throughput, and excellent reproducibility.

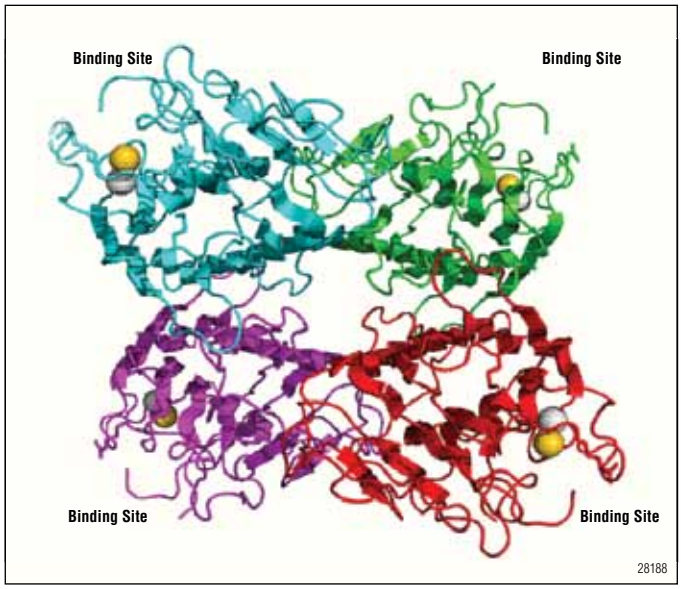

Figure 1. Structure of Concanavalin A at 2.4A resolution; Hardmad, K.D.; Ainsworth, C.F. Biochemistry, ***1972***, 11, *4910–4919.*

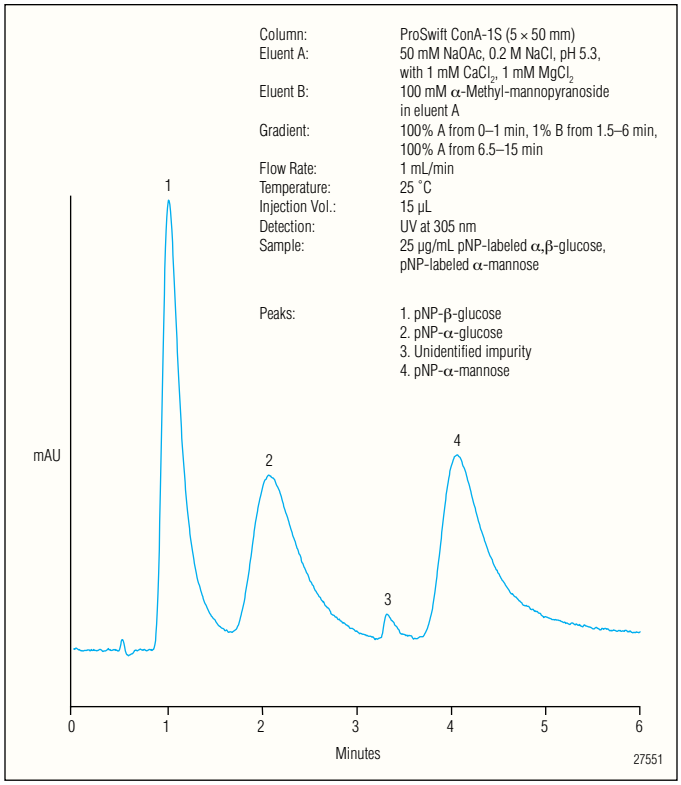

Figure 2. Specificity of the ProSwift ConA-1S column. Separation of three pNP-sugars.

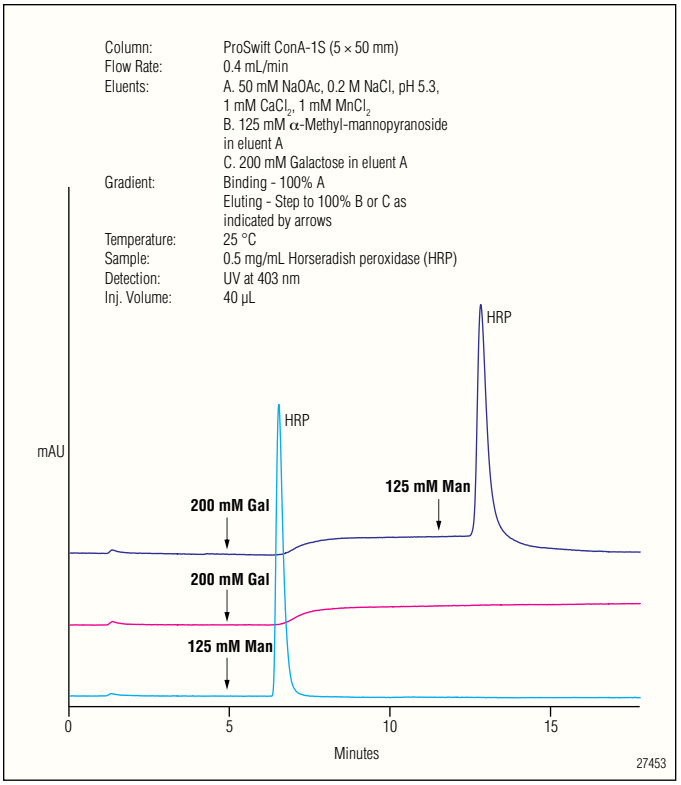

Figure 3. Specificity of the ProSwift ConA-1S column. Specific elution of horseradish peroxidase.

SYSTEM REQUIREMENTS

The ProSwift ConA-1S column can be used on any compatible HPLC system, which usually consists of a gradient pump; an autosampler; a thermal compartment; and a UV, fluorescence, or other type of detector, depending on the sample type.

COLUMN SPECIFICATIONS

Column Dimension:	5 × 50 mm
Protein Coated on Monolith:	Concanavalin A
Bimodal Monolith Pore Diameter:	3.25 and 0.62 µm
Binding Capacity:	~2 mg HRP/column
Operating Flow Rates:	Up to 2 mL/min
pH Range:	pH 5–8
Operating Temperature:	≤30 °C
Maximum Pressure:	2300 psi
Organic Solvent Limit:	10% Methanol

COLUMN CHEMISTRY AND SPECIFICITY

The ProSwift ConA-1S column is a polymeric monolith prepared by in-column polymerization, followed by functionalization with Con A. The monolith is a cylindrical polymer rod containing uninterrupted, interconnected, flow-through pores, with surface area greater than nonporous bead-based columns. The structure consists of small pores that contribute surface area, and larger pores that allow reduced backpressure at elevated flow rates. This approach results in short mass-transfer distances that produce improved efficiency, even at elevated flow rates. High quality Con A is covalently attached to the monolith column through the amine groups of Con A (see Figure 1). The sugar binding sites are protected during the conjugation process so the Con A activity is well maintained. Figures 2 and 3 show the specificity of the ProSwift ConA-1S column. Three pNP-labeled sugars were separated based on their different affinities towards Con A. Horseradish peroxidase (HRP), which is a glycoprotein with rich high-mannose type glycans, was bound to the Con A column and was eluted with α-methyl-mannopyranoside, which has high affinity for Con A.

LOADING CAPACITY OF ConA-1S COLUMN

The loading capacity for HRP is no less than 2.0 mg on the ProSwift ConA-1S column at a flow rate of 1 mL/min. Figure 4 shows the linearity of area to sample load for HRP when loaded onto the ProSwift ConA-1S column. This correlation allows the ProSwift ConA-1S to be used for quantitation of enriched species.

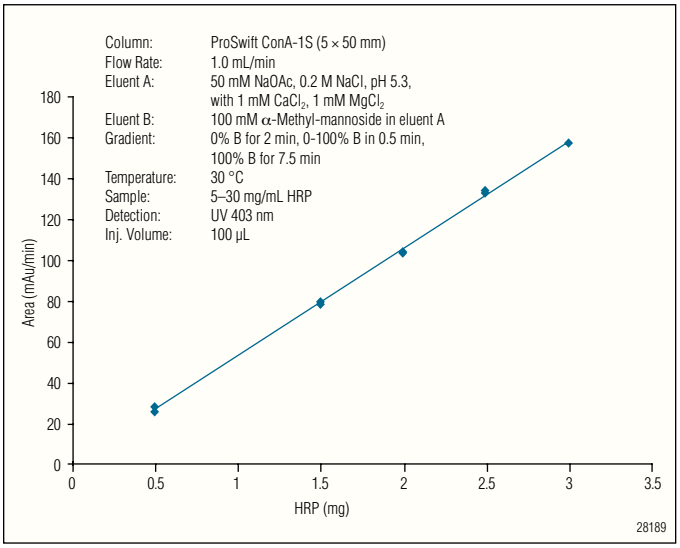

Figure 4. Linearity of area to sample load for HRP on ConA-1S column.

COLUMN RUGGEDNESS

The ProSwift ConA-1S column can be regenerated easily by washing with conditioning buffer after sample binding and elution. The rugged column chemistry allows hundreds of run cycles with minimal capacity loss. Figure 5 shows the ProSwift ConA-1S column maintains good capacity after 100 injections and elutions of HRP.

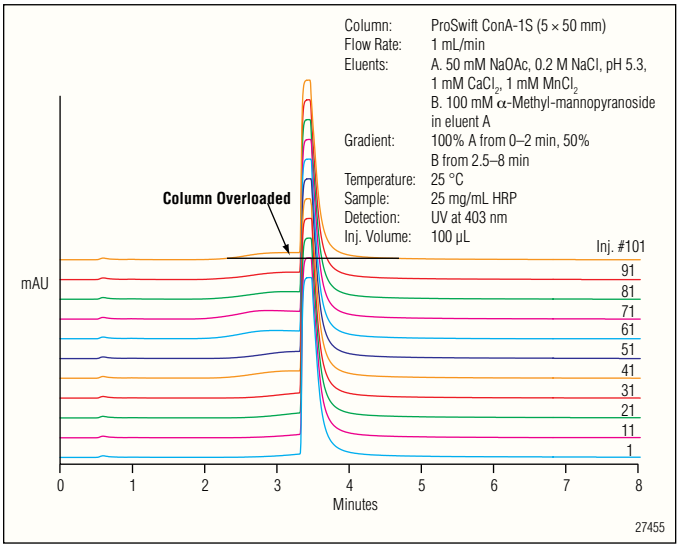

Figure 5. One hundred binding-elution cycles of HRP.

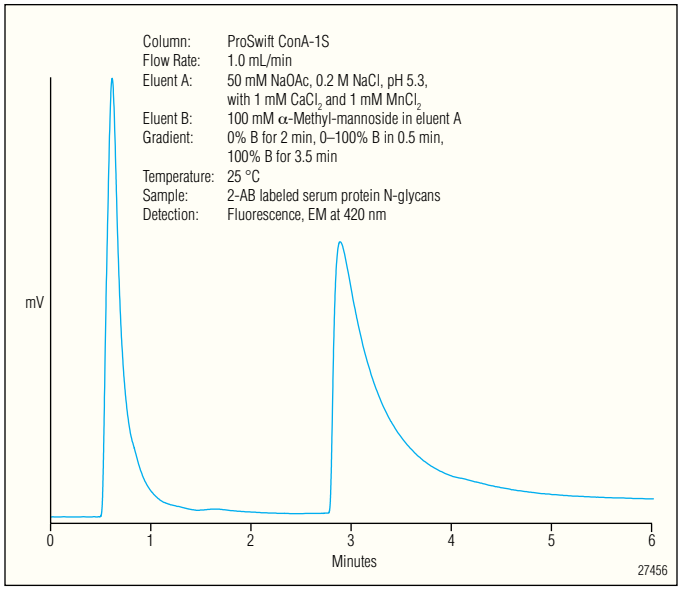

Figure 6. Purification of fluorescent-labeled glycans.

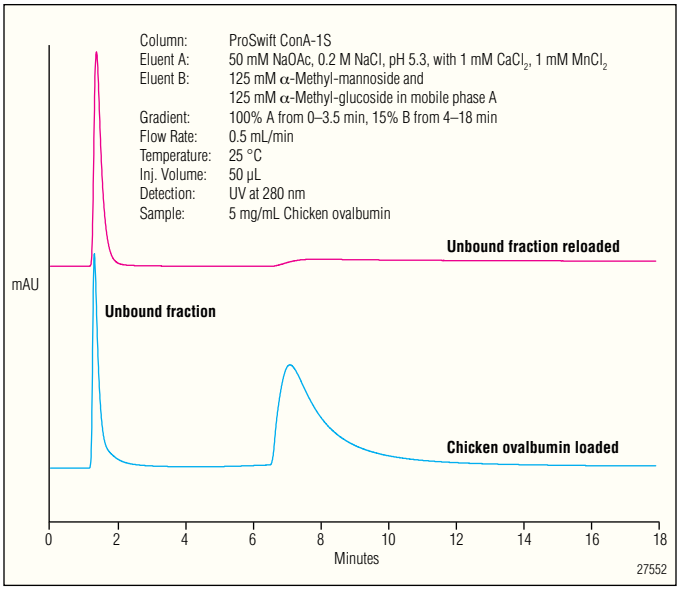

Figure 7. Separation of chicken ovalbumin glycoforms.

APPLICATIONS

Con A is one of the most well characterized and widely used lectins. It binds to β-mannose, and to β-glucose with weaker affinity. Usually high-mannose type glycans bind to Con A strongly, and some hybrid type glycans can also bind to Con A with good affinity, while complex type glycans usually have very weak affinity towards Con A. As β-mannose is commonly expressed on most glycoproteins, a Con A-affinity column is a useful tool for purification and enrichment for glycans, glycopeptides, and glycoproteins.

Fractionation of Glycans

Glycan samples can be fractionated on the Con A column based on their different affinities to Con A. Figure 6 shows fluorescence-labeled serum N-glycans were fractionated into two fractions on the ProSwift ConA-1S column.

Separation of Glycoprotein Glycoforms

Protein glycoforms with different affinities to Con A can be separated on the ProSwift ConA-1S column. In Figure 7, chicken ovalbumin was fractionated into an unbound and a bound fraction on this column. The unbound fraction was collected and reloaded onto the column. All of the previously unbound fraction again eluted in the flow-through. Elution of the unbound fraction indicates that it has different glycosylation pattens than the bound fraction.

Enrichment of Glycopeptides

HRP was digested with trypsin. The tryptic digest was fractionated on the ProSwift ConA-1S column. The bound and unbound fractions were collected and analyzed on a reversed-phase column (Figure 8).

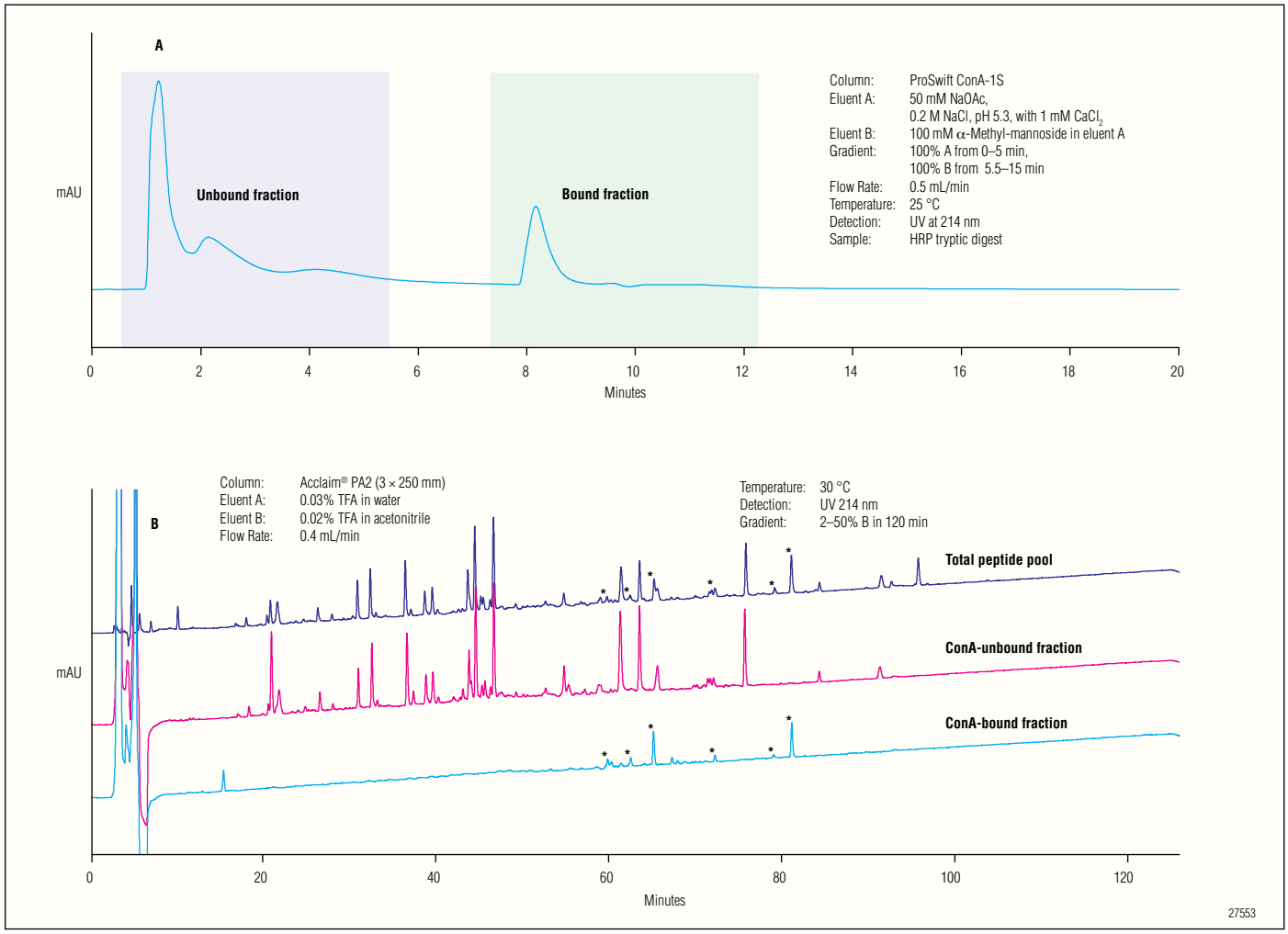

Figure 8. Enrichment of HRP glycopeptides. A) Enrichment of glycopeptides on ProSwift ConA column. B) Profiling of HRP peptide pools on a reversed-phase column.

Enrichment of Glycoproteins

Depleted human plasma proteins were fractionated on the ProSwift ConA-1S column (Figure 9).

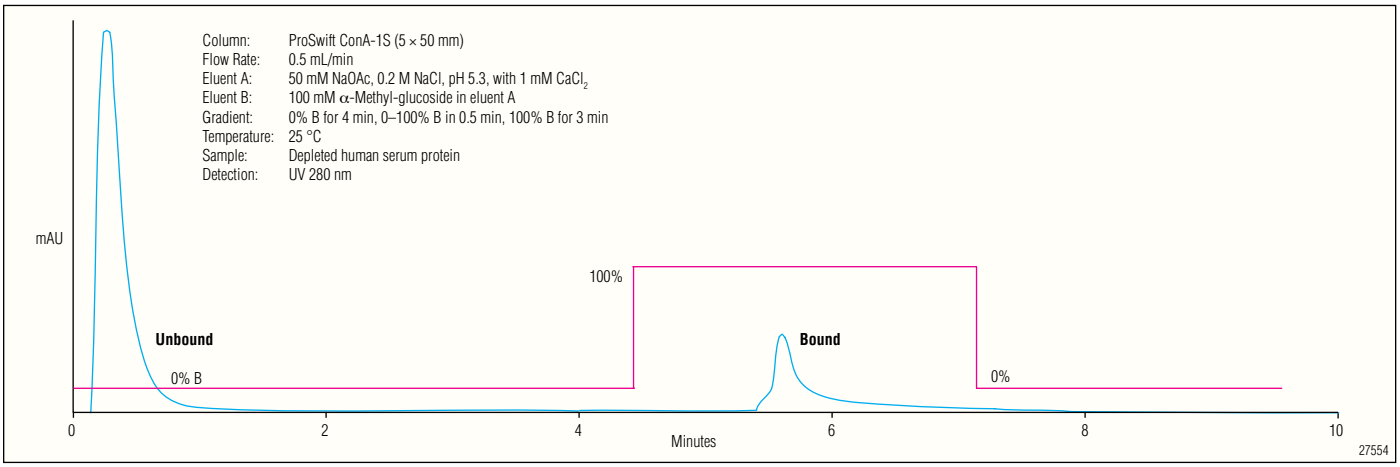

Figure 9. Enrichment of glycoproteins.

CONCLUSION

- A novel monolithic HPLC Con A-affinity column with high capacity and specificity was developed.
- The ProSwift ConA-1S column provides highly efficient enrichment, purification, and analysis on glycan, glycopeptide, and glycoprotein samples.
- The HPLC-compatibility of the ProSwift ConA-1S column allows automation, high throughput, and excellent reproducibility.

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