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Glutaraldehyde-Insolubilized Concanavalin A: an Adsorbent for the Specific Isolation of Polysaccharides and Glycoproteins

By EDWIN H. DONNELLY and I. J. GOLDSTEIN
Department of Biological Chemistry, University of Michigan, Ann Arbor, Mich. 48104, *U.S.A.*

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The use of water-insoluble polymers composed of or containing covalently bound biologically active substances such as antigens and enzyme substrates has proved to be an elegant procedure for the efficient isolation of antibodies and enzymes in pure form (Silman & Katchalski, 1966).

For several years this laboratory has been concerned with a study of the carbohydrate-binding protein of the jack bean, concanavalin A (Sumner & Howell, 1936). This phytohemagglutinin has been shown to possess many of the properties of immune antibodies and we have employed immunochemical techniques in its study (Goldstein & So, 1965; So & Goldstein, 1967*a*,*b*). Concanavalin A interacts to form a specific precipitate with branched α-glucans, α-mannans and certain β-fructans (Sumner & Howell, 1936; Cifonelli, Montgomery & Smith, 1956; Goldstein, Hollerman & Merrick, 1965*a*; So & Goldstein, 1969). Hapten inhibition studies demonstrated that the protein combining sites are most complementary to α-D-mannopyranosyl residues (Goldstein, Hollerman & Smith, 1965*b*; So & Goldstein, 1967*b*).

This communication reports the preparation and use of glutaraldehyde-cross-linked concanavalin A for the isolation of polysaccharides and glycoproteins that react with this protein.

Polymerization of concanavalin A with glutaraldehyde. The procedure of Avrameas & Ternynck (1969) was used. To a vigorously stirred solution (50ml) of concanavalin A (822mg; prepared by the procedure of Agrawal & Goldstein, 1965) in 0.1M-sodium phosphate buffer, pH 7.0, was added dropwise aq. 25% (w/v) glutaraldehyde (0.4ml; product of Eastman Organic Chemicals, Rochester, N.Y., U.S.A.) over a period of 1min. The reaction mixture, in an ice bath, was stirred magnetically for 1h. The solution became turbid in a few minutes and gelatinized after 15min. The suspension was centrifuged (3000rev./min) in a Sorvall RC-2 refrigerated centrifuge (SS-34 rotor) and the supernatant solution poured off. The extinction (at 280nm) of the supernatant solution revealed that 98% of the protein had been converted into the insoluble form. The cross-linked protein was washed by resuspension in 1M-NaCl solution and centrifuging. This operation was repeated several times.

Preparation of column. Bio-Gel P-10 (2.5g; product of BioRad Laboratories, Richmond, Calif., U.S.A.) was swelled in 1M-NaCl solution and added to the insoluble concanavalin A, which had been finely dispersed by trituration with a spatula. The suspension was poured into a column (2.7cm × 22cm) and the column washed with 1M-NaCl solution.

Binding and displacement of polysaccharides and proteins. Samples of polysaccharides and proteins were dissolved in 1M-NaCl solution and applied to the column. The column was eluted with 1M-NaCl solution. Fractions (approx. 6ml) were collected automatically and tested for carbohydrate (phenol–H_2SO_4 method of Dubois, Gilles, Hamilton, Rebers & Smith, 1956) or protein (extinction at 280nm). After all the carbohydrate or protein had been eluted, 1M-NaCl solution containing 0.1M-methyl α-D-gluco- or manno-pyranoside was added to the column. Protein was monitored as above. Fractions containing polysaccharide were recognized by their opalescence (glycogen, dextran) or estimated from their expected elution volume. Fractions containing polysaccharide or protein were combined (with non-opalescent substances several arbitrary pools were selected and combined), dialysed against several changes of distilled water and freeze-dried. Recovery was estimated by dissolving the recovered material in water and employing the phenol–H_2SO_4 method (Dubois *et al.* 1956) or measuring the extinction at 280nm.

Binding of glycogen. Rabbit liver glycogen (45mg) was dissolved in 1M-NaCl (1.0ml) and added to the column. No carbohydrate material was eluted until fractions 9 and 10, when less than 1mg of total glycogen was detected. A this point 0.1M-methyl α-D-mannopyranoside (or glucoside) in 0.1M-NaCl solution was added to the column. The glycogen was eluted in fractions 4–7 (distinctly opalescent). Fractions 4–10 were combined, dialysed against distilled water and freeze-dried. The yield was 40mg. In a second experiment 72.2mg of 79.5mg of glycogen was recovered by elution with α-methyl glucoside.

Binding of macroglobulin (*immunoglobulin M*). A sample (10.6mg) of purified monoclonal macroglobulin (Goldstein, So, Yang, & Callies, 1969) was completely bound by the concanavalin A column.

Elution with 0.1M-α-methyl glucoside in 1M-NaCl gave 8.22mg (yield 77%).

Binding of dextrans. Dextran B-1355-S (50mg; gift from Dr Allene Jeanes, Peoria, Ill., U.S.A.) was added to the column. A small portion (4.1mg) passed through the column in fractions 2 and 3. No further sugar emerged and α-methyl glucoside was added to the column at fraction 11. The dextran was eluted in fractions 13–20. The yield after dialysis and freeze-drying was 41mg.

Only a small proportion of dextran B-512F and dextran B-1299 (gift from Dr Allene Jeanes) was bound to the cross-linked concanavalin A column; 18mg of 20.8mg of dextran B-512F and 10.7mg of 15.6mg of dextran B-1299 passed through the column without being bound. It was also found that the dextran from *Streptococcus* sp. was essentially eluted with the void volume, 18.6mg of 22.9mg added passing through the column.

Control experiments with unreactive polysaccharides and proteins. As control polysaccharides, soluble laminaran and α-cyclodextrin (α-Schardinger dextrin) were passed through the concanavalin A column. The laminaran emerged with the void volume of the column, 16.2mg of 19mg added being recovered without the addition α-methyl glucoside. Similarly α-cyclodextrin was eluted as a single peak, 20mg of 20.7mg being recovered. Of 25mg of bovine serum albumin added, 23.3mg was recovered in the void volume.

Discussion. The above experiments demonstrate that concanavalin A, insolubilized by treatment with glutaraldehyde, displays the same specificity in the solid phase as when in solution. Thus the cross-linked protein specifically binds certain dextrans, glycogen and a glycoprotein. These substances are readily displaced from the concanavalin A column by the same glycosides (methyl α-D-gluco- and manno-pyranoside) that are potent inhibitors of concanavalin A–polysaccharide interaction (Goldstein *et al.* 1965*b*; So & Goldstein, 1967*b*). Further, laminaran (a β-glucan) and α-cyclodextrin (a cyclic polymer composed of six α-glucopyranosyl residues), two polysaccharides that do not conform to the class of polymers that react with concanavalin A (Goldstein *et al.* 1965*a*), are not bound by the concanavalin A column. Bovine serum albumin, a protein devoid of carbohydrate residues, is also not bound by the column.

The almost complete adsorption of dextran B-1355-S as opposed to the failure of the dextran B-512F, dextran B-1299 and dextran from *Streptococcus* sp. to be significantly bound to the concanavalin A column can probably be attributed to structural features. Dextran B-1355-S has been shown to react almost quantitatively with concanavalin A and hence has been employed in this laboratory as the polysaccharide for assaying concanavalin A (So & Goldstein, 1967*a*). On the other hand, dextran B-512F was shown to be far less reactive with concanavalin A (Goldstein, Poretz, So & Yang, 1968). It has been suggested that approx. 70% of the branches in dextran B-512F are only one unit long (Jeanes, 1957). This fact could account in part for the limited interaction of this dextran with the insoluble concanavalin A.

The differential behaviour towards insoluble concanavalin A of the various dextrans used in this study suggests the possibility of employing columns of cross-linked concanavalin A for fractionating mixtures of polysaccharides and glycoproteins.

It is evident that cross-linked columns of concanavalin A may be employed for the specific isolation of biopolymers containing the sugar residues requisite for interaction with this protein.

While this communication was in preparation, we learned that similar studies were being conducted in other laboratories. Lloyd (1970) prepared two insoluble forms of concanavalin A by procedures other than the one described in this communication with essentially the same results.

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