Cross-linked arabinogalactan: A new affinity matrix for the purification of *Ricinus communis* lectins

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Summary. Arabinogalactan has been cross-linked to give a new high capacity affinity matrix for the purification of *Ricinus communis* lectins. It shows a capacity which is 50 times greater than that of the conventional affinity matrices.

The lectins from *R. communis* have been successfully used as macromolecular probes for cell surface carbohydrate architecture and for the affinity chromatography of glycoproteins and glycolipids[2-8]. *R. communis* contains 2 proteins, a toxic protein (RCA_2, mol.wt 60,000), with an affinity for N-acetylgalactosamine and D-galactose, and another protein (RCA_1, mol.wt 120,000), which has an affinity for D-galactose only and can agglutinate erythrocytes[4]. The toxic protein RCA_2 inhibits protein synthesis and prevents the growth of tumors[10]. The lectins have earlier been purified by alcohol precipitation and ion exchange, chromatography[11] and more recently by affinity chromatography on a sepharose matrix[12] or a ligand bound to sepharose[13]. However, the capacity of sepharose for these lectins has been reported to be only 0.3–0.4 mg/ml of the gel[4]. The nonavailability of methods for large scale purification has hampered detailed physicochemical studies on these proteins of biological interest. Here we report a simple method for the rapid purification of the lectins in large amounts utilizing cross-linked arabinogalactan as an affinity matrix.

Materials and method. Larchwood arabinogalactan was obtained from Sigma Chemical Co. and Epichlorohydrin from BDH, England. Castor beans were purchased locally. Arabinogalactan was cross-linked with epichlorohydrin according to the method of Flodin[14]. Proteins were estimated according to the method of Lowry et al.[15]. A dialyzed 60% ammonium sulphate fraction of *R. communis* seed extract was subjected to affinity chromatography on the cross-linked arabinogalactan column. The purity of the eluted lectins was checked by polyacrylamide disc gel-electrophoresis[16].

Results. The elution profile of *R. communis* lectins adsorbed on cross-linked arabinogalactan and eluted by lactose is shown in figure 1, and the profile for separation of the lectins RCA_2 and RCA_1 on this column by elution with GalNAc and lactose, respectively, is shown in figure 2. 2 bands of equal intensity are shown in the electrophoretic pattern of *R. communis* lectins eluted by lactose (figure 3a). The GalNAc (figure 3b) and lactose (figure 3c) aluted fractions show single bands corresponding to RCA_2 and RCA_1, respectively. The binding capacity of cross-linked arabinogalactan for lectins was checked by loading a known amount of purified lectin on a small column

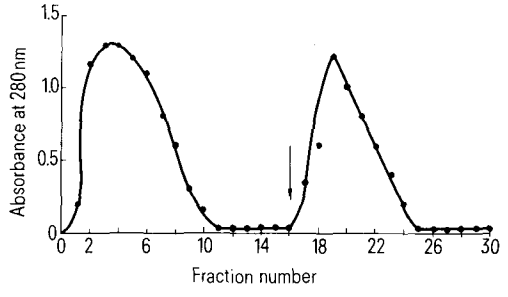

Fig. 1. The elution of *Ricinus communis* lectin bound to cross-linked arabinogalactan column. The dialyzed ammonium sulphate fraction was loaded on the arabinogalactan column (1.6 × 20 cm) at 4 °C. After washing the column exhaustively with 160 ml of PBS at a flow rate of 20 ml/h the adsorbed proteins were eluted with 0.1 M lactose in the buffer. Absorbance at 280 nm was recorded for each 10 ml fractions for the washings and each 2 ml fractions for the elutions. The position of the arrow indicates where the elution with lactose was commenced.

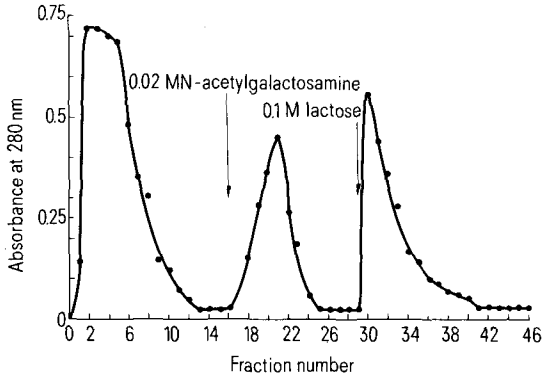

Fig. 2. The elution of 2 lectins RCA_2 and RCA_1 bound to cross-linked arabinogalactan column. The dialyzed ammonium sulphate fraction was loaded on the arabinogalactan column (1.6 × 20 cm) at 4 °C. After washing the column exhaustively with 160 ml of PBS, the adsorbed RCA_2 was eluted with 26 ml of 0.02 M N-acetylgalactosamine in PBS. It was further eluted with 34 ml of 0.1 M lactose in the buffer. Absorbance at 280 nm was recorded for each 10-ml fractions for the washings and each 2-ml fractions for the elutions.

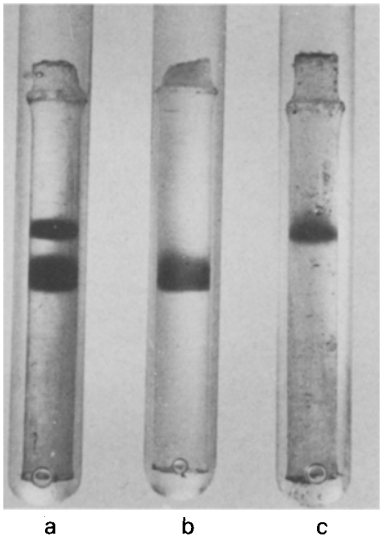

Fig. 3. Electrophoretic patterns of lectins purified on cross-linked arabinogalactan. The electrophoresis was performed in 7.5% polyacrylamide gel at pH 4.5 according to the method of Davis[16]. Staining was done with Coomassie brilliant blue according to the method of Chrambach et al.[17]. 50 µg of each of the lectins was electrophoresed in 0.075 M β-alanine-acetic acid buffer, pH 4.5, for 4 h using 3 mA/tube. The mobility is from top to bottom. Electrophoretic patterns for a) *Ricinus communis* lectin eluted with lactose alone, b) N-acetylgalactosamine eluted protein (RCA_2), c) lactose eluted protein (RCA_1).

(0.8×2 cm) and estimating the protein in the dialyzed lactose eluate. The binding capacity of arabinogalactan for these lectins was found to be 15 mg/ml of the gel.

Discussion. The binding capacity of *R. communis* lectins was very low for sepharose[4] or a ligand bound to sepharose[13]. Hence we investigated several β-galactosyl containing polysaccharides, and found arabinogalactan to be the most suitable as an affinity material. Arabinogalactan is a copolymer of D-galactose and L-arabinose in the ratio of 6:1. The main chain is β-(1→3) linked D-galactose with highly substituted 2-β-(1→6) linked D-galactose side chains and occasional side chains of 3-O-β-L-arabinopyranosyl-L-arabinofuranose[18]. The 2 proteins RCA_2 and RCA_1 have been separated effectively on this column utilizing their affinity for different sugars. Both the lectins have been obtained to electrophoretic purity. The capacity of arabinogalactan column to bind *R. communis* lectin in the present study was found to be 50 times higher than that of the conventional affinity matrices for these lectins. Studies reported here demonstrate that cross-linked arabinogalactan provides a high capacity affinity matrix for isolating *R. communis* lectins. It is hoped that cross-linked arabinogalactan will also find widespread applications in the purification of other galactose binding lectins.

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Inhibition of phosphodiesterase activity of human spermatozoa by spermine

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Summary. Sperm motility and metabolism are dependent upon the levels of cyclic AMP. Our studies have demonstrated that spermine by inhibiting sperm phosphodiesterase activity could regulate sperm physiology.

Cyclic AMP has been demonstrated to play an important role in the metabolism and motility of human spermatozoa[1-3]. Levels of cyclic AMP are regulated by 2 processes: a) Synthesis from adenosine triphosphate through the action of enzyme adenyl cyclase, and b) degradation of the cyclic AMP by phosphodiesterase. Studies carried out in our laboratory demonstrated that spermine stimulated the adenyl cyclase activity of human spermatozoa[4,5]. The present investigation examines the effect of this compound on the sperm phosphodiesterases.

Material and methods. The semen samples used for the study were obtained from fertile volunteers. Soon after liquefaction, the semen was centrifuged at 800×g for 30 min to sediment spermatozoa. The spermatozoa were then washed with the Krebs-Ringer bicarbonate buffer, recentrifuged and reconstituted to the original volume with Tris-HCl buffer (240 mM, pH 7.5). The spermatozoal suspension was sonicated at 0 °C every 30 sec for 10 sec during 4-5 min at 20 kc. The sonicate was then centrifuged at 2000×g for 10 min and the enzyme activity was determined in the supernate according to the method of Butcher and Sutherland[6]. The incubation mixtures consisted of cyclic AMP (1.0 mM), $MgSO_4$ (12.0 mM) Tris-HCl buffer (240 mM, pH 7.5) and the sonicated sperm supernate (40-60 µg protein per 0.1 ml). The total volume of the incubation mixture was 0.9 ml. Control tubes did not contain any spermine, whereas, in experimental tubes, different amounts of spermine were added. To 1 set of incubation tubes 100 µM theophylline was added to study its action on sperm phosphodiesterases under our experimental conditions. The enzyme activity is expressed in terms of ng of phosphate liberated per mg protein per 30 min. Determination of protein content of sperm supernate was carried out according to the method of Lowry et al.[8].

The data has been analyzed by the Student t-test and the levels of significance were read from 2-tailed table.

Effect of spermine on the cyclic AMP phosphodiesterase activity of human spermatozoa homogenate (ng of phosphate liberated per mg protein per 30 min)

No.	Spermine (mM)	Number of observations	Mean ± SE
1	0	7	158 ± 16
2	1.9	6	107 ± 17
3	3.8	5	59 ± 6
4	7.6	3	18 ± 5
5	Theophylline (100 µM)	4	109 ± 4

Level of significance: between 1 and 2 $p<0.05$; 1 and 3 $p<0.002$; 1 and 4 $p<0.001$. The incubation mixture contained: Sperm homogenate (supernatant) in 240 mM-Tris, cyclic AMP (1.0 mM), $MgSO_4$ (12.0 mM) and spermine (0-7.6 mM).