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Cell-surface heparan sulfate: An intercalated membrane proteoglycan

(glycosaminoglycans/rat liver/liposomes)

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ABSTRACT **Two pools of heparan sulfate proteoglycans have been selectively solubilized from rat liver plasma membranes by successive incubations with heparin and detergent. The two populations of proteoglycans have similar polyanionic properties as indicated by identical elution positions on ion-exchange chromatography on DEAE-Sephacel but differ in buoyant density in CsCl when analyzed by density gradient centrifugation in the presence of 4 M guanidine. The detergent-extracted proteoglycan has a lower buoyant density (≤1.40 g/ml) and is, as determined by gel chromatography, slightly larger than the heparin-released proteoglycan (buoyant density, ≥1.55 g/ml). Furthermore, in contrast to the heparin-released proteoglycan, the detergent-extracted proteoglycan is able to bind detergent micelles, shows affinity for the hydrophobic gel octyl-Sepharose, and can be inserted into liposomes. We conclude that the detergent-extracted heparan sulfate represents a proteoglycan species that has its core protein rooted in the lipid bilayer of the plasma membrane.**

Heparan sulfate is found in a wide variety of tissues (1, 2) and is synthesized *in vitro* by different cell types as first shown by Kraemer (3). Heparan sulfate is associated with the outer surface of cells and is a constituent of basement membranes (4) and of the extracellular matrix produced by cultured adherent cells (5). The location of heparan sulfate in the cellular microenvironment has led to speculations about its possible role in the regulation of cellular growth and transformation (6–8), in cell–substrate adhesion (9), and in differentiation processes (10).

We have characterized a high-density heparan sulfate proteoglycan from rat liver membranes (11). The M_r 80,000 proteoglycan contains four polysaccharide chains (M_r, ≈14,000) attached to a core protein (M_r, 20,000). A heparan sulfate proteoglycan isolated from an ascites hepatoma appears to be of equal size (12), whereas basement membrane proteoglycans have a higher M_r (13).

We have demonstrated that heparan sulfate proteoglycans are associated with the hepatocyte surface by two separate mechanisms (14). A portion of the proteoglycans is bound to surface receptors that recognize the polysaccharide chain (15), and these proteoglycans can be displaced by the addition of heparin (14). However, some of the proteoglycan molecules resist heparin treatment but are released from the cells after trypsin digestion (14). Here we present data suggesting that the latter (heparin-resistant) type of proteoglycan has its core protein embedded in the lipid bilayer of the plasma membrane.

MATERIALS AND METHODS

[^{35}S]Sulfate (carrier-free), [^{3}H]glucosamine (specific activity, 20 Ci/nmol; 1 Ci = 3.7 × 10^{10} becquerels), and [^{14}C]phosphatidylcholine (specific activity, 58 mCi/nmol) were purchased from Amersham Searle. Trypsin (type III; isolated from bovine pancreas) and phosphatidylcholine were obtained from Sigma, and chondroitinase ABC was from Miles. DEAE-Sephacel, Sepharose CL-4B, Sephadex G-50, and octyl-Sepharose were from Pharmacia. Heparin was obtained from Inolex (Park Forest South, IL) and purified as described (16). Sprague–Dawley rats were obtained from Charles River Breeding Laboratories.

Protein was determined by the method of Lowry *et al.* (17). Incubations of the proteoglycans with papain and chondroitinase ABC were carried out as described (18), and nitrous acid deamination was performed at pH 1.5 as described (19). Radioactivity was determined in a Packard scintillation counter with Scintiverse as emulsifier. Density gradient centrifugation in CsCl was carried out as described (11).

Isolation of Radioactively Labeled Plasma Membranes. Rats were injected intraperitoneally with 1 mCi per rat of either [^{35}S]sulfate or [^{3}H]glucosamine. The animals were killed 2 hr later, and liver plasma membranes were prepared as described (20), except that the protease inhibitors benzamidine·HCl (1 mM) and phenylmethylsulfonyl fluoride ($PhMeSO_2F$) (0.1 mM) were included in the buffers. The membranes, which are purified from the nuclear pellet by centrifugation in a discontinuous sucrose gradient, are believed to be enriched in membranes originating from the contiguous face of the hepatocyte (21). An average of 15 mg of membrane protein was obtained from one rat liver weighing 7–8 g. When [^{35}S]sulfate was used as a label, more than 95% of the radioactivity in the plasma membranes was in heparan sulfate (18), as compared with approximately 20% in the ^{3}H-labeled membranes.

Solubilization of ^{35}S-Labeled Plasma Membranes. Membranes (corresponding to 1.2 mg of protein) were incubated at 4°C with the selected solubilizing agent in 1 ml of 0.35 M NaCl/0.05 M Tris·HCl, pH 8.0, containing benzamidine·HCl (1 mM) and $PhMeSO_2F$ (0.1 mM). After 30 min, the membrane residues were pelleted by centrifugation at 20,000 × *g* for 30 min, and the supernatants containing the solubilized heparan [^{35}S]sulfate were assayed for radioactivity (Table 1) or were used for CsCl density gradient centrifugation (Fig. 1). The pellet was dissolved in 10% $NaDodSO_4$ for scintillation counting (Table 1) or resuspended for a second solubilization (Table 1, Figs. 1 and 3).

For trypsin treatment, the membranes were incubated at 37°C in 0.13 M NaCl/0.01 M phosphate buffer, pH 7.4, containing 0.01% trypsin. After 5 min, soybean trypsin inhibitor (final concentration, 0.05%), benzamidine·HCl (1 mM), and $PhMeSO_2F$ (0.1 mM) were added. The membranes were then centrifuged as before.

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Abbreviation: $PhMeSO_2F$, phenylmethylsulfonyl fluoride.

Purification of Heparan Sulfate Proteoglycans. Plasma membranes corresponding to 30 mg of protein were suspended in 25 ml of 0.35 M NaCl/0.05 M Tris·HCl, pH 8.0, containing heparin (0.1 mg/ml) and the protease inhibitors benzamidine·HCl (1 mM) and $PhMeSO_2F$ (0.1 mM). These protease inhibitors were included in all buffers used in the purification. The suspension was incubated in an end-over-end mixer at 4°C for 30 min and then centrifuged at 20,000 × *g* for 30 min. The supernatant fraction was saved, and the pellet was suspended in 25 ml of buffer containing 1% Triton X-100. The suspension was incubated and centrifuged as before. The two supernatant fractions were applied separately to two 3-ml DEAE-Sephacel columns, equilibrated in 0.35 M NaCl/0.05 M Tris·HCl, pH 8.0, and in the same buffer containing 0.1% Triton X-100, respectively. [Triton X-100 (0.1%) was added to all buffers used in the further purification of the proteoglycan solubilized in this detergent.] The columns were washed with 0.35 M NaCl/0.05 M acetate buffer, pH 4.0, and eluted at a flow rate of 9 ml/hr with a linear gradient (75 + 75 ml) from 0.35 to 1.5 M NaCl in acetate buffer, pH 4.0. Fractions containing labeled proteoglycan were pooled and further purified by gel chromatography on Sepharose CL-4B columns (1.1 × 100 cm) in 0.35 M NaCl/0.05 M Tris·HCl, pH 8.0. The columns were eluted at a flow rate of 6 ml/hr, and 1.5-ml fractions were collected.

The two proteoglycans obtained after this purification procedure will be referred to as purified "heparin-released" and "detergent-extracted" proteoglycans, respectively.

After papain digestion of the ^3H- and ^{35}S-labeled proteoglycans (^3H- and ^{35}S-proteoglycans), all the radioactivity was present in polysaccharide chains eluted in the void volume of a Sephadex G-50 column. These polysaccharide chains were susceptible to nitrous acid treatment but resisted chondroitinase ABC digestion, demonstrating their heparan sulfate nature.

Change of Detergent. For the experiments involving hydrophobic chromatography or preparation of liposomes, the detergent present in the preparation of the purified detergent-extracted proteoglycan was changed from Triton X-100 to sodium cholate. This was achieved by adding proteoglycans in 0.1% Triton X-100/0.35 M NaCl/0.05 M Tris·HCl, pH 8.0, to a 1-ml column of DEAE-Sephacel. The column was washed with 25 ml of 2% (wt/vol) cholate in 0.1 M NaCl/0.02 M Tris·HCl, pH 7.3, and eluted with the same buffer containing 3 M NaCl. The heparin-released proteoglycans used for hydrophobic chromatography and preparation of liposomes were treated in the same way.

Hydrophobic Chromatography. Aliquots (3000 cpm each) of the purified detergent-extracted and heparin-released ^{35}S-proteoglycans were incubated separately overnight with 1 ml of octyl-Sepharose in 2% cholate/3 M NaCl/0.02 M Tris·HCl, pH 7.3. The gels were then poured into columns and washed with 0.1 M NaCl/0.02 M Tris·HCl, pH 7.3, to release molecules interacting with weak hydrophobic affinity. Subsequently the columns were washed with 3 M NaCl/0.02 M Tris·HCl, pH 7.3, to remove molecules unspecifically adsorbed to the gel matrix, and finally the columns were eluted with 1% Triton X-100/3 M NaCl/0.02 M Tris·HCl, pH 7.3.

Preparation of Liposomes. Liposomes were prepared by the method of Brunner *et al.* (22). In short, 25,000 cpm (corresponding to less than 5 μg of protein) of the two purified ^3H-proteoglycans, dissolved in 2% cholate/0.1 M NaCl/0.02 M Tris·HCl, pH 7.3, were added to tubes containing 10 mg of phosphatidylcholine and 0.2 μCi of [^{14}C]phosphatidylcholine evaporated to dryness with a stream of N_2. To induce liposome formation, the mixtures were passed through Sephadex G-50 columns (1.5 × 25 cm), eluted with 0.1 M NaCl/0.02 M Tris·HCl, pH 7.3. The small cholate micelles (M_r in H_2O, 900–1,800; see ref. 23) were hereby separated from the [^{14}C]phosphatidylcholine vesicles and the ^3H-proteoglycans, eluting in the void volume of the column.

Table 1. Solubilization of heparan [^{35}S]sulfate from untreated and heparin-treated plasma membranes

	Membrane heparan [^{35}S]sulfate			
	% solubilized		% in residues	
Solubilizing agent	Exp. 1	Exp. 2	Exp. 1	Exp. 2
Control (unsolubilized membranes)	—	—	100	70.9
0.35 M NaCl/0.05 M Tris·HCl, pH 8.0	18.6	12.4	81.4	58.5
+ heparin (0.1 mg/ml)	32.5	12.2	67.5	58.7
+ NaCl (2 M)*	37.0	17.4	63.0	53.5
+ heparin (0.1 mg/ml + NaCl (2 M)*	36.0	17.7	64.0	53.2
+ Triton X-100 (1%)	53.3	68.1	46.7	2.8
+ Triton X-100 (1%) + heparin (0.1 mg/ml)	90.1	61.2	9.9	9.7
+ Triton X-100 (1%) + NaCl (2 M)*	83.9	66.0	16.1	4.9

^{35}S-Labeled plasma membranes (corresponding to 1.2 mg of protein and 1.5 × 10^4 cpm) were solubilized as described. In Exp. 1, the membranes were used directly after isolation (untreated), whereas in Exp. 2 the membranes were first incubated with heparin, pelleted by centrifugation, and then suspended in buffer containing the solubilizing agent. After centrifugation, the percentage of ^{35}S radioactivity recovered in the supernatant and pellet, respectively, was determined.
* Final concentration of NaCl.

RESULTS

Solubilization of Two Species of Heparan Sulfate Proteoglycan. When isolated ^{35}S-labeled plasma membranes were incubated with heparin (0.1 mg/ml) or NaCl (2 M) alone or in combination, less than 40% of the heparan [^{35}S]sulfate was released‡ (Table 1). Similarly, incubation of the membranes with Triton X-100 solubilized only 50% of the labeled proteoglycans. However, when Triton X-100 (1%) was combined with either heparin (0.1 mg/ml) or NaCl (2 M), 80–90% of the total heparan [^{35}S]sulfate was solubilized. Incubations of heparin-treated plasma membranes with heparin or NaCl, or both, released very little additional heparan [^{35}S]sulfate, whereas after incubation with Triton X-100, only a few percent of the radioactivity remained associated with the membrane residues. These results indicate that Triton X-100 and either heparin or NaCl treatments of the membranes, respectively, result in the solubilization of two pools of heparan sulfate proteoglycans with different modes of association with the plasma membrane.

Density Gradient Centrifugation. When heparan [^{35}S]sulfate proteoglycans that were solubilized from plasma membranes by Triton X-100/2 M NaCl were subjected to CsCl density gradient centrifugation in 4 M guanidine, radioactive material was recovered both at the top and at the bottom of the gradient (Fig. 1*A*). Incubation of the membranes with heparin alone released the high-density proteoglycan (Fig. 1*B*), while the low-density proteoglycan remained associated with the membrane and was solubilized upon subsequent extraction of the membranes with Triton X-100/2 M NaCl (Fig. 1*C*). The proteoglycan resisting heparin treatment could be solubilized from the membrane not

‡ We have noticed that upon storage a larger proportion of the proteoglycans are released by incubations with physiological buffers, probably due to protease activity in the plasma membranes. This explains the higher percentage of ^{35}S radioactivity released by heparin or high concentrations of NaCl reported by Oldberg *et al.* (11).

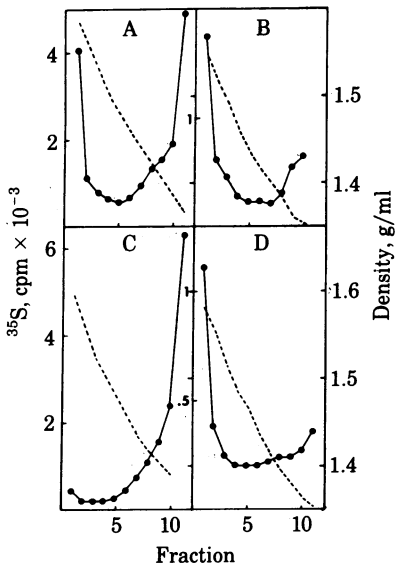

FIG. 1. Density gradient centrifugation in CsCl of ^{35}S-proteoglycans. The ^{35}S-proteoglycans used for centrifugation were obtained from isolated plasma membranes by solubilization with 1% Triton X-100/2 M NaCl (*A*) or 0.1 mg of heparin per ml (*B*). Alternatively, ^{35}S-proteoglycans remaining associated with the plasma membranes after heparin treatment were solubilized by incubation with 1% Triton X-100/2 M NaCl (*C*) or 0.01% trypsin (*D*). The samples were centrifuged in the presence of 4 M guanidine for 72 hr at 100,000 × *g* and 20°C. The bottom of the centrifuge tube was punctured, and fractions (≈1 ml) were collected and analyzed for radioactivity (●) and density (----).

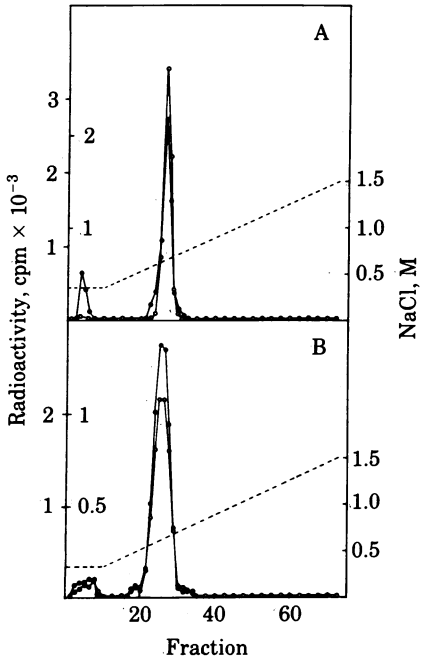

FIG. 2. Ion-exchange chromatography on DEAE-Sephacel of detergent-extracted ^{3}H-proteoglycans and heparin-released ^{35}S-proteoglycans. Purified detergent-extracted ^{3}H-proteoglycan (10,000 cpm) was mixed with purified heparin-released ^{35}S-proteoglycan (10,000 cpm) and chromatographed on a 2-ml DEAE-column before (*A*) and after (*B*) alkali treatment. The column was washed with 0.35 M NaCl/0.05 M acetate buffer, pH 4.0, and eluted with a linear gradient (150 ml) from 0.35 M to 1.5 M NaCl in acetate buffer, pH 4.0. In *A*, 0.1% Triton X-100 was included in the buffers. Radioactivity assays: ○, ^{3}H based on outside left ordinate scale; ●, ^{35}S based on inside left ordinate scale.

only with Triton X-100/2 M NaCl but also by mild trypsin treatment. This trypsin-released proteoglycan had a high buoyant density (Fig. 1*D*), demonstrating that the low-density proteoglycan may be converted to the high-density type by mild proteolysis. Taken together, these results demonstrate that two types of proteoglycans are present at the cell surface. The high-density proteoglycan is displaced from the membranes by heparin and presumably represents the receptor-bound proteoglycan (14). This proteoglycan can be classified as a peripheral-membrane glycoprotein.

The low-density proteoglycan resisted extraction with sodium chloride (2 M) and heparin (0.1 mg/ml) and was released in intact form only after solubilization with detergent. The possibility that this proteoglycan represents an intercalated membrane glycoprotein with its core protein embedded in the lipid interior of the plasma membrane was further investigated.

Structural Comparison of Heparin-Released and Detergent-Extracted Heparan Sulfate Proteoglycans. The polyanionic properties of the two proteoglycan species were analyzed by cochromatography of purified heparin-released ^{35}S-proteoglycan and detergent-extracted ^{3}H-proteoglycan on a column of DEAE-Sephacel, which was eluted by a linear NaCl gradient. The elution profiles of the ^{3}H and ^{35}S radioactivities coincided (Fig. 2*A*). Also individual polysaccharide chains, released from the core proteins by treatment with alkali, had identical elution positions (Fig. 2*B*), identicating that the two populations of proteoglycans have the same charge density.

The size distribution of the two proteoglycans in the presence of different detergents was investigated by gel chromatography on Sepharose CL-4B. The samples consisted of purified heparin-released ^{3}H-proteoglycans mixed with ^{35}S-proteoglycans obtained after solubilization of heparin-treated plasma membranes with successive 1% solutions of $NaDodSO_4$, deoxycholate, and Triton X-100.§ The columns were eluted in the presence of the detergent used for solubilization of the ^{35}S-proteoglycan. The $NaDodSO_4$-extracted ^{35}S-proteoglycan was eluted somewhat ahead of the heparin-released ^{3}H-proteoglycan (Fig. 3*A*). The apparent difference in molecular size between the two proteoglycan species was also observed when the column was eluted with the nondenaturing detergent deoxycholate (Fig. 3*B*). The difference in elution position was even more pronounced when the detergent in the eluting buffer was Triton X-100 (Fig. 3*C*). Although Triton X-100 and deoxycholate bind only to hydrophobic proteins, $NaDodSO_4$ binds in a cooperative mode to virtually all proteins. Therefore, the difference in elution position between the heparin-released and $NaDodSO_4$-extracted proteoglycans most likely reflects a difference in molecular size. The large difference in apparent size between the heparin-released and Triton X-100-extracted proteoglycans could be explained by the binding of detergent micelles to hydrophobic sites present in the detergent-extracted proteoglycan. The association of one micelle of Triton X-100 molecules (M_r in H_2O, 90,000; see ref. 23) with the proteoglycan would lead to a considerable increase in size.¶ On the other hand, binding of a deoxycholate micelle, which is much smaller (M_r in H_2O, 1700–4200; see ref. 23), would not significantly

§ After serial extraction of the plasma membranes with the three detergents, less than 15% of the radioactivity present in the intact membranes remained in the pellets obtained after centrifugation of the extract.

¶ The increase is to an apparent M_r larger than 90,000, but can be explained by the high Stokes radius commonly found for protein–Triton X-100 complexes (23).

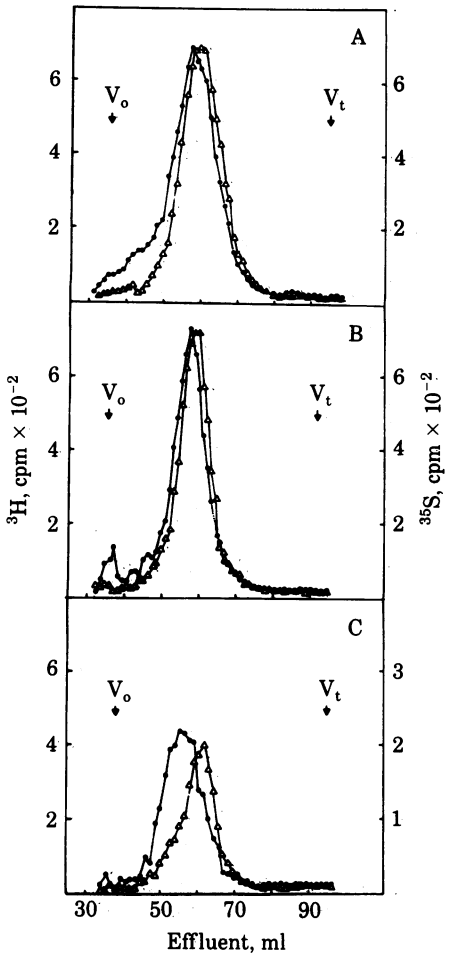

FIG. 3. Gel chromatography on Sepharose CL-4B in different detergents. Purified heparin-released ^3H-proteoglycans were cochromatographed with ^{35}S-proteoglycans solubilized from heparin-treated plasma membranes by incubations with 1% $NaDodSO_4$ (*A*), 1% deoxycholate (*B*), and 1% Triton X-100 (*C*). The columns (1.1 × 100 cm) were eluted with 0.35 M NaCl/0.05 M Tris·HCl, pH 8.0, with the addition of 0.1% of the detergent used for the solubilization of the ^{35}S-proteoglycan. The flow rate was 6 ml/hr; 1.5-ml fractions were collected and analyzed for radioactivity: △, ^3H; ●, ^{35}S.

alter the molecular size of the proteoglycan.

Hydrophobic Properties of the Proteoglycans. Purified proteoglycans of the two types were incubated with the hydrophobic gel octyl-Sepharose in 2% cholate/3 M NaCl/0.02 M Tris·HCl, pH 7.3. The high concentration of NaCl was chosen to enhance hydrophobic interactions. The heparin-released proteoglycan had no affinity for the gel, whereas the major portion of the detergent-extracted proteoglycan was firmly bound and was eluted only in the presence of Triton X-100 (Fig. 4). These results demonstrate that the detergent-extracted proteoglycan contains a hydrophobic region, presumably located in the core protein, which is absent in the heparin-released proteoglycan.

Experiments to incorporate the two species of heparan sulfate proteoglycans into liposomes were also carried out. ^{14}C-Labeled liposomes (^{14}C-liposomes) were formed in the presence of purified heparin-released and detergent-extracted ^3H-proteoglycans, respectively. To investigate if the proteoglycans had been incorporated into the liposomes, the samples were analyzed by anion-exchange chromatography on DEAE-Sephacel. ^{14}C-Liposomes formed in the absence of proteoglycans had no affinity

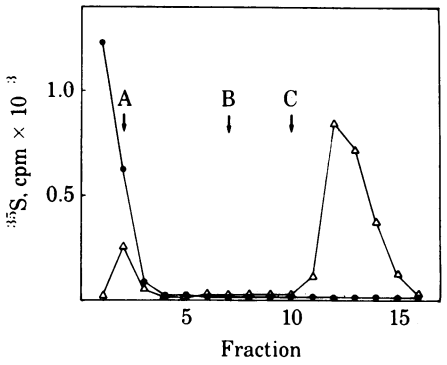

FIG. 4. Affinity chromatography on octyl-Sepharose. Purified detergent-extracted (△) and heparin-released (●) ^{35}S-proteoglycans, respectively, were incubated overnight with 1 ml of octyl-Sepharose in 2% cholate/3 M NaCl/0.02 M Tris·HCl, pH 7.3. The incubation mixtures were transferred to columns and washed with 0.1 M NaCl/0.02 M Tris·HCl, pH 7.3 (buffer A) and 3 M NaCl/0.02 M Tris·HCl, pH 7.3 (buffer B) and eluted with 1% Triton X-100/3 M NaCl/0.02 M Tris·HCl, pH 7.3 (buffer C).

for the ion exchange resin (Fig. 5*A*). Likewise, ^{14}C-liposomes formed in the presence of heparin-released proteoglycan were not bound to the column (Fig. 5*B*); no ^{14}C radioactivity coincided with the peak of ^3H-proteoglycans, eluted in the gradient. On the other hand, when ^{14}C-liposomes formed in the presence

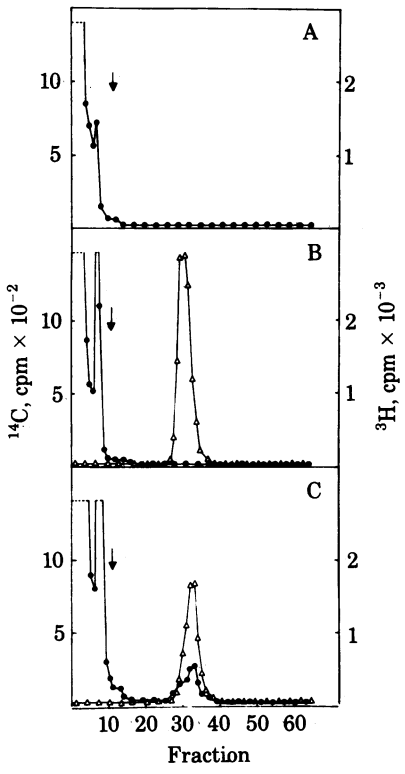

FIG. 5. Ion-exchange chromatography on DEAE-Sephacel of ^{14}C-liposomes formed in the presence of ^3H-proteoglycans. Samples containing ^{14}C-liposomes (*A*), ^{14}C-liposomes formed in the presence of heparin-released ^3H-proteoglycans (*B*), and ^{14}C-liposomes formed in the presence of detergent-extracted ^3H-proteoglycans (*C*) were applied to 2-ml DEAE-columns in 0.1 M NaCl/0.02 M Tris·HCl, pH 7.3. The columns were washed with 0.35 M NaCl/0.05 M acetate buffer, pH 4.0, and eluted in the same buffer with a linear gradient ranging from 0.35 M to 1.5 M NaCl. The gradient start is indicated by an arrow. The fractions were assayed for radioactivity: ●, ^{14}C; △, ^3H.

of detergent-extracted proteoglycan were analyzed, some of the ^{14}C radioactivity coeluted with the ^{3}H-proteoglycan peak, demonstrating that at least some of the detergent-extracted proteoglycans had been incorporated into the liposomes, with the polysaccharide chains facing the outside of the vesicles, still able to interact with the ion exchange resin.

The incorporation of the detergent-extracted proteoglycan into a phospholipid liposome suggests that the properties of the hydrophobic segment would allow the proteoglycan to be inserted into the lipid bilayer of a biological membrane.

DISCUSSION

Previous reports from our and other laboratories have demonstrated that heparan sulfate proteoglycans occur bound to the cell surface through an interaction between the polysaccharide chains and membrane receptors (15, 24, 25). This type of receptor-associated proteoglycan is displaced from the hepatocyte cell surface on addition of heparin (14, 26). A portion of the cell-associated heparan sulfate proteoglycans resists incubation with heparin but is released from the cell surface by mild trypsin treatment, indicating a second mechanism of proteoglycan–cell association (14).

In this study, the heparin-resistant proteoglycan is shown to be a proteoglycan species having its core protein anchored in the lipid bilayer of the membrane. The evidence for a membrane-intercalated proteoglycan includes the following. (*i*) Disintegration of the plasma membrane with detergent is required to solubilize the proteoglycan in an intact form. (*ii*) The detergent-solubilized proteoglycans are associated with detergent micelles as indicated by their larger apparent size when chromatographed in Triton X-100 (large micelles) compared with their elution position in deoxycholate (small micelles). (*iii*) Detergent-extracted but not heparin-released proteoglycans are retained on octyl-Sepharose, indicating the presence of hydrophobic domains in the detergent-extracted proteoglycan. (*iv*) Detergent-extracted but not heparin-released proteoglycans can be inserted into liposomes.

Some cell types appear to lack receptor-bound heparan sulfate at their cell surfaces (27). Yet, also these cells have heparan sulfate associated with their cell surfaces. Thus, a membrane-intercalated heparan sulfate proteoglycan may occur generally. A membrane-intercalated proteoglycan may represent a transition form in the secretion of heparan sulfate. A proteolytic enzyme like trypsin could cleave the protein core near the cell surface, leaving behind a hydrophobic segment and releasing the proteoglycan into the medium.

The heparan sulfate proteoglycan also could be biologically active in its intercalated form by participating in various interactions at the cell surface. With the core protein anchored in the membrane of one cell, the proteoglycan could bind through its polysaccharide chains to receptors present at the surface of a neighboring cell and, thus, mediate cell–cell contacts. The intercalated proteoglycan also could participate in cell–substrate adhesion by linking the cell to extracellular matrix components. Polysaccharides structurally related to heparan sulfate have been shown to interact with both fibronectin (28) and laminin (29). Finally, a membrane-intercalated proteoglycan with its polysaccharide chains exposed to the extracellular environment could serve as a "receptor" for circulating molecules. Thus, cell-associated heparan sulfate has been suggested to serve as a cellular binding site for the enzyme lipoprotein lipase (30), which is displaced by heparin from the endothelial cells lining the blood vessel wall.

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