Glycoconjugate Journal (1993) **10**:453–460

The growth promoter spermine interacts specifically with dermatan sulfate regions that are rich in L-iduronic acid and possess antiproliferative activity

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Received 21 April 1993

We have investigated interactions between spermine, a member of the growth promoting polyamine family, and various glycosaminoglycans. By using gel chromatography and equilibrium dialysis experiments we found that spermine binds to L-iduronic acid-rich dermatan sulfate (K_d, approximately 3.9×10^{-4} M) with an affinity similar to that between spermine and DNA. By digesting spermine–dermatan sulfate complexes with chondroitin ABC lyase, the formation of oligosaccharide fragments (tetra-to-decasaccharides) was demonstrated by polyacrylamide gel electrophoresis. Chondroitin sulfate, which is deficient in L-iduronic acid, generates no spermine-protected fragments. Analysis of protected dermatan sulfate oligosaccharides indicates that the majority of the L-iduronic acid residue is non-sulfated and in a periodate-resistant conformation. The oligosaccharides also possess antiproliferative activity.

Keywords: dermatan sulfate; interaction; spermine; cell-growth inhibitor

Glycosaminoglycans (GAG) are linear, often sulfated polysaccharides which usually are covalently linked to proteins, forming proteoglycans. These are found extracellularly in connective tissue matrices, both interstitially as well as at cell surfaces; some forms also occur intracellularly [1–4]. A common GAG is dermatan sulfate (DS) which is composed of disaccharide units consisting of galactosamine (GalNAc) and hexuronic acid (HexA). There are two kinds of HexA in DS; D-glucuronic acid (GlcA) and L-iduronic acid (IdoA) together with sulfated L-iduronic acid (IdoA-S).

A number of investigations (see [4–8]) have shown that GAGs have many biologically important functions, including regulation of cell proliferation and differentiation and control of enzyme activity. There are also a number of proteins in the extracellular space that bind GAGs selectively. These proteins, which include matrix proteins and plasma proteins and growth factors, often contain short basic amino acid consensus sequences, like BBXB (B = lysine or arginine) [4]. Although binding between basic peptides and acidic GAGs is electrostatic in nature it is not only related to the charge density of the GAG. Dermatan sulfate binds, for example, with much higher affinity to certain plasma proteins than does chondroitin sulfate (CS), in spite of similar charge density and molecular mass. The latter contains only GlcA, whereas dermatan sulfate contains both IdoA and GlcA [1]. The former HexA, but not the latter, can occur in several equi-energetic conformations, which results in a more flexible GAG chain where sulfate- and carboxylate groups can change their orientation. For that reason the GAG chain can adopt a 3-dimensional conformation which may be especially suitable for the interaction with a certain polycation. Conformational versatility of IdoA has been proposed as the basis for strong binding of IdoA-containing GAGs to polycationic molecules [9]. Moreover, it is known that IdoA-rich GAGs, like dermatan sulfate and heparan sulfate, inhibit fibroblast proliferation and that the antiproliferative activity is directly related to IdoA content [10].

Most cells contain a family of intracellular, polycationic growth promoters called polyamines which include putrescine, spermidine and spermine. Polyamines are essential for cell growth and concentrations are high in malignant cells and rapidly growing tissues [11]. When cells are depleted of polyamines by specific inhibitors of their synthesis, they cease to proliferate. As the polyamines are positively charged, their effect on many cellular processes is believed to be mediated through electrostatic binding to DNA. It has become evident in recent years that cells can import polyamines from the extracellular space by specific membrane transport systems [12]. In the extracellular environment, polyamines may thus have the opportunity to interact

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with GAGs which, like DNA, are polymers with repetitive negative charges.

As certain GAGs inhibit cell growth we have started to examine interactions between polyamines and GAGs. Here we describe a specific binding of spermine to dermatan sulfate that is rich in IdoA. By using equilibrium dialysis and gel filtration experiments, we find that spermine binds to dermatan sulfate and DNA with the same affinity and with significantly lower affinity to the IdoA-deficient chondroitin sulfate. By digesting dermatan sulfate–spermine complexes with chondroitin ABC lyase, protected saccharide fragments with antiproliferative activity have been isolated.

Materials and methods

Materials

Dermatan sulfate from pig skin was the same preparation as described elsewhere [13, 14]. After conversion to calcium salts DS was fractionated by stepwise ethanol precipitation (with 18%, 36%, 50% ethanol) into preparations DS-18, DS-36 and DC-50, respectively [15]. CS from bovine nasal cartilage was a gift from Professor D. Heinegård and hyaluronan, dermatan and chondroitin (both desulfated) gifts from Dr A. Malmström of this department. Calf thymus DNA was purchased from Pharmacia. The different lyases used were: chondroitin ABC (EC 4.2.2.4), AC-I (EC 4.2.22.5) and B lyase (EC 4.2.2.6) all from the Seikagaku Corporation (Japan). Spermine and [^{14}C]spermine (110 mCi mmol^{-1}) were products of Sigma Chemical Co. and Amersham International, respectively. Dialysis tubing with a mol. wt cut-off of 6000–8000 was purchased from Spectrum Medical Industries, Inc. The prepacked columns and column media used were: fast-desalting (FD) Sephadex G-25 10/10 (Pharmacia-LKB), DE-53 DEAE-cellulose (Whatman) and Bio-Gel P-6 (Bio-Rad). Electrophoresis equipment was from Pharmacia-LKB, supplemented by materials from a local workshop.

Degradation methods

Cleavage of GalNAc-HexA bonds in DS was achieved by using chondroitin ABC lyase and specific cleavage of GalNAc-GlcA bonds by using chondroitin AC-I lyase. These degradations were performed at 37 °C in 0.1 M Tris-HOAc-10 mM EDTA, pH 7.3. Selective scission of bonds between GalNAc and sulfated or non-sulfated IdoA was obtained by digestion with chondroitin B lyase in 50 mM Tris-HCl. pH 8.0, containing 0.05% bovine serum albumin, at 30 °C. Unsaturated uronosyl residues were detected by measuring the absorption at 232 nm in 0.05 M KCl:HCl, pH 1.8. The digestion was stopped when A_{232} had reached a constant value [16]. Non-sulfated IdoA was oxidized with periodate overnight at pH 3.0 and 4 °C and subsequently cleaved by alkaline elimination [17].

Separation methods

DS fragments were separated either by gel chromatography or by gradient polyacrylamide gel electrophoresis (PAGE). Gradient PAGE of oligosaccharides was executed as described in detail elsewhere [13, 14]. Gels were stained with Azure A and sometimes also with silver [18]. For gel chromatography (FPLC mode) we used a fast-desalting (FD) Sephadex G-25 column eluted either with 0.2 M NH_4HCO_3 or 125 mM Na-phosphate, pH 6.8, or with 0.15 M NaCl-0.01 M Tris, pH 7.4, at a rate of 1 ml min^{-1}. The effluent was collected in fractions of 0.5 ml. We also used Bio-Gel P-6 (18 mm × 1000 mm) eluted with 0.5 M NH_4HCO_3 at a rate of 8 ml h^{-1} (LC mode). The elution position of larger fragments of DS (>decasaccharides) could be monitored by the dimethylmethylene blue (DMB) method, whereas smaller fragments were detected by their A_{232} (see above). A Multiscan photometer, type 310 C, was used in the dimethylmethylene blue method [19]. Effluents were assayed for [^{14}C]spermine using an LKB Wallac 1214 Rackbeta Counter with ReadySafe (Beckman) as scintillator liquid. Products obtained after complete digestion with chondroitin ABC lyase (unsaturated disaccharides) were separated by HPLC on a Lichrosorb-NH_2 column connected to a Varian Vista chromatograph as described [20, 21].

Equilibrium dialysis

Dialysis tubing with a mol. wt cut-off of 6000–8000 was used as dialysis bag. Only spermine molecules, which are small and diffusable, could pass between the medium (outside the bag) and the bag, to which GAG was confined. The buffer consisted of 0.15 M NaCl-0.01 M Tris, pH 7.4. By using a constant concentration of GAG (or DNA) and varying concentrations of spermine/[^{14}C]spermine, it was possible to measure the binding parameters by constructing a Scatchard plot giving us the value of the dissociation constant (K_d) and the number of moles of spermine bound to each mole of GAG (r) according to the following equation:

$$[PL]/[L] = -[PL]/K_d + r[P^0]/K_d$$

where [PL] = molar concentration of bound spermine, [L] = molar concentration of unbound spermine, K_d = dissociation constant, r = number of moles of spermine bound to each mole of GAG, and [P^0] = molar concentration of GAG with an M_r of 15000. The slope of a plot of the equation is $-1/K_d$. As [P^0] is known and [PL] is measured, we obtain the value of r from $r = [PL]/[P^0]$ when [PL]/[L] = 0.

Using defined volumes inside (1 ml) and outside (10 ml) the dialysis bag and a 6000-fold excess of spermine with a specific activity of 17.4 mCi mol^{-1}, the values of [PL] and [L] were determined by liquid scintillation. Both [^{14}C]spermine and GAG were checked for binding to the

membrane, since this must be minimal for reliable results. The dialysis bag was slowly shaken for 24–48 h at constant temperature to reach equilibrium. As the concentration of spermine in the medium is considered to be equal to the concentration of unbound spermine inside the bag ([L]), the value of bound spermine ([PL]) could be calculated by subtracting the amount of unbound spermine from the total amount of spermine in the bag.

Isolation of undegraded, spermine-protected dermatan sulfate fragments

Dermatan sulfate preparations DS-18 or DS-36 (40 mg) and spermine (200 mg) were dissolved in 20 ml of digestion buffer and treated with 100 munits of chondroitin ABC lyase overnight followed by 25 munits each day until degradation was complete as monitored by A_{232}. The reaction mixture was dissolved in 5 ml 0.1 M glycine:NaOH buffer, pH 10.0 and applied to a column containing 5 ml DE-53 DEAE-cellulose equilibrated in the same buffer. The column was washed with 5 bed vol. of the equilibration buffer followed by elution with 5 bed vol. of 4 M guanidinium chloride:50 mM NaOAc, pH 5.8. Fractions from washes and elutions were assayed for [^{14}C]spermine and oligosaccharides by liquid scintillation and the DMB method, respectively. Non-degraded oligosaccharides were subsequently separated from disaccharides by FPLC on an FD Sephadex G-25 column eluted in 0.2 M NH_4HCO_3. The yield of these undegraded products was approximately 20–25% of the starting material.

Assay of growth effects

Dermatan sulfate oligosaccharides generated by digestion with chondroitin ABC lyase in the presence of spermine and then separated from spermine were assayed for growth effects according to methods described previously [10]. Fibroblasts seeded at a density of 4000–5000 cells per well (96-well microplates) were serum-starved for 24 h and then grown in Ham's F12 medium supplemented with insulin (10 μg ml^{-1}) and transferrin (25 μg ml^{-1}) in the absence or presence of dermatan sulfate oligosaccharides. The growth factor used was epidermal growth factor (recombinant human form obtained from Genzyme Corp., Boston, USA). Cell number was determined from the amount of adsorbed crystal violet as measured in a microplate photometer. Mean values ± standard error of the mean were calculated. Student's *t*-test was used to evaluate differences between groups. *P*-Values < 0.05 were considered significant.

Results and discussion

Gel chromatography of spermine and dermatan sulfate

A mixture of dermatan sulfate preparation DS-18 and [^{14}C]spermine (specific activity, 17.4 mCi (mol^{-1}) was chromatographed on an FD Sephadex G-25 column in 125 mM sodium phosphate, pH 6.8 (Fig. 1(a)). Approximately 40% of the spermine became associated with dermatan sulfate under these conditions. Further experiments with spermine and enzymatically or chemically pre-treated forms of DS-18 were performed. Digestion with chondroitin AC-I lyase which cleaves at GlcA (Fig. 1(b)), with chondroitin B lyase which cleaves at sulfated and non-sulfated IdoA (Fig. 1(c)) or cleavage of non-sulfated IdoA through periodate-oxidation/alkali treatment (Fig. 1(d)) gave products that did not associate with spermine under these conditions. These results suggest that all three types of repeating unit, i.e., those containing IdoA, IdoA(-S)

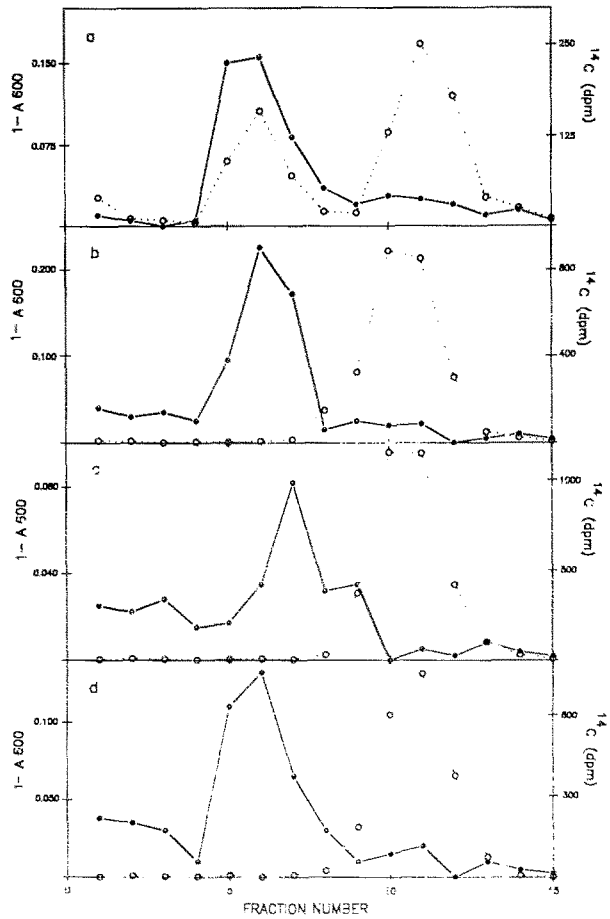

Figure 1. Gel chromatography of intact or pre-treated forms of dermatan sulfate preparation DS-18 and spermine. Samples, of 1 mg were mixed with 5 mg spermine (specific activity, 17.4 mCi mol^{-1}) and chromatographed on a FD Sephadex G-25 column in 125 mM sodium phosphate, pH 6.8. Aliquots of the fractions were analysed ●, for dermatan sulfate by using the DMB method and ○, for [^{14}C]spermine by liquid scintillation. The chromatograms are: (a) intact dermatan sulfate, (b) dermatan sulfate pre-treated with 4 × 10 munits of chondroitin AC-I lyase, (c) dermatan sulfate pre-treated with 4 × 0.7 munits of chondroitin B-lyase and (d) dermatan sulfate subjected to periodate-oxidation/alkali treatment. The elution positions for dermatan sulfate and free spermine were determined in separate experiments.

and GlcA, are necessary for a strong interaction. To estimate the actual strength of binding, equilibrium dialysis experiments were performed.

Equilibrium dialysis

The results of equilibrium dialysis experiments with various GAGs (or DNA) and spermine are summarized in Fig. 2. The DS-18 preparation (IdoA/GlcA = 90:10) contains approximately 5 binding-sites for spermine with a K_d of approximately 3.9×10^{-4} M (Fig. 2a). Spermine binds with 2.5-fold lower affinity to DS-36 (K_d, approximately 9.5×10^{-4} M) which is richer in GlcA (IdoA/GlcA = 75:25)

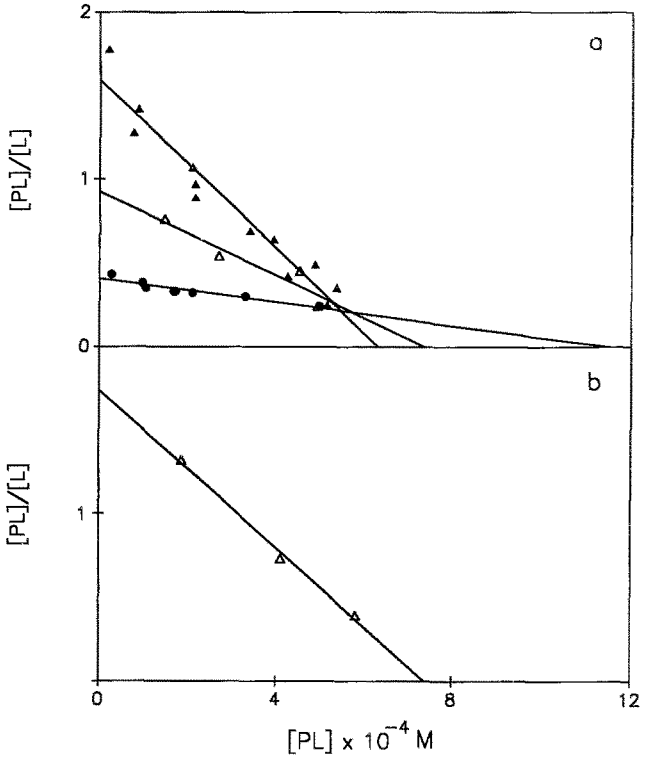

Figure 2. Scatchard plots of results obtained from equilibrium dialysis experiments. In (a) two forms of IdoA-rich dermatan sulfate (DS-18 or DS-36) or IdoA-deficient chondroitin sulfate (2 mg each) were dissolved in 1 ml 0.15 M NaCl:0.01 M Tris, pH 7.4, and injected into a dialysis bag. Spermine (specific activity, 17.4 mCi mol^{-1}) was added in amounts ranging from 0.05 mg to 8 mg (corresponding to 1.3×10^{-5}–2.1×10^{-3} M), either to the medium, the solution inside the bag or distributed equally between the two compartments. Measurements were performed as described in the Materials and methods section A plot of [bound]/[free] versus [free] was constructed. The DS-18 chains (▲) contain approximately five binding sites for spermine with a K_d of approximately 3.9×10^{-4} M, the DS-36 chains (△) approximately six binding sites with a K_d of approximately 9.4×10^{-4} M and the CS chains (●) approximately eight binding sites with a K_d of approximately 2.5×10^{-3} M. The results were each obtained with three different experiments, respectively. In (b) a similar experiment was carried out with DNA. Binding of spermine to calf thymus DNA yielded a K_d of approximately 4.1×10^{-4} M.

and with 10-fold lower affinity to the IdoA-deficient CS (K_d, approximately 2.5×10^{-3} M). DS-18 depleted of GlcA-units by chondroitin AC-I lyase digestion had approximately the same K_d as the starting material, but only half as many binding-sites. DS-18 depleted of IdoA-units by periodate oxidation/alkali treatment, hyaluronan (with GlcA-GlcNAc repeating units), dermatan (non-sulfated DS) and chondroitin (non-sulfated CS) do not bind spermine at all (results not shown). Furthermore, we also examined DNA in this system and obtained a K_d of approximately 4.1×10^{-4} M (Fig. 2(b)).

To examine further the strength of binding, samples from equilibrium dialysis experiments were chromatographed on FD Sephadex G-25 eluted in the same buffer. A solution from dialysis experiments with GAG and spermine (GAG-spermine mass ratio of 1:4) was directly applied to the column. The result showed that DS-18 and spermine co-eluted (as in Fig. 1a). However, DS-36 and CS, which have lower affinity for spermine (K_d approximately 9.5×10^{-4} and 2.5×10^{-3} M, respectively), dissociated entirely upon gel chromatography (results not shown).

Degradation of dermatan sulfate in the presence of spermine

Once an interaction between dermatan sulfate and spermine had been observed, it was of interest to find out if binding was restricted to a special region of the DS chain. Therefore we digested various DS preparations with chondroitin ABC lyase in the presence of spermine, postulating that spermine would protect binding sites from degradation. The general spermine/DS mass ratio was 5:1, which is beyond the saturation level observed in equilibrium dialysis experiments. As shown in Fig. (3a), digestion of DS-18 in the presence of spermine (○) yielded a lower A_{232} than with DS-18 alone (●), suggesting that spermine prevented complete degradation. Spermine also had an effect on the degradation of DS-36 (Fig. 3b), albeit, to a lower extent. The various pre-treated forms of DS (Fig. 3c–e) and CS (Fig. 3f) were completely degraded in the presence of spermine. These results show that spermine does not inhibit the enzyme *per se*. Instead, incomplete digestion of dermatan sulfate is correlated to high affinity binding of spermine. Hence, both DS-18 and DS-36 should generate protected oligosaccharides after digestion of spermine–DS complexes. Furthermore, the presence of all three types of HexA residues in the intact DS chain seem to be required to generate enzyme-resistant segments.

Characterization of spermine-protected dermatan sulfate segments

To generate protected dermatan sulfate chain-segments DS–spermine complexes were exhaustively digested with chondroitin ABC lyase. The DS fragments were separated from spermine by ion exchange chromatography at pH 10 (see the Materials and methods section). The protected fragments and the complete degradation products (di-

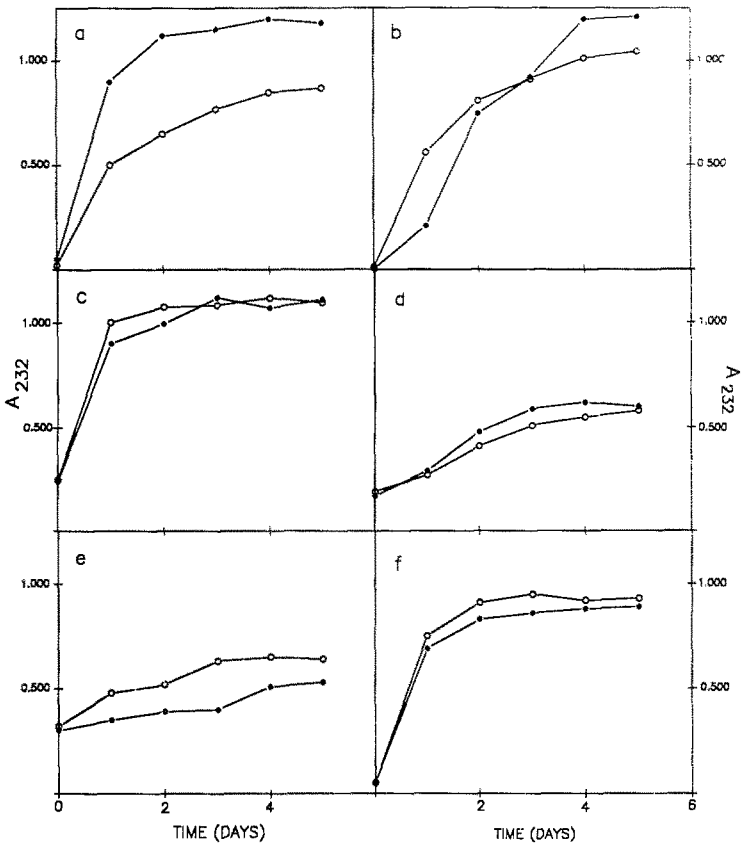

Figure 3. Enzymatic degradation of dermatan sulfate or chondroitin sulfate in the presence of spermine. (a–e) Two mg of intact preparations DS-18 or DS-36 or one of the pre-treated forms of DS-18 (see legend to Fig. 1) were digested with chondroitin ABC lyase in 1 ml of digestion buffer in the presence of 10 mg spermine (○) or without spermine (●). Aliquots (100 µl) were taken each day, diluted with 1 ml 0.05 M KCl/HCl, pH 1.8, and their A_{232} measured. Ten munits of enzyme were added each day until A_{232} was constant. The curves are: (a) intact DS-18, (b) intact DS-36, (c) DS-18 pre-treated with chondroitin AC-I lyase, (d) DS-18 pre-treated with chondroitin B lyase (the GlcA-containing fragments were isolated on an FD Sephadex G-25 column in 0.2 M NH_4HCO_3) and (e) DS-18 pre-treated with periodate-oxidation/alkali treatment; (f) is the same procedure as in (a) but with chondroitin sulfate.

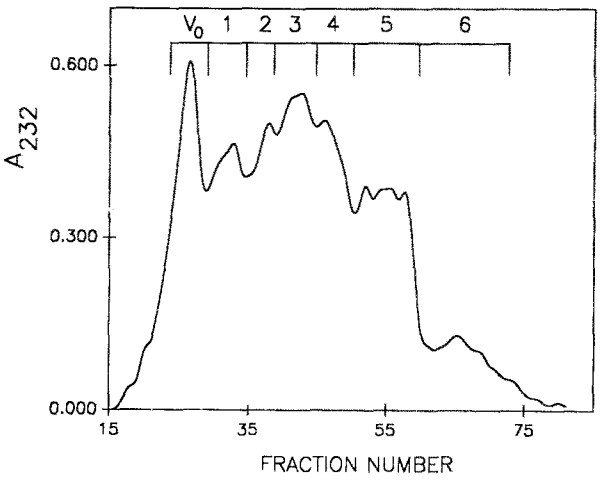

Figure 4. Gel chromatography on Bio-Gel P-6 of spermine-protected dermatan sulfate fragments. Preparation DS-18 (40 mg) and spermine (200 mg) were dissolved in 20 ml of digestion buffer and treated with 100 munits of enzyme overnight followed by 25 munits each day until degradation was complete, as monitored by A_{232}. The spermine-protected DS-fragments were then separated from spermine on a DE-53 DEAE cellulose column at pH 10 and from disaccharides–degradation products on an FD G-25 column as described in the Materials and methods section. Elution was performed in 0.2 M NH_4HCO_3. The void volume peak, which represented oligosaccharide fragments that had been protected from degradation, was pooled, freeze-dried and applied to the Bio-Gel P-6 column. Aliquots (400 µl) of the column effluent were analysed for A_{232}. Fractions were pooled as indicated by the bars (V_0–6). Pool 6 elutes in the position of disaccharide.

saccharides) were resolved by chromatography on a FD Sephadex G-25 column in 0.2 M NH_4HCO_3 (see the Materials and methods section). The protected DS fragments were applied to a Bio-Gel P-6 column eluted with 0.5 M NH_4HCO_3 (Fig. 4). The results from this experiment showed a wide range of fragment-sizes, even intact DS-18 chains (pool V_0). These DS chains, which could have a high number of spermine binding sites, were re-examined in a dialysis experiment (not shown). We obtained the same K_d value as for the original DS-18 preparation (approximately 3.9×10^{-4} M) but a larger number of binding-sites (approximately eight).

In order to examine further the various spermine-protected fragments of dermatan sulfate, gradient PAGE was performed. This method separates according to both size and charge. Pools 1, 3 and 5 from the Bio-Gel P-6 chromatography separation were subjected to electrophoresis together with similar oligosaccharides derived from preparation DS-36. As shown in Fig. 5(a), pools 1, 3 and 5 derived from DS-18 contained oligosaccharides with sizes ranging from tetra- to decasaccharides. Different charge variants were also apparent. Pool V_0 (not shown) consisted of almost intact chains. This banding pattern has been reproduced seven times.

Digestion of dermatan sulfate preparation DS-36 in the presence of spermine should also result in the formation of protected fragments (see previous results in Fig. 3b). DS-36 was subjected to the same procedure as DS-18, leading to the result shown in Fig. 5(b), lane 1. Intact DS-36 chains could not be detected, but we obtained oligosaccharides with a wider range of sizes as well as a larger number of charge variants than with DS-18. Protected fragments from DS-36 were further degraded with either chondroitin B (Fig. 5(b), lanes t = 3 and t = 6, Ch.B lyase) or AC-I lyase (Fig. 5(b), lanes t = 3 and t = 6, Ch.AC-I lyase). Whereas treatment with the B enzyme resulted in almost complete degradation, only limited effects were

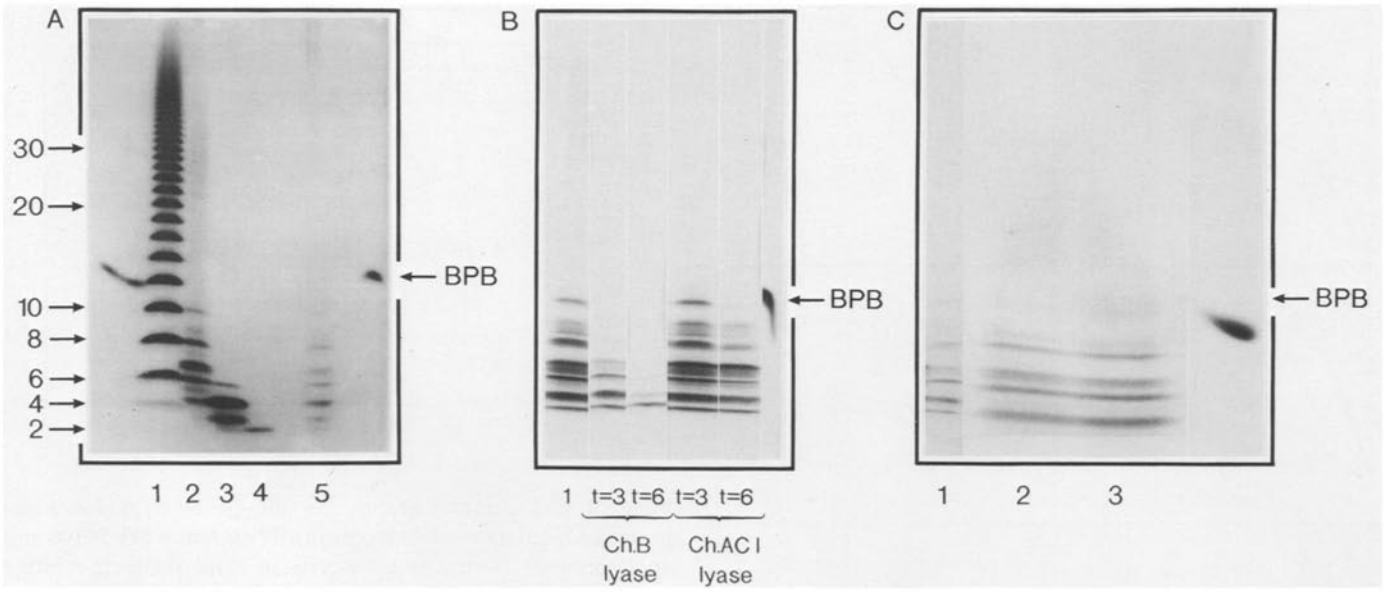

Figure 5. Gradient PAGE of spermine-protected fragments formed by digesting (a) and (c) dermatan sulfate preparation DS-18 or (b) DS-36 with chondroitin ABC lyase in the presence of spermine. Protected fragments were generated and isolated as described in the legend of Fig. 4. Samples of 100 μg from each pool were run. In (a) lane 1 is DS-36 degraded with chondroitin AC-I lyase which was used as a standard. The figures in the left margin indicate the sizes of the oligosaccharides. Lanes 2–4 are pools 1, 3 and 5 from the Bio-Gel P-6 separation, respectively; lane 5 is a mixed DS-18 oligosaccharide pool. In (b) lane 1 is the corresponding oligosaccharide material from DS-36. The other lanes are enzymatic digests of these oligosaccharides as indicated. Material corresponding to 375 μg was dissolved in 250 μl of digestion buffer and treated with either chondroitin B (for $t = 3$ and 6 days) or AC-I lyase (for $t = 3$ and 6 days). At the indicated time-intervals aliquots (100 μl) were taken, dissolved in 1 ml KCl/HCl, pH 1.8, their A_{232} determined and the samples then electrophoresced. Both degradations were complete on day 6. In (c) the material shown in lane 2 panel (a) (approximately 30 μg) was electrophoresced directly (lane 1) or oxidized with periodate in the presence (lane 2) or absence (lane 3) of spermine, then cleaved with alkali and electrophoresced. Staining was performed with Azure A (a and b) or with silver (c). BPB, bromophenol blue marker.

observed with the AC-I enzyme. The IdoA/GlcA ratio of the DS-36 oligosaccharides was estimated to be 90:10 by measuring A_{232} when both degradations were complete. Periodate oxidation (generally selective for non-sulfated IdoA) also resulted in limited degradation (Fig. 5(c)) suggesting that the IdoA residues were either largely sulfated or occupied a periodate-resistant conformation.

To determine the sulfation pattern in the spermine-protected dermatan sulfate oligosaccharides, unsaturated disaccharides generated by complete digestion with chondroitin ABC lyase were resolved by HPLC on Lichrosorb-NH_2. The results indicated that 85% consisted of 4-sulfated disaccharides and 15% corresponded to over-sulfated forms (including those with IdoA-S). No 6-sulfated disaccharides were found. These results suggest that most of the non-sulfated IdoA is in a periodate-resistant conformation.

Growth effects of spermine-protected fragments

Dermatan sulfate preparations DS-18 and DS-36 both have antiproliferative effects on cultured lung fibroblasts [10]. As shown in Fig. 6 both intact DS-18 and the spermine-protected oligosaccharides reduced growth to approximately 50–60% of normal at the highest concentration used. Apparently, the antiproliferative activity is retained in the spermine-protected fragments, but the specific activity is unchanged.

Conclusion

We have observed a specific interaction between spermine and dermatan sulfate. The most IdoA-rich DS preparation used (DS-18) bound to spermine with the same strength as did DNA (K_d, approximately 3.9×10^{-4} M and 4.1×10^{-4} M, respectively). The K_d value for DNA is in the same range as, but not identical to, results obtained in other studies [22]. Under the conditions used here (ionic strength 0.15 M) the deviation could not be due to adsorption of labelled spermine to the dialysis membrane or to the Donnan equilibrium. It could be explained by the absence of Mg^{2+} and K^+ in our system. Mono- and divalent cations are inhibitors of the binding of spermine to DNA. We were, however, mainly interested in a direct comparison between GAGs and DNA concerning binding of spermine under the same conditions.

Spermine does not bind to dermatan sulfate that has been pre-treated with specific enzymes (chondroitin B and AC-I lyases) or chemicals (selective periodate oxidation) in order

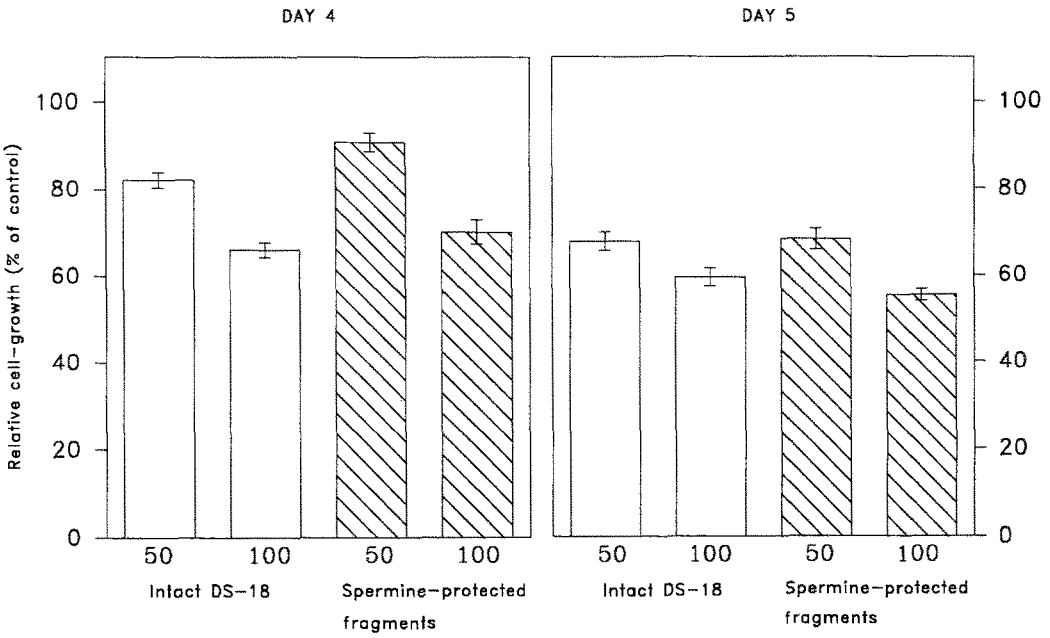

Figure 6. Effects of dermatan sulfate (open bars) and spermine-protected dermatan sulfate oligosaccharides (hatched bars) on growth of normal human lung fibroblasts in Ham's F12 medium (supplemented with insulin and transferrin) with epidermal growth factor (10 ng ml^{-1}). Cells were plated at a density of 4000–5000 cells per well and incubated either with intact dermatan sulfate preparation DS-18 or with spermine-protected fragments thereof at two concentrations (50 and 100 μg ml^{-1}), respectively. On each plate a set of control incubations was made. Cell number was determined at days 4 and 5 by the crystal violet method as described in the Materials and methods section. Values are means ± SEM, where $n = 4$ or 5. Figures below bars represent the concentration (μg ml^{-1}) of substances added.

to cleave at, or in, GlcA, IdoA or IdoA-S residues. Spermine-protected fragments, potentially including the actual binding regions, could be isolated after digestion of DS–spermine complexes with chondroitin ABC lyase. The fragments included two major pools, one with almost intact chains and another with oligosaccharides ranging from tetra- to decasaccharides. These results suggest that the minimum size for a binding site is a tetrasaccharide. The large-sized fragments derived from DS-18 contained eight binding sites for spermine. If all binding sites were of tetrasaccharide size, this would give a total molecular mass of approximately 8000 Da. As this is only half the size of an intact DS-18 chain (M_n 17 500 Da [15]), the remainder of the chain may have adopted a shape that is generally inaccessible to degradation by the ABC lyase. It was also noted that the spermine-protected fragments were resistant to periodate oxidation despite the presence of largely non-sulfated IdoA. Further studies of the various fragments are necessary to determine their exact sequences and the possible changes in IdoA conformation induced upon interaction with spermine.

The biological relevance of the interaction between spermine and dermatan sulfate may be related to the antiproliferative effects of the latter [10]. Polyamines are believed to act as intracellular growth-promoters. However, they are also present extracellularly and can be taken up by specific carrier mechanisms [12]. Sequestration of spermine to extracellularly fixed dermatan sulfate chains (as part of matrix-bound proteoglycans) would deplete the cells of growth-promoting polyamines. It was also found that spermine-protected dermatan sulfate oligosaccharides possessed antiproliferative activity. The identification of active species is a major future objective.

Investigations on the specificity of interactions between polycationic molecules and GAGs are relevant also from another standpoint. Polyamines and basic peptides of defined sequence could be used to probe proteoglycan side-chains for the presence of unique saccharide sequences with specific biological activities. Future studies are also aimed at isolating various homogeneous spermine-binding oligosaccharides in order to investigate their carbohydrate sequence and the possible conformational changes induced by spermine and other polyamines.

Acknowledgements

We acknowledge grants from the Swedish Medical Research Council, Cancerfonden, The Gustaf V:s 80-års fond, The JA Persson, Nilsson, Kock and Österlund Foundations and the Medical Faculty, University of Lund. We thank Birgitta Havsmark and Kristin Persson for technical assistance.

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