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Structural studies of a sulfated L-galactan from *Styela plicata* (Tunicate): analysis of the Smith-degraded polysaccharide.

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ABSTRACT

The tunic of the ascidian *Styela plicata* is rich in a high molecular weight sulfated L-galactan called the F-1 fraction. This polysaccharide is of complex structure and is highly branched. In this study we undertook detailed structural analysis of the F-1 fraction that was submitted to the Smith degradation procedure which, for this polysaccharide, results mainly in the elimination of the branches. By methylation, and one- and two-dimensional n.m.r. analysis of the Smith-degraded and desulfated Smith-degraded F-1 fraction, we determined the main structure of this polymer. It is composed of a core of α-L-galactopyranose units, sulfated at C-3 and glycosidically linked through position 1→4. The nonsulfated, nonreducing end units are branched at C-2 of the sulfated L-galactoses.

INTRODUCTION

The tunic of ascidians (Chordata, Tunicata) upon protease treatment releases sulfated polysaccharides composed mainly of α-L-galactopyranosyl units[1–3]. For all species studied, the major component was found to be a high molecular weight sulfated L-galactan, designated the F-1 fraction. In previous studies we demonstrated for the F-1 fraction of *Styela plicata*, using periodate oxidation, methylation, and n.m.r. spectral analysis, that most of the sulfate esters are O-3 linked, that the main glycosidic linkage present is 1→4, and that there is extensive branching in the molecule[3]. Minor amounts of D-glucopyranosyl residues are also found in this polysaccharide[2].

The branching point of this molecule, as well as the distribution of the various units, were not clear from these studies. Therefore, we undertook more detailed studies using a simplified molecule that was obtained by Smith degradation of the F-1 fraction.

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EXPERIMENTAL

Extraction of the sulfated polysaccharides from the tunic of Styela plicata. — The polysaccharides were extracted from the tunic by papain digestion and ethanol precipitation as previously described[4].

Purification of fraction F-1 from Styela plicata. — Fraction F-1 was purified by ion-exchange chromatography on a DEAE-cellulose column and by gel-filtration chromatography on Sepharose CL-4B as previously described[4].

(a) DEAE-cellulose column. About 200 mg of the polysaccharides extracted from the tunic were applied to a DEAE-cellulose column (7 x 2 cm), equilibrated with 0.1M sodium acetate buffer (pH 5.0) and washed with 100 mL of the same buffer. The column was eluted by a linear gradient, prepared by mixing 100 mL of 0.1M sodium acetate buffer (pH 5.0) with 100 mL of 2.0M NaCl in the same buffer. The fractions were analyzed for total hexose by the reaction described by DuBois *et al.*[5] and by absorbance of u.v. light (280 nm). The fractions containing the sulfated polysaccharides, as indicated by the DuBois-positive test, were pooled, dialyzed against distilled water, and lyophilized.

(b) Sepharose CL-4B. About 40 mg of the sulfated polysaccharides were applied to a Sepharose CL-4B column (115 × 1.5 cm) and eluted with 0.5M pyridine–acetate buffer (pH 6.0) at a flow rate of 6 $mL.h^{-1}$. Fractions were assayed by the reaction of DuBois *et al.* and by their metachromatic properties[4].

Chemical analysis. — Total hexose was measured by the method of DuBois *et al.*[5] After acid hydrolysis (4.0M trifluoroacetic acid for 6 h at 100°), total hexosamine was measured by a modified Elson–Morgan reaction[6] and sulfate by the $BaCl_2$–gelatin method[7]. The percentages of the various hexoses and hexosamines were estimated by gas–liquid chromatography (g.l.c.) of the corresponding alditol acetates[8]. The hexuronic acid content was estimated by the carbazole reaction[9]. Optical rotations were measured with a digital polarimeter (Perkin–Elmer model 243-B).

Agarose and polyacrylamide gel electrophoresis. — Sulfated polysaccharides were analyzed by agarose gel electrophoresis, as previously described[10]. About 25 μg of sulfated glycans was applied to a 0.5% agarose gel in 0.05M 1,3-diaminopropane–acetate buffer (pH 9.0). After electrophoresis, the glycans were fixed with cetyltrimethylammonium bromide in water and stained with 0.1% toluidine blue in 0.1:5:5 (v/v) acetic acid–ethanol–water. The molecular weights of the sulfated polysaccharides were determined by polyacrylamide gel electrophoresis[11]. About 25 μg of the sulfated glycans were applied to a 6% polyacrylamide slab gel, and, after electrophoresis, the gel was stained with 0.1% toluidine blue in 1% aq. acetic acid. After staining, the gel was washed for ~8 h in 1% aq. acetic acid. The molecular weight markers used were dextran sulfate, average M_r = 500 000 (St_1); chondroitin 6-sulfate from shark cartilage, average M_r = 40 000 (St_2) and dextran sulfate, average M_r = 8000 (St_3).

Periodate oxidation and Smith degradation. — The F-1 fraction (175 mg) was dissolved in 20 mL of 0.1M $NaIO_4$ and kept in the dark at room temperature. After 5 days, excess periodate was destroyed by the addition, in the dark, of a few drops of

ethylene glycol. The solution was then dialyzed against distilled water for 24 h and then lyophilized. The oxidized polymer was reduced with 0.1M $NaBH_4$ in 20 mL of 0.1M NaOH for 12 h at room temperature. Excess $NaBH_4$ was destroyed by careful, dropwise addition of glacial acetic acid (along with a few drops of butanol to prevent foaming) to the solution maintained in an ice bath. The resulting solution was dialyzed against distilled water for 24 h and lyophilized. The oxidized and reduced F-1 fraction (129 mg) was treated with 0.1M H_2SO_4 for 30 h at room temperature. The solution was cooled in an ice bath and neutralized with 0.1M NaOH. The Smith-degraded polysaccharide was precipitated by the addition of 3 vol. of abs. ethanol. After 12 h at $-10°$, the precipitate was collected by centrifugation, vacuum dried, dissolved in distilled water, dialyzed against distilled water for 24 h, and recovered by lyophilization (72 mg).

Formation of polyalcohols from the polysaccharides. — To analyze the formation of polyalcohols, the polysaccharides were hydrolysed with 3.0M trifluoroacetic acid for 4 h at 100°, reduced with $NaBH_4$, and the resulting alditols were acetylated. The products were analysed by g.l.c., using an AN-600 capillary column (30 m × 0.30 mm) from Thames Chromatography (Maidenhead, UK). The column was programmed to run at 140° for 10 min, then raised to 170° at $2°.min^{-1}$, and held at 170°. The carrier gas was hydrogen with a linear velocity of 20 $cm.s^{-1}$.

Desulfation. — Desulfation of the Smith-degraded F-1 fraction was performed as previously described[4]. The Smith-degraded F-1 (68 mg) in 10 mL of water was mixed with 1 g dry weight of Dowex-50W [H^+] (200–400 mesh) resin. After neutralization with pyridine, the solution was lyophilized. The resulting pyridinium salt was dissolved in 10 mL of 9:1 (v/v) methyl sulfoxide–methanol, maintained for 4 h at 80°, dialyzed against distilled water, and lyophilized to give 35 mg of the desulfated polymer.

Methylation. — The intact or chemically modified polysaccharides (10 mg) were methylated either by the Hakomori method[12], with the modification introduced by Conrad[13], or by the recently developed method that utilizes solid NaOH–MeI in Me_2SO[14–16]. Briefly, 4 mg of the polysaccharide was dissolved in 1.28 mL of Me_2SO (spectroscopic grade, Merck, A.G.), and to this was added 102 mg of powdered NaOH and 90 μl of MeI (Merck). The mixture was stirred for 60 min at room temperature, at the end of which time 3.2 mL of 1.0M acetic acid and 1.28 mL of distilled water were added to the mixture. The solution was then dialyzed against distilled water and lyophilized. This procedure was repeated three times. From 10 mg of the original polysaccharide was obtained 9.5 mg of its methylated derivative.

The methylated polysaccharides were hydrolyzed with 4.0M trifluoroacetic acid for 6h at 100°, reduced with $NaBH_4$, and the resulting alditols were acetylated. The alditol acetates from the methylated sugars were analyzed by g.l.c. as described above, and by gas-liquid chromatography–mass spectrometry (g.l.c.–m.s.) as previously described[2,3].

N.m.r. spectra. — Samples of SD F-1 and desulfated SD F-1 (about 20 mg) were exchanged with D_2O by repeated lyophilization and dissolved in 0.6 mL of 99.9% D_2O for n.m.r. spectroscopic analysis.

^{1}H-n.m.r. spectra at 500 MHz and ^{13}C-n.m.r. spectra at 125 MHz were recorded at

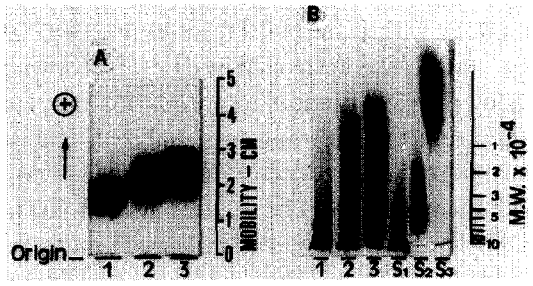


Fig. 1. Electrophoresis of the F-1 fraction from *S. plicata* before and after chemical modifications. The intact (1), periodate oxidized/borohydride reduced (2) and Smith-degraded (3) F-1 fraction were submitted to electrophoresis on agarose (A) and on polyacrylamide (B) gel (see Experimental section).

50° using a JEOL GSX-500 spectrometer. The COSY spectrum of SD F-1 was obtained using the standard pulse sequence supplied with the spectrometer's software. Chemical shifts in both ^{1}H- and ^{13}C-n.m.r. spectra are quoted in p.p.m. downfield (δ-scale) relative to internal 3-(trimethylsilyl)-1-propanesulfonic acid, sodium salt at $\delta = 0$ p.p.m.

RESULTS AND DISCUSSION

Chemical and electrophoretic analysis of intact and Smith-degraded F-1 fraction.— Figure 1 shows the electrophoretic migration of intact and chemically modified F-1 fraction from *Styela plicata* in two different gel systems. The electrophoresis on agarose gel (Fig. 1A) shows slight differences of the periodate-oxidized and Smith-degraded F-1 fraction (SD F-1) compared to the intact polymer. However, these chemical reactions did not produce extensive degradation of the molecule, as the modified polymers would otherwise have higher electrophoretic mobility on this gel. This conclusion is reinforced by examining the electrophoresis on polyacrylamide gel shown in Fig. 1B. Both the periodate-oxidized and SD F-1 migrate as a polydisperse molecule, and there is indeed considerable molecular weight reduction of the modified molecule as compared to the intact one. However, fragmentation into small oligosaccharides is not seen after Smith degradation, as would be the case if there were many intrachain, periodate-sensitive sites.

Conditions for the Smith degradation were explored by trying different acid concentrations and hydrolysis temperatures. Those found to cause the least molecular weight reduction and maximal polyalcohol removal were then chosen. The major polyalcohol obtained from fraction F-1 is glycerol, together with small amounts of threitol and erythritol[3,4].

The chemical analysis of the intact and SD F-1 is shown in Table I. Smith degradation of the F-1 fraction produces an increase in the molar ratios of L-galactose and sulfate ester, while the values for D-glucose decrease and amino sugars totally disappear (Table I). In addition, the optical rotation of SD F-1 is more negative than the intact polymer. In fact, its specific optical rotation of $-160°$ approaches more closely

TABLE I

Chemical composition and specific optical rotation of fraction F-1 from *Styela plicata*, before and after Smith degradation

F-1 treatment	*% of dry weight*[a]			*Molar ratios*[b]				$[\alpha]_D$ 20°
	Hex	*HexNH*	*Sulfate*	L-*Gal*[c]	D-*Glc*[d]	*HexNH*[e]	*Sulfate/total sugar*[f]	
Intact	61.5	3.2	25.5	0.84	0.10	0.06	0.50	−132°
Smith degradation	55.8	<0.1	35.1	0.97	0.03	<0.01	0.80	−160°

[a] Total hexose was estimated by the method of DuBois *et al.*[5], total hexosamine by a modified Elson–Morgan reaction[6], and sulfate by the $BaCl_2$/gelatin method[7]. [b] The relative proportions of galactose and glucose were determined by g.l.c. [c] Galactose occurs entirely in the L-enantiomeric form[2,17]. [d] Glucose occurs as the D-isomer[2]. [e] Approximately 80% of the hexosamine is galactosamine, as estimated by g.l.c. [f] Total sugar refers to the sum of hexoses and hexosamines, as determined by the DuBois et al.[5] and Elson–Morgan[6] reactions, respectively.

that of −179° reported for methyl α-L-galactopyranoside[17]. Besides those sugars listed in Table I, no other sugars were detected in the intact and SD F-1, the detection threshold of the methods used being ~0.02 mg per mg of polysaccharide.

Methylation studies. — Fraction F-1 before and after various chemical treatments was methylated by the Hakomori method or by the NaOH–MeI–Me_2SO method (Table II). The properties of the polysaccharide after three rounds of methylation indicate that the reaction was almost quantitative and did not remove sulfate groups. This finding is supported by the fact that the products were chloroform insoluble, and that the comparison of the infrared spectra of the original and methylated polysaccharide showed that the OH stretch band was nearly absent, with concomitant increase in intensity of the CH stretch bands at 3000–2800 cm^{-1}, with no apparent loss in the band at 1240 cm^{-1}.

Table II shows the molar ratios of the methylated sugars obtained from intact and SD F-1 before and after desulfation*. Clearly, a less complex polysaccharide results from Smith degradation of the F-1 fraction. The decrease of 2,3,4,6-tetra-*O*-methyl-L-galactose and enrichment of the 2,6-di-*O*-methyl-L-galactose, which after desulfation becomes 2,3,6-tri-*O*-methyl-L-galactose, confirms our initial proposition that, for the most part, a linear polysaccharide composed of L-galactose units linked 1→4 and sulfated at position-3 results from this chemical degradation. This conclusion is even more clear if we look at the methylation data for SD F-1 that was submitted to a second cycle of Smith degradation. In this molecule, the 2,6-di-*O*-methyl-L-galactose accounts for up to 78% of the total derivatives.

* Different times of desulfation were tested in order to minimize molecular weight reduction or loss of material. In our example, 4 h at 80° was found to work best. The loss of sulfate was monitored by the disappearance of the S=O band at 1240 cm^{-1} in the infrared spectra. In addition, the desulfation was estimated by the molar ratio sulfate: total sugar, which decreased from 0.80 in the SD F-1 to 0.07 in the desulfated SD F-1.

TABLE II

Methylation analysis of F-1 before and after various chemical treatments

O-Methylated sugars[a] *(as alditol acetates)*	*Molar ratios*[b]				
	Intact F-1		*Smith-degraded F-1 (one cycle)*		*Smith-degraded F-1 (two cycles)*
	Sulfated	*Desulfated*	*Sulfated*	*Desulfated*	*Sulfated*
2,3,4,6-L-Gal	0.12[c] (0.19)[d]	0.35[d]	0.12[c]	0.09[c]	0.05[c]
2,4,6-L-Gal	0.17 (0.09)	0.14	0.03	0.16	0.05
2,3,6-L-Gal	0.17 (0.08)	0.34	0.05	0.62	n.d.
2,6-L-Gal	0.38 (0.40)	0.03	0.55	0.04	0.78
3,6-L-Gal	n.d. (n.d.)	0.02	n.d.	0.09	n.d.
6-L-Gal	0.16 (0.06)	0.02	0.17	n.d.	0.12
2,3,4,6-D-Glc	n.d. (0.05)	0.05	n.d.	n.d.	n.d.
2,3,6-D-Glc	n.d. (0.13)	0.05	0.08	n.d.	n.d.

[a] The identity of each peak was established by mass spectrometry. [b] The molar ratios are based in the area of each peak compared with total area; n.d. = not detected. [c] Methylated by the NaOH–MeI–Me_2SO method. [d] Methylated by the Hakomori method. Values were taken from ref. 2 and 3.

Another interesting result which comes about is related to the unit in the central chain to which the branches are connected. We consistently obtain a 6-*O*-methyl derivative in equimolecular proportion with 2,3,4,6-tetra-*O*-methyl derivatives from intact and Smith-degraded F-1. This clearly indicates that the branches are attached to C-2 of the 3-sulfated and 1→4 linked units of the central core. This is more apparent when we look at the data for desulfated SD F-1, in which the 6-*O*-methyl derivative has been replaced by 3,6-di-*O*-methyl derivative. Possibly the branches do not occur at the non-sulfated and 1→4 linked units because the 3,6-di-*O*-methyl derivative is absent in both the intact F-1 and the sulfated SD F-1.

In previous studies smaller amounts of 6-*O*-methyl-L-galactose and 3,6-di-*O*-methyl-L-galactose were obtained by the standard Hakomori procedure from intact and desulfated F-1 fractions[2,3]. This underestimation of the branching units could be attributable to experimental protocol. In fact, larger amounts of the 6-*O*-methyl derivative were obtained by the solid NaOH–MeI–Me_2SO method when compared to the amounts produced by the Hakomori procedure (Table II).

Finally, the presence of small but reproducible amounts of a 2,4,6-tri-*O*-methyl derivative in the methylation of intact and SD F-1 could be ascribed to either non-sulfated 1→3 linked units, or to 3-sulfated L-galactose residues at the nonreducing end. However, the 2,4,6-tri-*O*-methyl derivative is not enriched after Smith degradation (Table II), as would be expected since both types of units are resistant to periodate oxidation. Therefore, our results do not allow any conclusions to be made concerning the origin of this 2,4,6-tri-*O*-methyl derivative. In addition, minor degradation of fraction F-1, besides those predicted by Smith degradation, cannot be excluded, especially due to the more drastic conditions that must be used for the periodate oxidation of sulfated sugars[18].

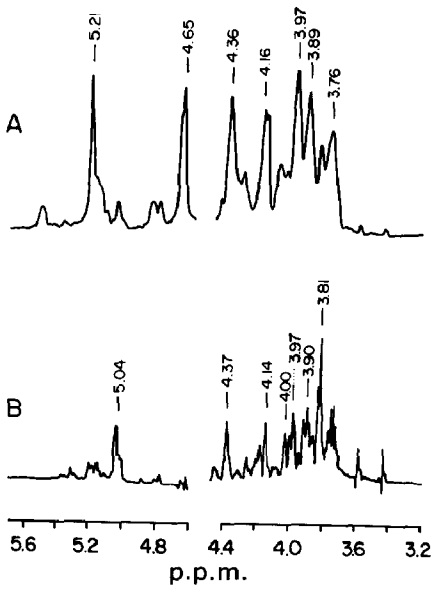
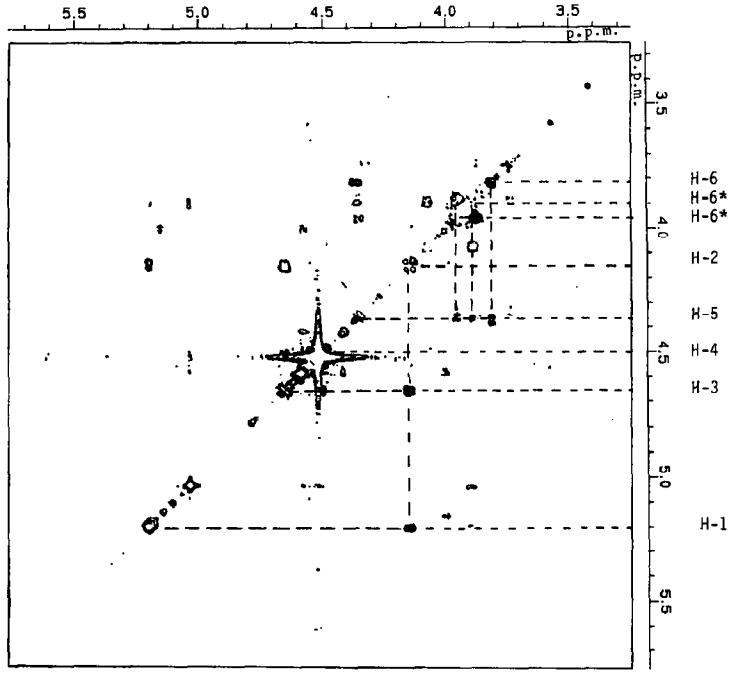


Fig. 2. Partial ^{1}H-n.m.r. spectrum (500 MHz, 50°, D_2O) of Smith-degraded F-1 before (A) and after (B) desulfation.



Assignments

Proton	δ (p.p.m.)
H-1	5.21
H-2	4.16
H-3	4.65
H-4	4.51
H-5	4.36
H-6,6'	3.79
H-6*	3.97
H-6'*	3.89

Fig. 3. ^{1}H–^{1}H COSY spectrum (500 MHz, 50°, D_2O) of Smith-degraded F-1.

^{1}H- and ^{13}C-n.m.r. spectroscopic studies. — SD F-1 before and after desulfation were also analysed by ^{1}H- and ^{13}C-n.m.r. spectroscopy. The ^{1}H-n.m.r. spectrum at 500 MHz for SD F-1 (Fig. 2A) shows a doublet at δ 5.21 (J 4.4 Hz) which is attributable to the anomeric proton of an α-L-galactopyranoside[19]. A peak at δ 4.65 disappears after desulfation (Fig. 2B), while a new peak appears at δ 3.81, which is close to the chemical shift reported in the literature for the non-substituted H-3 proton of α-D-galactopyranoside[19]. From the methylation data, it is therefore possible to assign this peak to H-3.

To confirm these assignments a ^{1}H–^{1}H COSY spectrum at 500 MHz of SD F-1 was run (Fig. 3), from which it is possible to assign the signals at δ 4.16 and at δ 4.51 to H-2 and H-4, respectively, and to confirm the assignments of the signal at δ 4.65 to H-3 by tracing connectivities from the H-1 signal at δ 5.12. No cross-peak appears for H-4–H-5 as the $^3J_{H,H}$ value for this coupling is small in D-galactopyranoside systems. The H-5 resonance is seen at δ 4.36, close to the chemical shift of the galactopyranoside H-5 reported[19], with cross-peaks to H-6 at δ 3.97 and 3.89. A signal at δ 3.79, also showing a cross-peak to H-5, is probably that of the coincident H-6s of desulfated F-1.

The ^{13}C-n.m.r. spectrum of the intact F-1 fraction (inset in Fig. 4A) shows an intense signal attributed to nonsubstituted C-6, which resonates at δ 62.4–60.3. This result agrees with the methylation studies (Table II) in showing the absence of substitution at C-6. However, the complexity of the ^{13}C spectrum obtained for intact F-1 fraction does not allow the identification of the glycosidically substituted or sulfated carbons. In contrast, SD F-1 affords a well resolved ^{13}C spectrum (Fig. 4A). Because of its distinctive chemical shift at δ 101.2, the signal is readily attributable to the α-anomeric carbon. The signal at δ 60.3 is typical of a nonsubstituted C-6, as suggested by the methylation studies. C-2 and C-5, which are neither sulfated nor substituted by glycosidic linkages (Table II), resonate in the region of nonsubstituted carbons. Two other signals ascribed to substituted secondary carbons resonate at δ 76.9 and 77.4. From the methylation data (Table II), it is possible to attribute them to C-3 and C-4, respectively. After desulfation (Fig. 4B), C-3 moves upfield by 6.9 p.p.m., while C-2 and C-4 move downfield by 1.9 and 2.3 p.p.m., respectively, as expected for a polysaccharide that was sulfated at C-3 (Table III)[19,20].

Table III compares the tentatively assigned ^{13}C-resonances of SD F-1, before and after desulfation, with those of known standards. The chemical shifts for SD F-1 are similar to those of standard methyl 3-*O*-sulfo-α-D-galactopyranoside, confirming that this unit is the main structural feature found in this polysaccharide. The chemical shifts obtained after desulfation strikingly resemble those of a methyl α-galactopyranoside glycosidically substituted at C-4 by another galactopyranoside unit[21]. This disaccharide is the main repeating structure found in SD F-1, as revealed by methylation analysis (Table II).

CONCLUSIONS

Figure 5 summarizes the main L-galactose units found in fraction F-1 from *S. plicata* and the reactions employed to determine these structures. The methylation

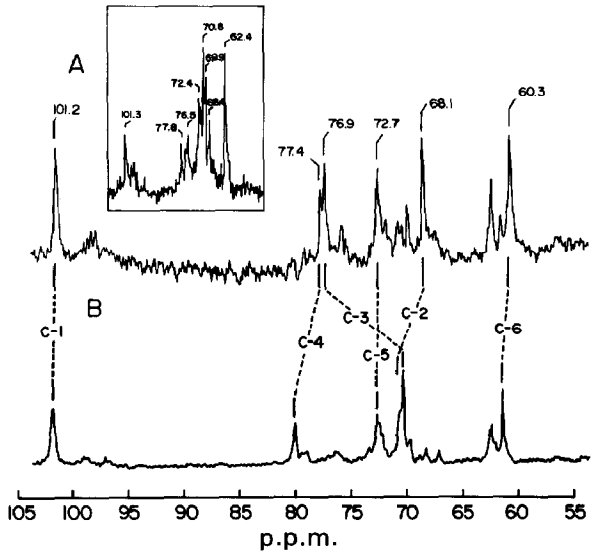


Fig. 4. ^{13}C-N.m.r. spectra (125 MHz, 50°, D_2O) of Smith-degraded F-1 before (A) and after (B) desulfation. The inset in A shows the ^{13}C-n.m.r. spectrum of the intact F-1 fraction.

TABLE III

^{13}C-N.m.r. chemical shifts (δ) for Smith-degraded F-1 (before and after desulfation) and for reference compounds

Compounds	*^{13}C-Chemical shifts (δ)*					
	C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*
Smith-degraded F-1	101.2	68.1	76.9	77.4	72.7	60.3
Smith-degraded and desulfated F-1	101.5	70.0	70.0	79.7	72.2	61.1
α-D-Gal*p*-α (1→4)-D-Gal*p*-Met[a]	100.4	69.3, 69.5	71.9	79.8	70.0, 70.1, 69.9	61.5
Methyl α-D-Gal*p*-3-SO_4[b]	100.4	67.4	79.0	68.8	71.6	62.2

[a] Data from ref. 21. [b] Data from ref. 19.

studies of the ascidian polysaccharides indicate that fraction F-1 is constituted mainly of a carbohydrate core of L-galactose linked glycosidically through position 1→4 and sulfated at the 3-position (**1a**, Fig. 5). Therefore, 2,3,6-tri-*O*-methyl L-galactose (**8**) is the major methyl ether derivative obtained from desulfated F-1 (**5**), whereas 2,6-di-*O*-methyl-L-galactose (**2**) is the predominant methyl ether derivative obtained from sulfated F-1 (**1**) (see data from Table II and ref. 2 and 3). In addition, we obtained 6-*O*-methyl-L-galactose in equimolar proportion with the 2,3,4,6-tetra-*O*-methyl derivative (**2**) through methylation of intact F-1 by the solid NaOH–MeI–Me_2SO method (Table II). This finding indicates that some of the 3-*O*-sulfated and 1→4 linked units of

the central polysaccharide core are substituted at O-2 by nonsulfated L-galactopyranose units (**1b**). Finally, the formation of 2,3,6-tri-*O*-methyl-L-galactose (**2**) from intact F-1 indicates that some of the 1→4 linked units are not sulfated (**1c**).

Smith degradation removes the nonsulfated L-galactopyranose units at nonreducing end (**1b**) and the nonsulfated 1→4 linked residues (**1c**), increasing the proportion of the 3-*O*-sulfated and 1→4 linked α-L-galactopyranoside units (**3**). This conclusion is supported by chemical analysis (Table I), methylation data (Table II), and ^{13}C-n.m.r. studies (Fig. 4A). The ^{13}C-n.m.r. spectrum of SD F-1 (Fig. 4A) contains two

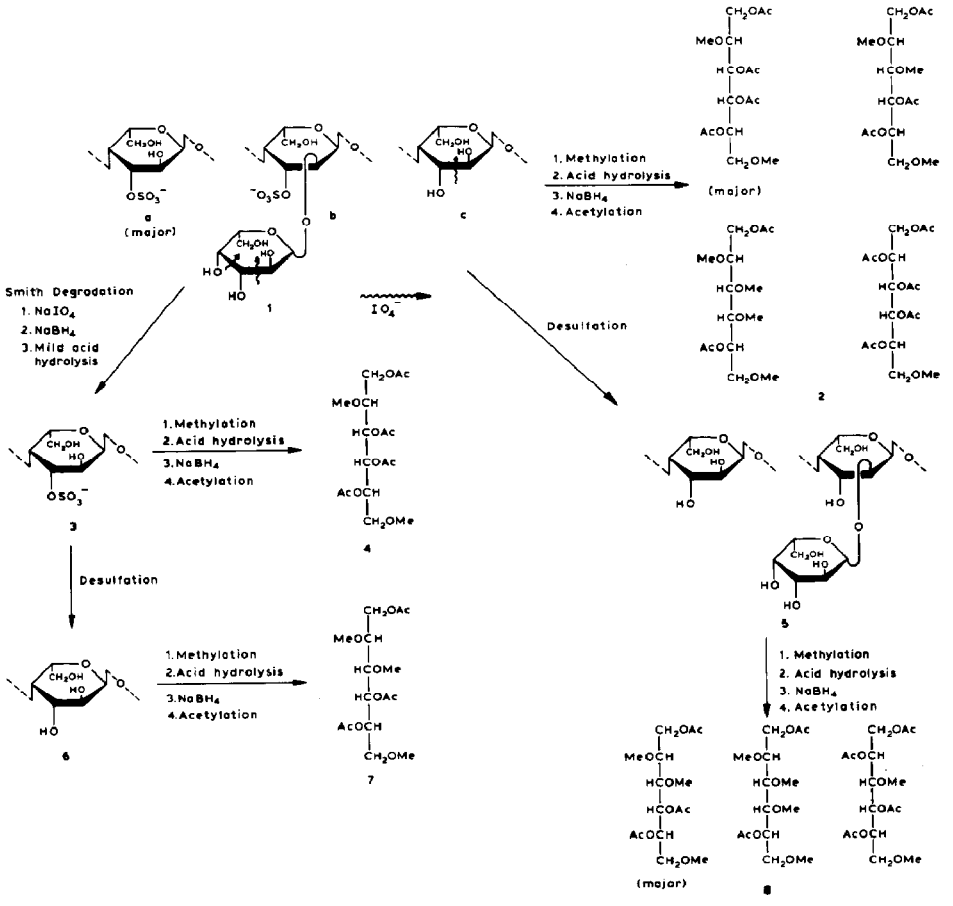


Fig. 5. Hypothetical structures for the main L-galactose units in the F-1 fraction from *S. plicata* and the reactions employed to determine these structures. Fraction F-1 from *S. plicata* contains large amounts of α-L-galactopyranose residues, sulfated at the 3-position and glycosidically linked through the 1→4 position (**1a**). Some of these units contain nonsulfated L-galactopyranose units substituted at the O-2 position (**1b**). In addition, nonsulfated α-L-galactopyranose units linked 1→4 also occur in the polymer (**1c**). Smith degradation, which removes the periodate-sensitive units of this polysaccharide, increases the proportion of the 3-sulfated α-L-galactopyranosyl units in the polymer (**3**). Desulfation of the SD F-1 produces a polysaccharide composed of α-L-galactopyranose glycosidically 1→4 linked (**6**). See Conclusions section for details.

carbon atoms which resonate in the region of substituted secondary carbons, as expected for a linear polysaccharide whose units are sulfated at O-3. Desulfation of SD F-1 produces a polysaccharide rich in nonsulfated α-L-galactopyranose units linked glycosidically through the 1→4 position (**6**) (Table II). The ^{13}C-n.m.r. spectrum of this desulfated polysaccharide shows a downfield shift of about 6.9 p.p.m. for the signal of the carbon atom bearing the sulfate ester and smaller upfield shifts for adjacent carbon atoms (Fig. 4, Table III), as already observed in the literature for other sulfated sugars[19,20].

A marked structural variation was observed among F-1 fractions from different species of ascidians[3]. F-1 from the ascidian *Clavelina* sp. is distinguished by its low content of nonreducing end units, while the F-1 fraction from other species are highly branched polymers. Interestingly, the F-1 fraction from *S. plicata* after Smith degradation, which removes mainly the nonreducing end residues, strongly resembles the intact F-1 fraction from *Clavelina* sp.[3] Overall, our results suggest that the sulfated L-galactan from different species of ascidians may have a similar glycan core, differing mainly in the proportion of nonsulfated nonreducing end units in the polymer.

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REFERENCES

1 R. M. Albano and P. A. S. Mourão, *Biochim. Biophys. Acta*, 760 (1983) 192–196.
2 P. A. S. Mourão and A. S. Perlin, *Eur. J. Biochem.*, 166 (1987) 431–436.
3 M. S. G. Pavão, R. M. Albano, A. M. Lawson, and P. A. S. Mourão, *J. Biol. Chem.*, 264 (1989) 9972–9978.
4 R. M. Albano and P. A. S. Mourão, *J. Biol. Chem.*, 261 (1986) 758–765.
5 M. DuBois, K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith, *Anal. Chem.*, 28 (1956) 350–354.
6 C. J. Rondle and W. T. J. Morgan, *Biochem. J.*, 61 (1955) 586–589.
7 H. Saito, T. Yamagata, and S. Suzuki, *J. Biol. Chem.*, 243 (1968) 1542–1563.
8 H. W. Kircher, *Anal. Chem.*, 32 (1960) 1103–1106.
9 Z. Dische, *J. Biol. Chem.*, 167 (1947) 189–198.
10 C. P. Dietrich and S. M. S. Dietrich, *Anal. Biochem.*, 70 (1976) 645–647.
11 J. C. Hilborn and P. A. Anastassiadis, *Anal. Biochem.*, 31 (1969) 51–55.
12 S. Hakomori, *Biochem. J. (Tokyo)*, 55 (1964) 205–208.
13 H. E. Conrad, *Methods Carbohydr. Chem.*, 6 (1972) 361–364.
14 J. Cincanu and F. Kerek, *Carbohydr. Res.*, 131 (1984) 209–217.
15 A. Gunnarsson, *Glycoconj. J.* 4 (1987) 239–245.
16 A. Dell, M. E. Rogers, J. E. Thomas-Oates, T. N. Huckebery, P. N. Sanderson, and I. A. Nieduszynski, *Carbohydr. Res.*, 179 (1988) 7–19.

17 M. S. G. Pavão, R. M. Albano, and P. A. S. Mourão, *Carbohydr. Res.*, 189 (1989) 374–379.
18 H. U. Choi and K. Meyer, *Carbohydr. Res.*, 40 (1975) 77–88.
19 R. R. Contreras, J. P. Kamerling, J. Breg, and J. F. G. Vliegenthart, *Carbohydr. Res.*, 179 (1988) 411–418.
20 P. J. Archbald, M. D. Fenn, and A. B. Roy, *Carbohydr. Res.*, 93 (1981) 177–190.
21 J. H. Bradbury and G. A. Jenkins, *Carbohydr. Res.*, 126 (1984) 125–156.