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SPECIFIC BINDING OF SULFATED PROTEOGLYCANS TO CONCANAVALIN A - SEPHAROSE 4B

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Summary: More than 90 % of [^{35}S]proteoglycans isolated from the secretions of human skin fibroblasts bind to Concanavalin A-Sepharose 4B (Con A-Sepharose) in the presence of 1 M NaCl. Above pH 5.0 1 M concentrations of methyl-α-D-mannoside and other haptenic inhibitors for Con A-sugar interaction prevent binding of [^{35}S] proteoglycans, whereas equimolar concentrations of non-haptenic carbohydrates do not effect binding. Below pH 5.0 [^{35}S] proteoglycans bind to Con A-Sepharose in the presence of both methyl-α-D-mannoside and galactose. About 60 % of the proteoglycans bound at pH 4.0 are eluted at pH 7.5 in the presence of 1 M methyl-α-D-mannoside. [^{35}S] Glycosaminoglycans prepared from [^{35}S] proteoglycans do not bind to Con A-Sepharose in the presence of 1 M NaCl.

These results indicate a [^{35}S] proteoglycan-Con A interaction via the protein core of the proteoglycan and the sugar binding sites of Con A.

Introduction: Sulfated glycosaminoglycans appear to be generally occuring cell surface constituents on cultured mammalian cells (1-6). The amount of chondroitin-4/6-sulfate, dermatan sulfate and heparan sulfate associated as proteoglycans with the cell surface varies with the cell type, cell cycle and transformation (4,6-8). Con A is widely used as a probe for cell surface associated carbohydrates. It is generally assumed that Con A interacts with the membraneous glycoproteins and glycolipids. Several investigations have established that sulfated glycosaminoglycans do not interact with the sugar binding sites of Con A (9-11). However, the interaction of sulfated proteoglycans with Con A has so far not been studied.

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We examined the interaction of proteoglycans isolated from the secretions of human skin fibroblasts with Con A. Secretory [^{35}S] proteoglycans that stem at least in part from the cell surface (3,4) were used for this study, since they can be isolated under mild non-proteolytic conditions.

Materials and Methods: The monosaccharides were obtained from Serva, Heidelberg, Con A-Sepharose from Pharmacia, Freiburg.
[^{35}S]proteoglycans were isolated from the secretions of human skin fibroblasts as described (4). The relative amounts of chondroitin-4/6-sulfate, dermatan sulfate and heparan sulfate in this preparation were 11:83:6 (4).
[^{35}S]glycosaminoglycans were prepared from these proteoglycans by treatment with 0.15 M NaOH for 4 h at 37°C and subsequent neutralisation.
Radioactivity was determined in a liquid scintillation counter (Packard, Modell 3390/TT) using Unisolve (Zinsser, Frankfurt) as scintillation medium.

Results: Proteoglycans isolated from the secretions of human skin fibroblasts and [^{35}S]glycosaminoglycans prepared from the secreted [^{35}S]proteoglycans were loaded on Con A-Sepharose columns equilibrated in 1 M NaCl buffered at pH 7.5 (Table 1). More than 90 % of the applied [^{35}S]proteoglycans bound to Con A-Sepharose, whereas less than 5 % of the [^{35}S]glycosaminoglycan chains were bound under these conditions. The latter finding is in agreement with earlier reports in the literature (9-11). Inclusion of 1 M methyl-α-D-mannoside into the eluent desorbed more than 70 % of the applied [^{35}S]proteoglycans from the Con A-Sepharose column.

The interaction of [^{35}S]proteoglycans with Con A-Sepharose could be quantified in less time and with higher reproducibility, when the samples were applied to Con A-Sepharose columns equilibrated and eluted in solutions allowing or preventing binding. Several carbohydrates were tested for their inhibitory effect on the [^{35}S] proteoglycan-Con A interaction. Only those carbohydrates inhibited the [^{35}S]proteoglycan binding to Con A-Sepharose that are known to interact with the sugar binding sites of Con A. Thus glucose,

Table 1: Elution of [^{35}S]proteoglycans and [^{35}S]glycosaminoglycans from Con A-Sepharose at pH 7.5

(in 50 mM Tris/HCl, pH 7.5)	[^{35}S]Proteoglycans eluted (% of applied)	[^{35}S]Glycosaminoglycans eluted
1.0 M NaCl	7.6	92.7
1.0 M Galactose in 1.0 M NaCl	1.9	1.0
1.0 M Methyl-α-D-mannoside in 1.0 M NaCl	56.1	1.8
1.0 M Methyl-α-D-mannoside in 1.0 M NaCl	15.2	1.3
0.1 M Sodium borate, pH 7.5 in 1.0 M NaCl	2.3	0.4

Samples of 0.2 ml (containing 20 000 cpm [^{35}S]radioactivity) were applied to 0.5 x 2.0 cm Con A-Sepharose columns, equilibrated in 1 M NaCl in 50 mM Tris/HCl, pH 7.5. After incubation for 2 h at room temperature the columns were stepwise eluted in fractions of 3 ml with the eluents listed above. Between the first and the second elution with methyl-α-D-mannoside the column was kept at 20°C for 16 h.

mannose, their α-methyl derivatives and N-acetylglucosamine, but not galactose, glucuronic acid, L-rhamnose and xylose were found to inhibit the binding (Table 2).

The effect of pH on the binding of [^{35}S]proteoglycans to Con A-Sepharose was studied between pH 4.0 to 8.0. In the presence of 1 M galactose in 1 M NaCl more than 90 % of the applied [^{35}S]proteoglycans bound to Con A-Sepharose over the whole pH range tested. Presence of 1 M methyl-α-D-mannoside prevented binding only above 5.0 (Fig. 1 A). Elution with 1 M methyl-α-D-mannoside in 1 M NaCl buffered at pH 7.5 desorbed up to 60 % of [^{35}S]proteoglycans bound at pH 4.0 in the presence of either 1 M methyl-α-D-mannoside or 1 M galactose. At high salt concentrations [^{35}S]glycosaminoglycan chains did not bind to Con A-Sepharose over the whole pH range tested (Fig. 1 B).

Discussion: The present results clearly demonstrate the specific binding of [^{35}S]proteoglycans to immobilized Con A via the sugar binding sites of the lectin. Interaction with the Con A sugar

Table 2: Sugar specific inhibition of the [^{35}S]proteoglycan-Con A interaction

Sugar (1 M)	[^{35}S] Proteoglycans eluted (% of applied)
-	12.9
Glucose	104.4
Mannose	102.8
N-Acetylglucosamine	104.5
Galactose	14.7
Glucuronic acid	6.0
L-Rhamnose	15.9
Xylose	19.3
Methyl-α-D-mannoside	96.5
Methyl-α-D-glucoside	103.5

[^{35}S]Proteoglycans from fibroblast secretions (20 000 cpm) were applied to 0.5 x 2.0 cm Con A-Sepharose columns, equilibrated and eluted with solutions containing the indicated sugars (1 M) in 1 M NaCl, 50 mM Tris/HCl, pH 7.5. The elution volume was 3 ml.

binding sites requires that in proteoglycan molecules sugar residues are present with unsubstituted hydroxyl groups at C-3, C-4 and C-6 configurated as in α-D-glucopyranose (12). Since glycosaminoglycan chains prepared from secreted proteoglycans do not interact with the sugar binding sites of Con A, it is likely that the carbohydrates mediating the binding of proteoglycans to Con A are located on the protein core.

Below pH 5.0 the interaction between proteoglycans and Con A appears to be stronger than above pH 5.0. The binding at pH 4.0 can be reversed by raising the pH to 7.5, thus excluding irreversible denaturation of the proteoglycans on the Con A-Sepharose column. The pI of Con A is reported to be around pH 5.1 (13). This may indicate, that above pH 5.1 the negative charge of both proteoglycans and Con A may weaken the attractive forces, whereas below pH 5.1 their opposite charge may strengthen the interaction. Involvement of

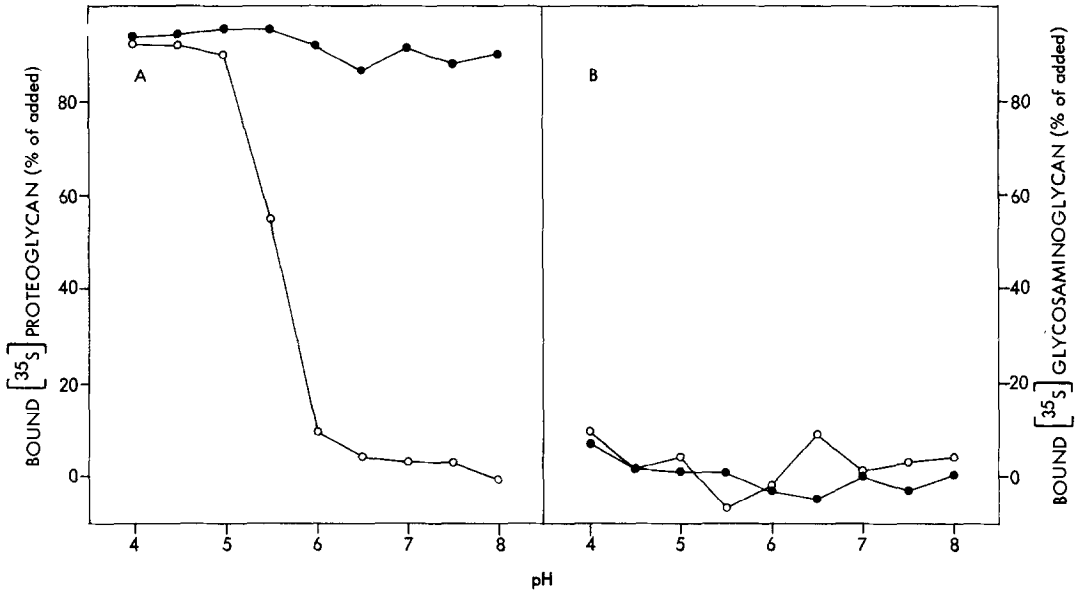

Fig. 1: Effect of pH on the interaction of $[^{35}S]$proteoglycans and $[^{35}S]$glycosaminoglycans with Con A-Sepharose.

A: $[^{35}S]$Proteoglycans from fibroblast secretions (20 000 cpm) were loaded onto 0.5 x 2.0 cm Con A-Sepharose columns equilibrated and eluted with 1 M methyl-α-D-mannoside, 1 M NaCl in 50 mM buffers (O—O) or with 1 M galactose, 1 M NaCl in 50 mM buffers (●—●). The elution volume was 3 ml. Sodium acetate was used for pH 4.0-5.5, sodium phosphate for pH 6.0-7.0 and Tris/HCl for pH 7.5-8.0.

B: $[^{35}S]$Glycosaminoglycans were loaded onto Con A-Sepharose columns, equilibrated and eluted as described above.

electrostatic forces in Con A-polyelectrolyte interaction has been suggested earlier (14).

The specific interaction of Con A with proteoglycans isolated from the secretions of cultured fibroblasts at physiological pH raises the question whether cell surface associated proteoglycans are part of the cell surface receptors for Con A. By inference a number of the biological effects exerted by the interaction of Con A with cell surface receptors may be mediated by Con A binding to cell surface associated proteoglycans.

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