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Note

Rapid resolution of nucleotide sugars by lectin affinity high-performance liquid chromatography

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Lectins are highly specific carbohydrate-binding proteins with haemagglutinating activity[1]. Since each lectin has a characteristic carbohydrate-binding property, its specificity makes it a useful and important tool in various fields of research such as biochemistry, cell biology, immunology and serology. From recent biochemical research, cell surface specificity may be expected to play an important role in cell interactions and cell recognition. Lectins immobilized on agarose are employed in purifying glycoproteins, glycopeptides and oligosaccharides. For example, Blake and Goldstein[2] performed a very efficient separation of nucleotide sugars on *Bandeireae simplifolia* I lectin-Sepharose, whereas it is very difficult to resolve epimeric UDP-glucose and UDP-galactose (UDP = uridine diphosphate) using a paper chromatographic system in which each migrates with an R_F value of 0.84. Recently, lectin affinity high-performance liquid chromatography (HPLC) has been applied to fractionation of glycoprotein in serum[3].

In this note we report the application of lectin affinity HPLC to the rapid separation of nucleotide sugars. We have taken advantage of the specificities of RCA_{120}, PNA and SBA lectins for D-galactopyranosyl and 2-acetamido-2-deoxy-D-galactopyranosyl residues, and WGA for the 2-acetamido-2-deoxy-D-glucopyranosyl residue, in order to perform separations of UDP-glucose and UDP-galactose, UDP-N-acetyl-D-glucosamine and UDP-N-acetyl-D-galactosamine, monitored by the UV adsorbance of uridine at 261 nm.

MATERIALS AND METHODS

Chemicals

A poly(acrylic ester) gel (WG003) for HPLC which contained epoxy residues was obtained from Hitachi Chemical Co. (Tokyo, Japan). Lectins (RCA_{120}, SBA, WGA, PNA) were obtained from Hohnen Oil Company (Tokyo, Japan). UDP-glucose, UDP-galactose, UDP-N-acetyl-D-glucosamine and UDP-N-acetyl-D-galactosamine were purchased from Sigma (St. Louis, MO, U.S.A.), and were dissolved in 0.01 *M* phosphate buffer, pH 7.4, containing 0.15 *M* sodium chloride.

Immobilization of lectins to epoxy-activated gel (WG003)

The coupling of lectins to gel was performed according to Matsumoto *et al.*[4].

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The final lectin gels, RCA_{120}-WG003, PNA-WG003, WGA-WG003 and SBA-WG003, contained *ca.* 8.87 mg of RCA_{120}, 5.86 mg of PNA, 7.26 mg of WGA and 3.55 mg of SBA per gram of suction-dried gel, respectively, as determined from the absorbance at 280 nm of the original and filtered lectin solutions.

Chromatographic conditions

The lectin-immobilized gels were suspended in phosphate-buffered saline (PBS), *i.e.*, 10 m*M* phosphate buffer, pH 7.4, containing 0.15 *M* sodium chloride and packed into 250 × 4.6 mm I.D. columns by the slurry packing method at a flow-rate of 1.5 ml/min using the same buffer, which was throughly degassed under vacuum and sonication before use. An HPLC system comprising a Shimazu LC-4A pump system, a SPD-2AS UV spectrophotometric detector (Shimazu Seisakusho Co., Kyoto, Japan) and an injector (Rheodyne, Cotatai, CA, U.S.A.) with a 20-μl loop was used of a flow-rate of 1.0 ml/min at room temperature.

RESULTS AND DISCUSSION

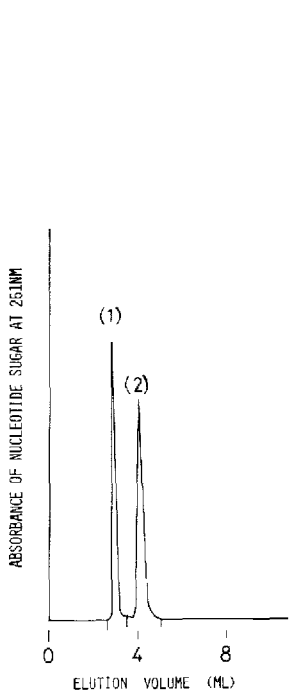
Table I shows the elution volumes of nucleotide sugars on the various lectin affinity columns. The RCA_{120} affinity column was effective for the separations of UDP-glucose and UDP-galactose and of UDP-N-acetyl-D-glucosamine and UDP-N-acetyl-D-galactosamine. The SBA column was effective only for separation of the latter pair. Neither the PNA nor the WGA column allowed the separation of these nucleotide sugars. The elution profiles of UDP-N-acetyl-D-glucosamine and UDP-N-acetyl-D-galactosamine from the RCA_{120}-WG003 and SBA-WG003 columns are shown in Figs. 1 and 2, respectivley. The profile of UDP-glucose and UDP-galactose eluted from the RCA_{120}-WG003 column is shown in Fig. 3. The SBA-WG003 column yielded a broad peak of UDP-N-acetyl-D-galactosamine. It seems that the four kinds of isolectins in SBA, which have different specific haemagglutinating activities[5], have different effects on the elution of UDP-N-acetyl-D-galactosamine.

TABLE I

ELUTION VOLUMES (ml) OF NUCLEOTIDE SUGARS ON VARIOUS LECTIN AFFINITY COLUMNS

Lectin	*Group I*		*Group II*	
	UDP-GlcNAc	*UDP-GalNAc*	*UDP-Glc*	*UDP-Gal*
RCA	3.05	4.23	3.06	3.95
PNA	3.04	3.04	3.04	3.06
WGA	3.09	3.12	3.04	3.06
SBA	3.06	3.81	3.06	3.09

Fig. 4 shows the effect of sample concentration on separations of nucleotide sugars. On the RCA_{120}-WG003 column the elution of UDP-N-acetyl-D-glucosamine did not change, while UDP-N-acetyl-D-galactosamine was eluted more rapidly as its concentration increased.



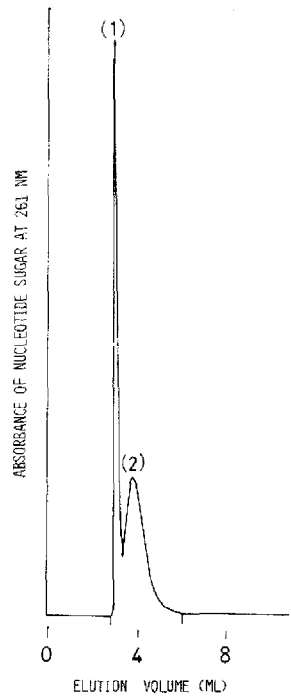
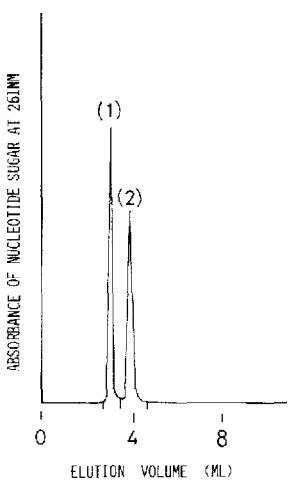


Fig. 1. Separation of UDP-N-acetyl-D-glucosamine (0.05 mg/ml) (1) and UDP-N-acetyl-D-galactosamine (0.05 mg/ml) (2) on the RCA_{120}-WG003 column.

Fig. 2. Separation of UDP-N-acetyl-D-glucosamine (0.10 mg /ml) (1) and UDP-N-acetyl-D-galactosamine (0.10 mg/ml) (2) on the SBA-WG003 column.



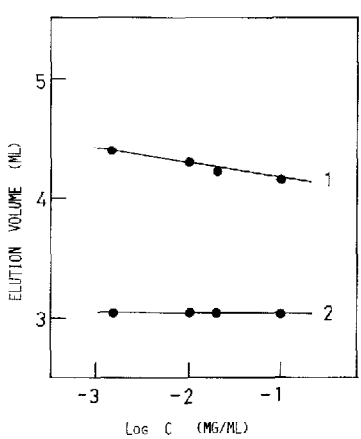
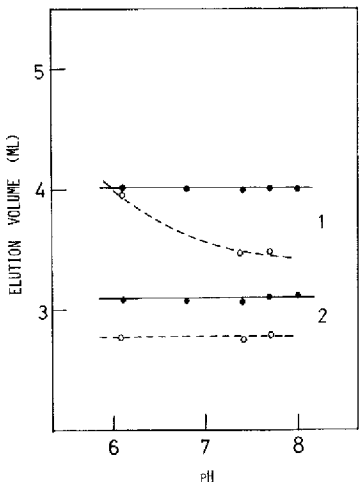


Fig. 3. Separation of UDP-glucose (0.05 mg /ml) (1) and UDP-galactose (0.05 mg/ml) (2) on the RCA_{120}-WG003 column.

Fig. 4. Effect of sample concentration on the separation of UDP-N-acetyl-D-galactosamine (1) and UDP-N-acetyl-D-glucosamine (2).

As the affinity of lectins has been reported to be dependent on pH[6], we have estimated the effect of pH on the separation of nucleotide sugars by the RCA_{120}-WG003 column (Figs. 5 and 6). In the case of the mobile phase containing 0.15 *M* NaCl, the pH of the buffer has a slightly negative effect on the separation of UDP-N-acetyl-D-glucosamine and UDP-N-acetyl-D-galactosamine under the acidic conditions. In contast, in the case of a phosphate buffer without sodium chloride, the RCA_{120}-WG003 column gave a more efficient separation of nucleotide sugars under acidic conditions.



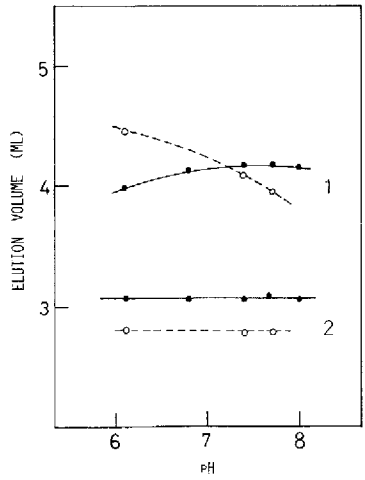


Fig. 5. Effect of pH and salt concentration on the separation of (1) UDP-galactose (0.05 mg/ml) and (2) UDP-glucose (0.05 mg/ml) on the RCA_{120}-WG003 column. ●—●, PBS containing 0.15 *M* NaCl; ○----○, PBS without NaCl.

Fig. 6. Effect of pH and salt concentration on the separation of (1) UDP-N-acetyl-D-galactosamine (0.05 mg/ml) and (2) UDP-N-acetyl-D-glucosamine (0.05 mg/ml) on the RCA_{120}-WG003 column. Details as in Fig. 5.

The results presented here demonstrate that the RCA_{120}-WG003 column is a very effective affinity adsorbent for rapid separations of nucleotide sugars containing terminal D-galactopyranosyl or 2-acetamido-2-deoxy-D-galactopyaranosyl residues. This column can facilitate the determination of the purity of commercial and synthetic UDP-D-galactose and UDP-N-acetyl-D-galactosamine. This column may also be effectively used to separate reactants from products in a fixed-time assay of UDP-galactose-4-epimerase activity.

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