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Increased calcium affinity of a fucosylated chondroitin sulfate from sea cucumber

João Ruggiero [a], Ricardo P. Vieira [b], Paulo A.S. Mourão [b,*]

[a] *Departamento de Física, Instituto de Biociências, Letras e Ciências Exatas, Universidade Estadual Julio de Mesquita Filho, Caixa Postal 136, São José do Rio Preto, SP, 15054-000, Brazil*

[b] *Departamento de Bioquímica, Instituto de Ciências Biomédicas, Universidade Federal do Rio de Janeiro, Caixa Postal 68041, Rio de Janeiro, RJ, 21941-590, Brazil*

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Abstract

Calcium binding and charge distribution on a fucosylated chondroitin sulfate and a standard chondroitin 6-sulfate have been studied using a metallochromic indicator and conductimetric titrations. The fucosylated chondroitin sulfate has a ~ 5-fold greater affinity for calcium ions than the standard chondroitin 6-sulfate. Possibly, this increased affinity for calcium ions is due to the branches on the fucosylated chondroitin sulfate, since the calcium affinity of an unbranched, sulfated fucan is similar to that of the standard chondroitin 6-sulfate. More charged groups per disaccharide unit (and a shorter distance between these groups) also distinguish the fucosylated chondroitin sulfate from standard chondroitin 6-sulfate. Comparison between native and chemically modified (desulfated or carboxyl-reduced) polysaccharides suggests that the sulfate esters are responsible for the increased charge density of the fucosylated chondroitin sulfate and that the presence of the fucose branches does not alter the length of the repetitive units which compose the central core of chondroitin from sea cucumber. These results are consistent with the chemical studies of these two polysaccharides.

1. Introduction

Recently, we have isolated sulfated polysaccharides from different invertebrate connective tissues and compared them with the well known glycosaminoglycans that occur in vertebrate tissues. Novel sulfated polysaccharides were found in the tunic of ascidians [1–7] and the body wall of a sea cucumber [8–10].

* Corresponding author.

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The presence of fucose-rich sulfated polysaccharides have been reported in the body wall of sea cucumber [8–14]. We found that the main fraction of these sulfated polysaccharides has a chondroitin sulfate-like structure, containing unexpectedly large numbers of sulfated α-L-fucopyranose branches linked to position 3 of the β-D-glucuronic acid residues [9,10]. Methylation analysis and NMR spectroscopy revealed that the position of the glycosidic linkage and the site of sulfation in the fucose branches were extremely heterogeneous. We proposed a preponderance of disaccharide units formed by 3,4-di-*O*-sulfo-α-L-fucopyranosyl units glycosidically linked through position 1 → 2 to 4-*O*-sulfo-α-L-fucopyranose (**1**, Fig. 1).

The ability of glycosaminoglycans to complex divalent cations, especially calcium, is an important biological function of this class of polysaccharide. Comparison of the calcium affinities of different glycosaminoglycans has been useful in correlating biological function with physicochemical properties [15–18]. Different experimental approaches have been used to study calcium-glycosaminoglycan interactions, and the order of their affinity for calcium ions is heparin > chondroitin sulfate > keratan sulfate > hyaluronic acid [18–20]. Values of association constants determined by equilibrium dialysis vary from 31 900 M^{-1} (heparin) to 710 M^{-1} (hyaluronic acid) [18]. These results suggest that the affinity for calcium decreases with the decrease in number of charged groups per disaccharide unit.

The main purpose of the present work is to compare the charge distributions of the fucosylated chondroitin sulfate and of the standard chondroitin 6-sulfate, in order to determine the contribution of the sulfated fucose branches to the binding of calcium ions.

2. Experimental

Native and chemically modified polysaccharides.—Standard chondroitin 6-sulfate from shark cartilage and hyaluronic acid from umbilical cord were purchased from Sigma Chemical Company (St. Louis, MO, USA). Fucose-branched chondroitin sulfate and sulfated fucan were extracted from the body wall of the sea cucumber *Ludwigothurea grisea* by papain digestion, and purified by procedures previously described [10]. Purification procedures of the fucosylated chondroitin sulfate involve two subsequent columns of DEAE-cellulose, as described [10]. Desulfation of the glycosaminoglycans by solvolysis in Me_2SO [21] and reduction of the hexuronic acid carboxyl groups in the polysaccharide by 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide–$NaBH_4$ [22] were performed as described previously. Yield and integrity of the polysaccharides obtained after these two procedures were the same as in our previous study [9,10].

Chemical analysis of the polysaccharides.—Hexuronic acid was measured by the carbazole reaction [23], total methyl pentose by the method of Dische and Shettles [24], and total hexose by the method of Dubois et al. [25]. After acid hydrolysis of the polysaccharide (6.0 N HCl at 100°C for 6 h), total hexosamine was measured by a modified Elson–Morgan reaction [26], and sulfate by the $BaCl_2$–gelatin method

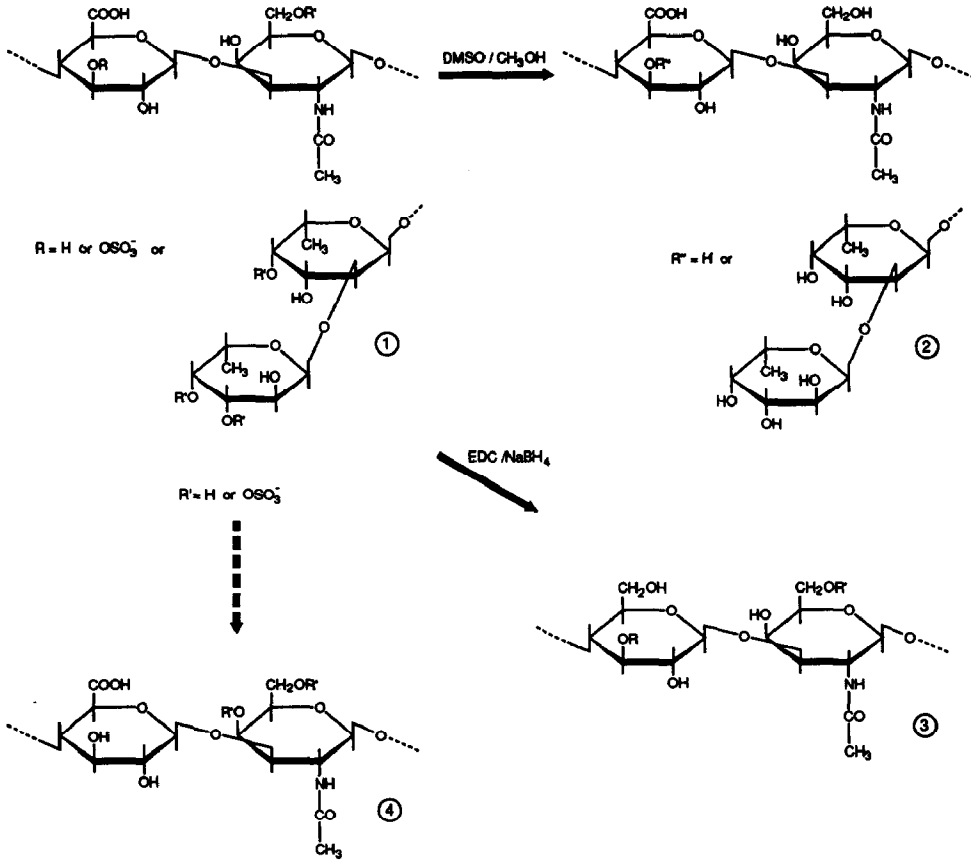

Fig. 1. Proposed structure of the major components of the fucosylated chondroitin sulfate of the sea cucumber before (1) and after chemical modifications (2–4). The polymer contains side chains of disaccharide units of sulfated α-L-fucopyranosyl linked to approximately one-half of the β-D-glucuronic acid moieties through the 0-3 position of the acid (1). Desulfation of this polysaccharide by solvolysis in Me_2SO–methanol yields the structures shown in 2, whereas reduction of hexuronic acid carboxyl groups with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC)–$NaBH_4$ transforms the glucuronic acid residues of the polysaccharide in glucose (3). The sulfated fucose branches can be removed from the sea cucumber polysaccharide by mild hydrolysis with acid to give a polymer similar to standard chondroitin 4- or 6-sulfate (4). See text for other comments.

[27]. The percentages of hexoses, methyl pentoses and hexosamines in the acid hydrolyzates were estimated by gas–liquid chromatography of the corresponding alditol acetates [28] and by paper chromatography in (3:2:1) *n*-butanol–pyridine–water for 48 h.

Conductimetric and potentiometric titrations.—Native and chemically modified polysaccharides were converted to the acid form by passage through a Dowex 50W-XB cation exchange column (Sigma). The hydroxides used in both potentiometric and conductimetric titrations were purchased as standardized solutions from E. Merck A.G. (Darmstadt, Germany).

Polymer concentrations (in mequiv/L) were determined by potentiometric titrations. In these experiments increasing volumes of 0.1 N NaOH were added to a 5 mL solution of polysaccharide (acid form) and the pH was measured on a Micronal/Metrohm pH meter using a Metrohm pH electrode.

In the conductimetric titration, increasing volumes of 0.1 N KOH or LiOH were added to a 5 mL solution of polysaccharide (~ 5 mequiv/L, acid form, pH ~ 2.9), and the conductivity was measured with a CD21 Digimed conductimeter equipped with platinized electrodes. The cell constant (1 cm) was determined periodically using a standard KCl solution.

Both titrations were carried out at 25.0 ± 0.01°C using a thermostatted cell.

Calcium titration and analysis of the binding data.—Calcium titrations were performed with the use of tetramethylmurexide (TMM) as a metallochromic indicator, purified and generously donated by Professor R. Kohn (Bratislava University, Czechoslovakia). The method is a slight modification of that proposed by Kohn and Furda [29], as described elsewhere [30,31].

In aqueous solution TMM binds Ca^{2+} reversibly in a 1:1 stoichiometry, and when calcium is complexed it undergoes a shift from 530 to 490 nm in the peak of its absorption spectrum [32]. Thus, the thermodynamics of the complex formation and the spectral properties of the free and bound TMM allow the determination of free and bound calcium in a given solution.

The titration experiments were run at 17°C in 10 mM NaCl solutions, containing polysaccharide (1 to 5 mequiv/L), 10 to 30 μM TMM and increasing amounts of $CaCl_2$. The absorbances at 490 and 530 nm were measured on a double-beam spectrophotometer (Hitachi, model U-2000).

The stability constant (K_{app}) of the complex between TMM and Ca^{2+} can be written as:

$$K_{app} = \frac{[TMM]f_{TMMCa}}{[TMM](1-f_{TMMCa})([Ca]_t - [TMM]f_{TMMCa})} \quad (1)$$

where [TMM], $[Ca]_t$, and f_{TMMCa} are the total concentrations of TMM and calcium and the mole fraction of TMM complexed with calcium, respectively.

The quantity f_{TMMCa} can be determined from titrations in 10 mM NaCl, using the experimental values of the ratio (R) of absorbances at 490 and 530 nm:

$$R = \frac{A_{490}}{A_{530}} = \frac{f_{TMMCa}\epsilon^{490}_{TMMCa} + (1-f_{TMMCa})\epsilon^{490}_{TMM}}{f_{TMMCa}\epsilon^{530}_{TMMCa} + (1-f_{TMMCa})\epsilon^{530}_{TMM}} \quad (2)$$

K_{app} can then be calculated from Eq. 1. The values of the extinction coefficients (ϵ) for the metallochromic indicator were determined from measurements in the absence and in the presence of a large excess of $CaCl_2$. The values obtained were: $\epsilon^{530}_{TMM} = 13800$, $\epsilon^{490}_{TMM} = 8400$, $\epsilon^{530}_{TMMCA} = 8420$, and $\epsilon^{490}_{TMMCA} = 20000$. K_{app} is an apparent stability constant, dependent on the ionic strength. Nevertheless, we found a nearly constant value of 650 ± 30 M^{-1} at 17°C, since the slight dilution of NaCl upon addition of $CaCl_2$ was nearly counterbalanced by the increase in ionic strength due to the $CaCl_2$. These observations are in accord with Manzini et al. [31].

Table 1
Chemical composition and average molecular mass [a] of native and chemically modified polysaccharides

Polysaccharide	Chemical modification	Molar ratios					Average molecular mass (kDa) [c]
		GlcUA	GalNH [b]	Fuc	Glc	Sulfate	
Fucosylated chondroitin sulfate	Native	0.30	0.39	0.31	< 0.01	1.03	40
	Desulfated	0.35	0.46	0.19	< 0.01	0.05	12
	Carboxyl-reduced	0.06	0.30	0.31	0.33	0.99	32
Chondroitin 6-sulfate	Native	0.48	0.50	0.01	< 0.01	0.51	40
Sulfated fucan	Native	< 0.01	< 0.01	1.00	< 0.01	1.10	30

[a] Data in the Table are mean values obtained from four replicates, and variations were found not to exceed 5%. [b] Galactosamine is *N*-acetylated [8–10]. [c] Average molecular masses were determined by gel filtration on Sephacryl S-400 columns and compared with glycosaminoglycans standards.

The knowledge of K_{app} allows calculation of the free calcium concentration, $[Ca^{2+}]_f$, from the absorbance ratio R in the presence of the polysaccharides, as follows:

$$[Ca^{2+}]_f = \frac{R\epsilon^{530}_{TMM} - \epsilon^{490}_{TMM}}{K_{app}(\epsilon^{490}_{TMMCa} - R \cdot \epsilon^{530}_{TMMCa})} \quad (3)$$

$[Ca]_b$ is then calculated from:

$$[Ca]_b = [Ca]_t - [Ca^{2+}]_f - f_{TMMCa} \cdot [TMM] \quad (4)$$

The binding data are plotted according to the usual Scatchard diagram:

$$[Ca]_b/[Ca^{2+}]_f \text{ vs. } [Ca]_b \quad (5)$$

3. Results and discussion

Chemical modifications of the fucose-branched chondroitin sulfate.—Fig. 1 shows a comparison of the proposed structure of the fucosylated chondroitin sulfate from sea cucumber before (**1**) and after (**2** and **3**) various chemical modifications, and the structure of standard chondroitin sulfate (**4**). Solvolysis in Me_2SO removes most of the sulfate esters (**2**, Fig. 1) and slightly reduces the fucose content (Table 1). The glucuronic acid carboxyl groups in the polysaccharide were reduced with 1-ethyl-3-(3-dimethylamino propyl)carbodiimide–$NaBH_4$ (**3**, Fig. 1) and the extent of the reaction was estimated by the decrease in the glucuronic acid and the formation of glucose (Table 1). The sulfated fucose branches can be removed from the polymer by mild hydrolysis with acid (broken line, Fig. 1), since fucose forms a

glycosidic linkage that is more sensitive to acid than that formed by glucuronic acid or by hexosamine. The proposed structure of the defucosylated polymer is similar to that of standard chondroitin sulfate (**4**, Fig. 1). In the present study we compare the properties of the fucosylated chondroitin sulfate with those of standard chondroitin 6-sulfate, because of the similarity of the latter polymer with a defucosylated chondroitin sulfate from sea cucumber [9,10].

Another sulfated polysaccharide isolated from the sea cucumber is a sulfated fucan (Table 1), mostly a linear polymer composed of disulfated and monosulfated α-L-fucopyranosyl units [33].

Conductimetric titrations of the sulfated polysaccharides.—Conductimetric titrations of anionic polymers have been used to determine their charge distribution [34]. In these experiments, a strong polyacid shows a sharp decrease in conductivity (σ) with an increase in degree of neutralization (α_n). This behavior is ascribed to the replacement of hydrogen ions by alkali metal ions during the course of the titration, since alkali metal ions associated with strong acid groups are less mobile than hydrogen ions [34,35]. With a weak polyacid, on the other hand, hydrogen ions are more tightly bound and their mobility is lower than for alkali metal ions associated with these groups [34,35]. Thus, the replacement of hydrogen ions during a titration with KOH or LiOH leads to a slow increase in the conductivity.

Conductimetric titrations of fucosylated chondroitin sulfate (Fig. 2A) and of standard chondroitin 6-sulfate (Fig. 2B) show curves with a mixed behavior that can be ascribed to the presence of both strong (sulfate) and weak (carboxyl) groups. A sharp decrease in conductivity with neutralization (to **a**, Figs. 2A and 2B) is followed by a slow increase in this parameter (from **a** to **b**), up to the point where complete neutralization of the polymer occurs (**b**). This interpretation for the titrations of the native chondroitin sulfates is confirmed by the titrations of their carboxyl-reduced and desulfated derivatives. Thus, in the titration of a carboxyl-reduced derivative from fucosylated chondroitin sulfate (Fig. 2C), the initial sharp decrease in conductivity ascribed to sulfate groups is accentuated, and in the titration of the desulfated derivative from the same polymer (Fig. 2D), the slow increase in conductivity ascribed to carboxyl groups is prominent *.

From the conductivity values (σ) obtained at the end point corresponding to the neutralization of the polymers by KOH or LiOH, transport factor (f) can be calculated [36] as follows:

$$f = \frac{\sigma_K - \sigma_{Li}}{10^3 \cdot C_p \cdot (\lambda_K - \lambda_{Li})} \quad (6)$$

where the σ_K and σ_{Li} are the experimental values of conductivities at **a** (Fig. 2)

* Some sulfate esters resist desulfation (Table 1) and may account for the initial decrease in conductivity observed in Fig. 2D.

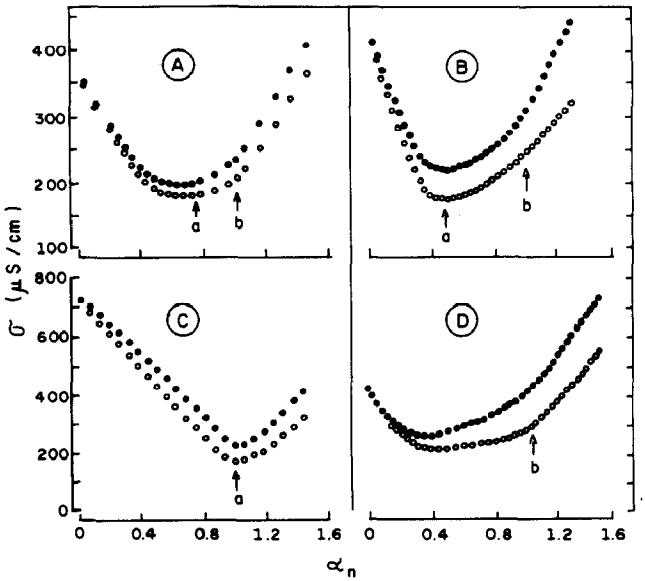

Fig. 2. Conductimetric titrations of native (A), carboxyl-reduced (C), and desulfated (D) chondroitin sulfate from sea cucumber and of standard chondroitin 6-sulfate (B). The conductimetric titrations of the polysaccharides, acid form, were performed as described under "Methods", with increasing amounts of KOH (●) or LiOH (○). The arrows **a** and **b** indicate the end points of titration of the sulfate and carboxyl groups, respectively. These points were extrapolations of the ascending and descending conductances, with **a** being the intersection, and **b** the point at which the conductance deviates from the straight line. The degree of neutralization (α_n) is the concentration of KOH or LiOH divided by the concentration of the polymer (C_p), both as equiv/L.

obtained from the titrations with KOH and LiOH, λ_K and λ_{Li} are the reduced ion conductances for K and Li and C_p is the polymer concentration in equiv/L.

In the counterion condensation model proposed by Manning [37,38] the transport factor is related to the linear charge density (ξ) as:

$$f = \frac{0.87}{|z|\xi} \qquad |z|\xi > 1 \tag{7a}$$

$$f = 1 - \frac{0.55|z|^2\xi^2}{3.14 + |z|\xi} \qquad |z|\xi < 1 \tag{7b}$$

where z is the valence of the counterion and ξ, for a rigid rod polyelectrolyte, is given by:

$$\xi = \frac{e^2}{\epsilon \cdot k \cdot T \cdot b} \tag{8}$$

e being the electrical charge of the titratable groups, ε the solution dielectric constant, k the Boltzmann constant, and b the distance between charged groups in the polymer.

Table 2
Transport factor (f), linear charge density (ξ), distance between charged groups (b), and average number of charged groups per disaccharide unit of native and chemically modified chondroitin sulfates

Polysaccharide	Chemical modification	f	Equation [a]	ξ	b (Å)	Average number of charged groups per disaccharide unit [b]
Fucosylated chondroitin sulfate	Native	0.34±0.01	*7a*	2.56	2.79	3.65
	Carboxyl-reduced	0.45±0.01	*7a*	1.93	3.70	2.75
	Desulfated	0.92±0.02	*7b*	0.75	9.52	1.07
Standard chondroitin sulfate	Native	0.62±0.02	*7a*	1.40	5.10	2.00
	Carboxyl-reduced	0.93±0.02	*7b*	0.70	10.20	1.00
Hyaluronic acid	Native	0.94±0.02	*7b*	0.64	11.10	0.91

[a] ξ were calculated from Eqs. *7a* or *7b*, as indicated in the Table.
[b] These values were calculated based on the length of the repeating unit of the carboxyl-reduced chondroitin sulfate (10.20 Å) divided by the distance between charged groups of the other acidic polysaccharides (b). See text for details.

The experimental data shown in Fig. 2 and the calculations described in Eqs. *6* and *8* were used to determine the transport factor, the linear charge density and the distance between charged groups of the various polysaccharides (Table 2). The linear charge density is higher (and the distance between charged groups is lower) in the fucosylated chondroitin sulfate than in standard chondroitin 6-sulfate. Therefore, the presence of highly sulfated fucose branches markedly increases its anionic charge density. After reduction of the glucuronic acid carboxyl groups, the charge density of standard chondroitin 6-sulfate decreases by half (the distance between charged groups doubles). However, the same chemical modification only slightly affects these same parameters of the fucosylated chondroitin sulfate since most of its anionic charges are due to sulfate esters. Accordingly, desulfation of the fucosylated chondroitin sulfate dramatically reduces its charge density, which now resembles that of a carboxyl-reduced chondroitin 6-sulfate. In fact, both the carboxyl-reduced chondroitin 6-sulfate and the desulfated chondroitin sulfate from sea cucumber have a single anionic charge per disaccharide unit (Table 1). This is attributed to sulfate esters at the O-6 position of galactosamine residues and to carboxyl groups of the glucuronic acid units, respectively (Fig. 1). The distances between the anionic groups of these two chemically modified chondroitins are similar, indicating that the repeat units of the two polymers have approximately the same length (Table 2). The values obtained are in agreement with the length of the repetitive unit of other glycosaminoglycans determined by different methodologies. For example, the length of a tetrasaccharide unit of heparin was reported as 20.4 Å [39] or 21.1 Å [40]. This result confirms that the fucose residues in the fucosylated chondroitin sulfate are branches and not intercalated among the

repetitive disaccharide units of the central chondroitin core. This inference is in agreement with our previous chemical studies (Fig. 1) [9,10].

In order to estimate the average number of charged groups per disaccharide unit we assume that the several sulfate esters and carboxyl groups are distributed within the 'space' of this disaccharide unit, thus reducing the distance between charged groups. According to this proposition the length of the repeating unit of the carboxyl-reduced chondroitin 6-sulfate (10.20 Å, Table 2) divided by the distance between charged groups of other acidic polysaccharides should indicate the average number of charged groups per disaccharide units (Table 2). In fact, these values are in good agreement with the chemical analyses of these polysaccharides (Table 1), which show 3.41 and 1.98 charged groups per disaccharide units (expressed as galactosamine residues) for the fucosylated chondroitin sulfate and standard chondroitin 6-sulfate, respectively.

Binding of calcium to fucosylated chondroitin sulfate.—Scatchard plots of calcium binding to the native fucosylated chondroitin sulfate and to standard chondroitin 6-sulfate are biphasic (Figs. 3A and B) indicating the presence of two class of sites. Desulfation and carboxyl reduction each eliminate one class of sites (Figs. 3C and D), so that the low-affinity sites can be assigned to the carboxyl groups and the high-affinity sites to the sulfate groups.

The apparent association constants (K) estimated by TMM complexation, to these sites as M^{-1} can be obtained from the Scatchard plots as $[Ca]_b/[Ca^{2+}]_f \times [Ca]_b$, and are shown in Table 3. Simply drawing tangents to the two ends of the Scatchard plots may give erroneous results [41]. However, we calculated K for the sulfate and carboxyl groups using a computer program that was developed to resolve such plots [41] (see continued lines in Figs. 3A and B).

One of the major observations of this study is that the calcium affinity of the sulfate groups is ~ 5-fold greater in the fucosylated chondroitin sulfate than in the standard chondroitin 6-sulfate (Table 3). This higher affinity for calcium is not a specific property of the sulfated fucoses, since a sulfated fucan from sea cucumber has a low calcium affinity compared with the other polysaccharides (Table 3). The higher affinity for calcium also cannot be attributed to the higher sulfate content of the fucosylated polymer, since the sulfated fucan has a low affinity but a higher sulfate–total sugar ratio than the fucosylated chondroitin sulfate (Table 1). Finally, the calcium affinity is not a simple function of linear charge density of the polymer since this latter value increases about 1.8-fold from the standard chondroitin 6-sulfate to the fucosylated chondroitin sulfate (Table 2) whereas the affinity for calcium increases ~ 5-fold. It may be that the higher calcium affinity of the fucosylated polysaccharide is related to the position occupied by the sulfated fucose branches on the chondroitin sulfate from sea cucumber *.

* K_{app} has a strong dependence on NaCl concentration. Low concentrations of NaCl were used to assure precise measurement of this association constant. This raises a question concerning the affinities of carboxyl and sulfate groups for Ca^{2+} under physiological conditions. Possibly, this point has to be clarified using a different methodology.

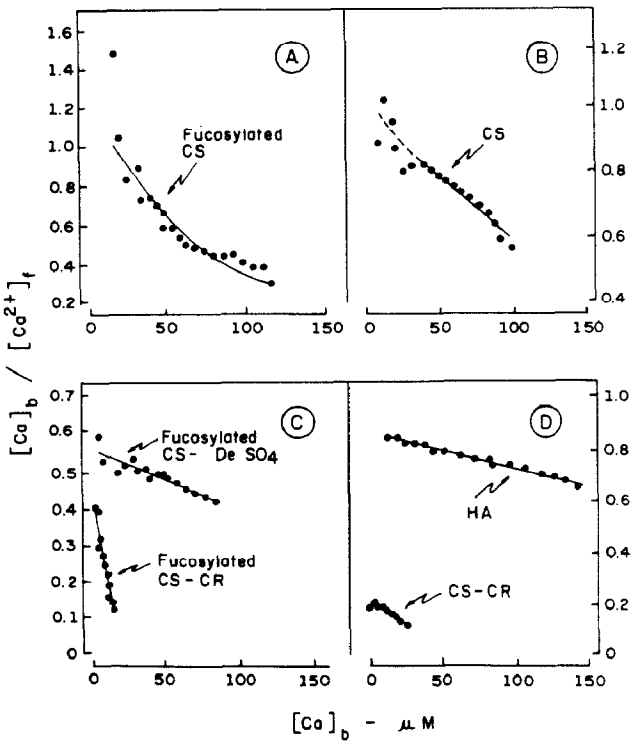

Fig. 3. Scatchard plots of calcium binding to the native or chemically modified chondroitin sulfates and to the hyaluronic acid. Calcium binding to fucosylated chondroitin sulfate (Fucosylated CS), to standard chondroitin 6-sulfate (CS) and to hyaluronic acid (HA), before and after desulfation ($DeSO_4$) or carboxyl reduction of the glucuronic acid units (CR) was performed as described in "Methods". The free calcium concentration ($[Ca^{2+}]_f$) and the concentration of calcium complexed with the polysaccharide ($[Ca]_b$) were calculated as described in the text and binding data were plotted as usual Scatchard diagram. See additional comments on Table 3.

The association constant for the high-affinity sites of the fucosylated chondroitin sulfate (Table 3) is very similar to values obtained for heparin using either a similar experimental procedure (20 000 M^{-1}) [42] or equilibrium dialysis (31 900 M^{-1}) [18], although neither of these previous studies distinguished between sulfate and carboxyl groups. Since heparin and fucosylated chondroitin sulfate possess completely distinct chemical structures *, the calcium-binding sites in these polymers do not appear to have specific structural requirements.

* Another distinguishing characteristic of these two sulfated polysaccharides is the very low anticoagulant activity of fucosylated chondroitin sulfate compared with standard heparin. In the USP assay, standard heparin and fucosylated chondroitin sulfate have anticoagulant activities of 140–180 and 7 USP/mg, respectively.

Table 3
Apparent association constants (K) as M^{-1} for native and chemically modified chondroitin sulfates

Polysaccharide	Chemical modification	Anionic group	
		Sulfate	Carboxyl
Fucosylated chondroitin sulfate	Native [a]	15000 ± 1200	1000 ± 130
	Native [b]	21000 ± 2000	1600 ± 180
	Desulfated	–	1600 ± 260
	Carboxyl-reduced	21500 ± 2200	–
Standard chondroitin 6-sulfate	Native [c]	5000 ± 700	–
	Native [d]	5100 ± 1000	1100 ± 300
	Carboxyl-reduced	3600 ± 400	–
Sulfated fucan	Native	3200 ± 300	–
Hyaluronic acid	Native	–	1400 ± 100

[a] Least square fitting with two binding sites and a weight factor sufficient to neglect the leftmost point (Fig. 3A).
[b] Same fitting as in [a] without weight factor.
[c] Least square fitting with one binding site (Fig. 3B).
[d] Least square fitting with two binding sites (Fig. 3B).

4. Conclusions

Calcium binding and charge distribution of a fucosylated chondroitin sulfate and of a standard chondroitin 6-sulfate have been studied using a metallochromic indicator and conductimetric titrations. Measurements on chemically modified polymers show that a high-affinity class of sites can be assigned to sulfate groups, and low affinity sites to carboxyl radicals. The sulfate groups of the fucosylated chondroitin sulfate have a ~ 5-fold higher affinity for calcium than the standard chondroitin 6-sulfate. Possibly, this increased affinity for calcium may be due to the branches on the fucosylated chondroitin sulfate.

Data from the conductimetric titrations were interpreted using a polyelectrolyte theory proposed by Manning [37,38]. This theory has been applied mostly to linear polymers, containing a single class of charged groups with a uniform distribution along the polymer. Our results demonstrate that the theory can be applied to branched polysaccharides, containing different charged groups (sulfates and carboxylates), since linear charge density, distance between charged groups and average number of charged groups per disaccharide unit determined by conductimetric titrations (Table 2) are in agreement with the data from chemical studies (Table 1, Fig. 1). In addition, data from conductimetric titrations make it possible to estimate the length of the disaccharide unit of the standard and fucosylated chondroitin sulfates. Similar values were obtained for both polysaccharides (Table 2, see text). This result indicates that the fucose residues in the chondroitin sulfate from sea cucumber are branched and not intercalated among the repetitive disaccharide units of the central core. This inference is in agreement with our previous chemical studies [9,10].

5. Acknowledgments

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