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Structure of the sulfated α-L-fucan from the egg jelly coat of the sea urchin *Strongylocentrotus franciscanus*: patterns of preferential 2-*O*- and 4-*O*-sulfation determine sperm cell recognition

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The egg jelly coats of sea urchins contains sulfated polysaccharides responsible for inducing the sperm acrosome reaction which is an obligatory event for sperm binding to, and fusion with, the egg. Here, we extend our study to the sea urchin *Strongylocentrotus franciscanus*. The egg jelly of this species contains a homofucan composed of 2-*O*-sulfated, 3-linked units which is the simplest structure ever reported for a sulfated fucan. This polysaccharide was compared with other sulfated α-L-fucans as inducers of acrosome reaction in conspecific and heterospecific sperm. Although all these fucans are linear polymers composed of 3-linked α-L-fucopyranosyl units, they differ in the proportions of 2-*O*- and 4-*O*-sulfation. The reactivity of the sperm of each species is more sensitive to the egg jelly sulfated fucan found in their own species. The reactivity of the sperm does not correlate with the charge density of the fucan, but with the proportion of 2-*O*- and 4-*O*- sulfation. The pattern of sulfation may be an important feature for recognition of fucans by the sperm receptor contributing to the species-specificity of fertilization.

Key words: sea urchin/fucan/acrosome reaction/polysaccharide/sulfation

Introduction

Sea urchin eggs are surrounded by a transparent gelatinous layer that induces the exocytotic sperm acrosome reaction. The acrosome reaction is an obligatory event for sperm binding to, and fusion with, the egg. It is a signal transduction event linked to ion fluxes, membrane depolarization and internal pH changes (Vacquier, 1986a; Darszon *et al*., 1996).

A major macromolecule of egg jelly coat, the one responsible for inducing the sperm acrosome reaction, is a sulfated polysaccharide (Mulloy *et al*., 1994; Alves *et al*., 1997, 1998; Vacquier and Moy, 1997). These compounds have simple, well-defined repeating structures and each species represents a particular pattern of saccharide chain and/or sulfate substitution. For example, the sea urchin *Echinometra lucunter* contains a homopolymer of 2-*O*-sulfated, 3-linked α-L-galactan. The species *Arbacia lixula* and *Lytechinus variegatus* contain linear sulfated α-L-fucans with regular tetrasaccharide repeating units; the specific pattern of sulfation varies in the two species (Mulloy *et al*., 1994; Alves *et al*., 1997). These sulfated polysaccharides are extremely species-specific as inducers of the sperm acrosome reaction (Alves *et al*., 1997) and represent an unusually simple example of ligand-induced signal transduction leading to exocytosis (Alves *et al*., 1997; Vacquier and Moy, 1997).

More recently we reported two structurally distinct sulfated fucans in the egg jelly of the sea urchin *Strongylocentrotus purpuratus* (Alves *et al*., 1998). Approximately 90% of individual females of this species spawn eggs having only one of two possible fucans. Both purified fucans have equal potency in inducing the sperm acrosome reaction. The reason that eggs from this species possess two sulfated fucans isotypes remains unknown.

We now extend our study to another species, *Strongylocentrotus franciscanus*. The egg jelly layer of this sea urchin contains a homofucan composed of 2-*O*-sulfated, 3-linked units which is the simplest structure ever reported for a sulfated fucan. It contrasts with the very heterogeneous sulfated fucans from brown algae (Nishino *et al*., 1991; Patankar *et al*., 1993; Mulloy *et al*., 1994) and with similar compounds from other echinoderms in which the oligosaccharide repeating units differ in specific patterns of sulfation (Mulloy *et al*., 1994; Alves *et al*., 1997; Alves *et al*., 1998).

The purified sulfated α-L-fucan from *S.franciscanus* was tested as an inducer of acrosome reaction in homospecific and heterospecific sperm. Our results suggest that the 2-*O*- and 4-*O*-sulfate esters may constitute a structural feature for recognition of fucans by the receptor on the sperm surface.

Results and discussion

Purification of a sulfated α-L-fucan from the egg jelly coat of the sea urchin S.franciscanus

Sulfated polysaccharides extracted from the egg jelly coat of *S.franciscanus* were purified by anion exchange chromatography on DEAE-cellulose (Figure 1). A peak rich in sialic acid was eluted completely by 0.5 M NaCl and denominated as a "sialic acid–rich glycoconjugate" in analogy with similar compounds described in other species of sea urchin (SeGall and Lennarz, 1979). A second peak, eluted at higher salt concentration, corresponds to the sulfated fucan.

The sulfated fucan and the sialic acid-rich glycoconjugate migrate on agarose gel electrophoresis and stain with toluidine blue (Figure 2), indicating both are highly anionic charged polymers. Interestingly, the unfractionated samples have a slightly retarded electrophoretic mobility when compared with the isolated compounds. This observation suggests an interaction between the sulfated fucan and the sialic acid-glycoconjugate.

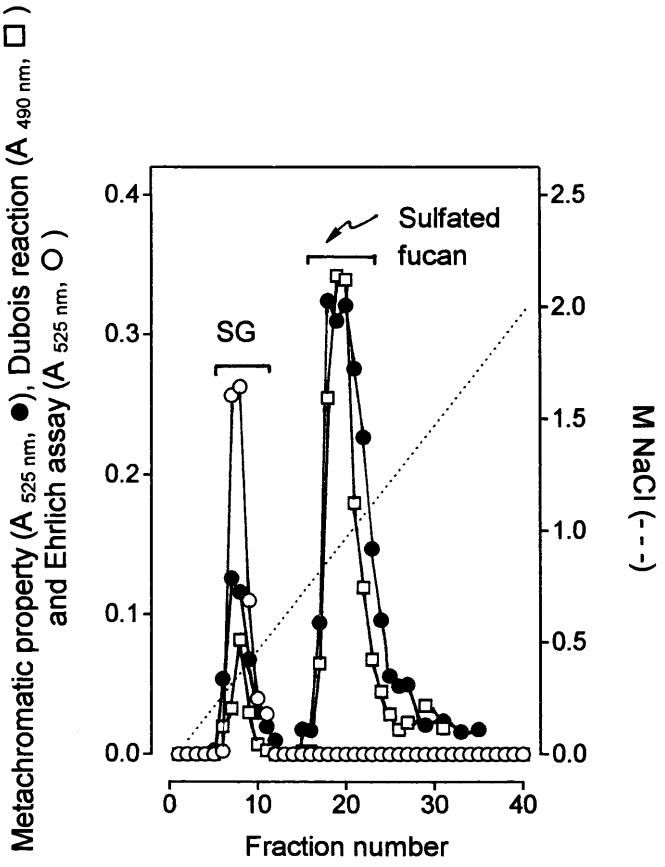


Fig. 1. Purification of the sulfated α-L-fucan from the egg jelly coat of *S.franciscanus*. The crude polysaccharides (50 mg) were purified on a DEAE-cellulose column as described under *Materials and methods*. Fractions containing the sulfated α-L-fucan, as indicated by the Dubois and methachromatic positive tests (horizontal bar) were pooled, dialyzed against distilled water, and lyophilized. SG indicates sialic acid-glycoconjugate.

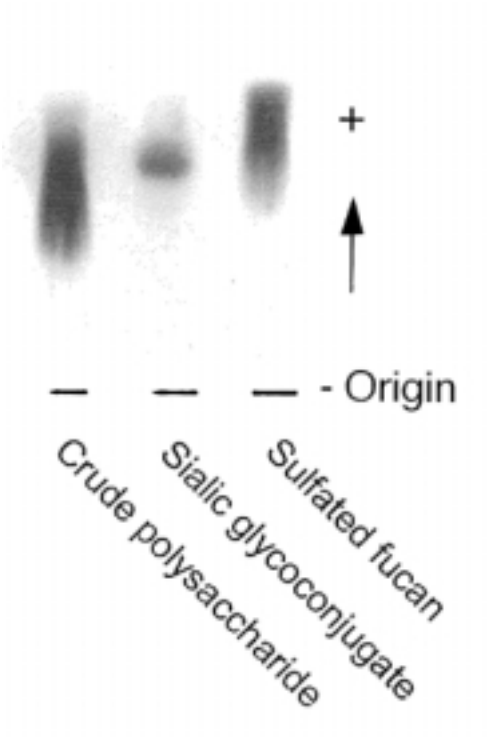


Fig. 2. Agarose gel electrophoresis of the sulfated polysaccharides purified from *S.franciscanus*. The crude polysaccharides and the purified sulfated α-L-fucan and the sialic acid-glycoconjugate (~15 μg of each) were applied to a 0.5% agarose gel, and the electrophoresis was run for 1 h at 110 V in 0.05 M 1,3-diaminopropane:acetate buffer (pH 9.0). The gel was fixed with 0.1% *N*-cetyl-*N*,*N*,*N*-trimethylammonium bromide solution. After 12 h, the gel was dried and stained with 0.1% toluidine blue in acetic acid:ethanol:water (0.1:1:5,v/v).

Table I. Chemical composition and specific optical rotation of the sulfated fucan from the egg jelly coat of *S.franciscanus*

Chemical composition (molar ratio)		$[\alpha]_D^{20\circ C}$
Fucose	Sulfate	
1.00	1.14	-57°

Chemical analysis of the purified sulfated fucan revealed fucose as the only sugar with a content of ~1.1 sulfate ester groups per residue (Table I). The strongly negative specific rotation is compatible with residues of α-L-fucopyranose.

Structure of the sulfated α-L-fucan

Methylation analysis. Three rounds of methylation of the native sulfated α-L-fucan from *S.franciscanus* yields mainly 4-O-methyfucose, whereas 2,4-di-O-methylfucose is the predominant methyl ether derivative from the desulfated α-L-fucan (Table II). These results are consistent with a linear polysaccharide composed mainly of 3-linked and 2-O-sulfated L-fucose residues. Possibly 2-O-methylfucose and non-methylated fucose obtained from the desulfated fucan are products of incomplete methylation, whereas the formation of 4-O-methylfucose indicates some 2-O-sulfated residues are still present after the solvolytic desulfation process. One round of methylation of the desulfated fucan yields almost the same proportions of methyl ether derivatives as in Table II, except for a higher proportion (45%) of 2-O-methylfucose and a lower proportion (35%) of 2,4-di-O-methylfucose. Thus, apparently position 4 is more resistant to methylation than position 2 in the case of this polysaccharide. This observation suggest that the small proportion (6%) of 4-O-methylfucose is a product of 2-O-sulfated residues that are still present after desulfation reaction rather than a result of incomplete methylation. The small proportion (5%) of unmethylated fucose obtained from the native fucan is not merely a product of an incomplete reaction since its proportion remains unchanged after an additional methylation (not shown). It may indicate small amounts of 2,4-di-*O*-sulfated residues in the native fucan.

Table II. Methylated fucose derivatives obtained from native and desulfated fucan from the egg jelly coat of *S.franciscanus*

		% of total peak area	
Alditol[a]	t_R[b] (min)	Native	Desulfated
2,4-Met$_2$-Fuc	26.9	7	64
2-Met-Fuc	29.1	<1	15
4-Met-Fuc	30.8	88	6
Fuc	32.4	5	15

[a]The identity of each peak was established by mass spectrometry.
[b]Retention time on a DB-1 capillary column.

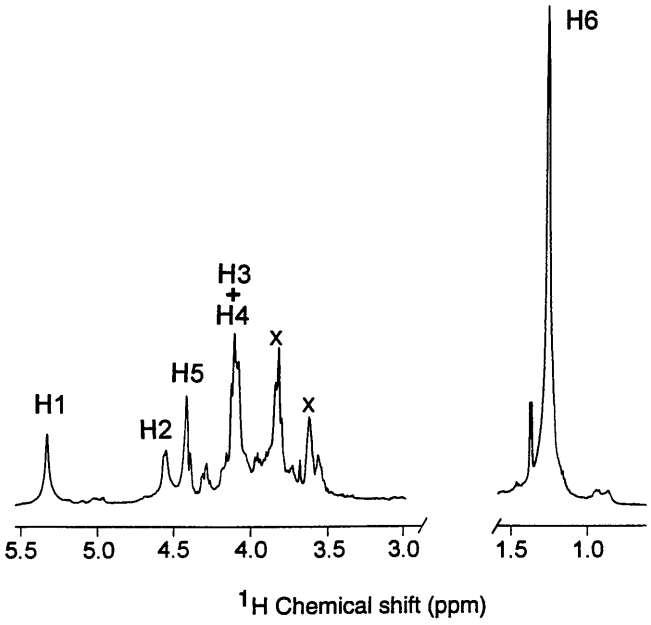


Fig. 3. Expansions of the 5.5–3.0 and 1.5–1.0 p.p.m. regions of the ^{1}H spectrum at 600 MHz of the sulfated α-L-fucan from *S.franciscanus*. The spectrum was recorded at 60°C for sample in D_2O solution. Chemical shifts are relative to internal trimethylsilylpropionic acid at 0 p.p.m.. The HOD signal was partially suppressed by presaturation. × are signals from noncarbohydrate contaminants.

NMR spectroscopic analysis. The one-dimensional ^{1}H spectrum of the sulfated fucan has a single anomeric signal at 5.33 p.p.m. and agreeing with the presence of a homopolymer of sulfated α-L-fucan (Figure 3) as is the methyl signal at 1.25 p.p.m.. It was not possible to record NMR spectra of the desulfated fucan since it has low solubility in water.

The assignment of peaks was achieved by analysis of ^{1}H COSY (Figure 4A), ^{1}H TOCSY (Figure 4B), and ^{1}H/^{13}C HMQC (Figure 5) spectra. All spectra have the simplicity compatible with the presence of a homopolymer.

All fucan protons, with the exception of H4, could be assigned in the COSY spectrum (Figure 4A). The cross peaks show unambiguously the correlation between the spin systems. The TOCSY shows the same result with strong correlation between H1 to H2, H2 to H3, and H5 to H6 (Figure 4B). The ^{1}H chemical shifts are presented in Table III.

Table III. Proton chemical shifts (p.p.m.) for residues of α-L-fucose in the sulfated α-L-fucan from *S.franciscanus* and in standard compounds

Compound	Chemical shifts[a]					
	H1	H2	H3	H4	H5	H6
Present work	5.33	**4.55**	4.10	4.07	4.42	1.25
-3-α-L-Fuc*p*-(2,4SO_3-)-1-[b]	5.40	**4.58**	4.39	**4.91**	4.37	1.25
-4-α-L-Fuc*p*-(2SO_3-)-1-[c]	5.31	**4.56**	4.23	4.01	4.52	1.35
-3-α-L-Fuc*p*-1-[b]	5.03	3.96	4.01	3.96	4.35	1.21

[a]The ^{1}H spectrum was recorded at 600 MHz in 99.9% D_2O. Chemical shifts are referenced to internal trimethylsilylpropionic acid. Values in boldface indicate positions bearing sulfate ester.
[b]Data from a sea cucumber sulfated α-L-fucan (Ribeiro *et al*., 1994).
[c]Data from a sulfated α-L-fucan from the sea urchin *Arbacia lixula* (Alves *et al*., 1997).

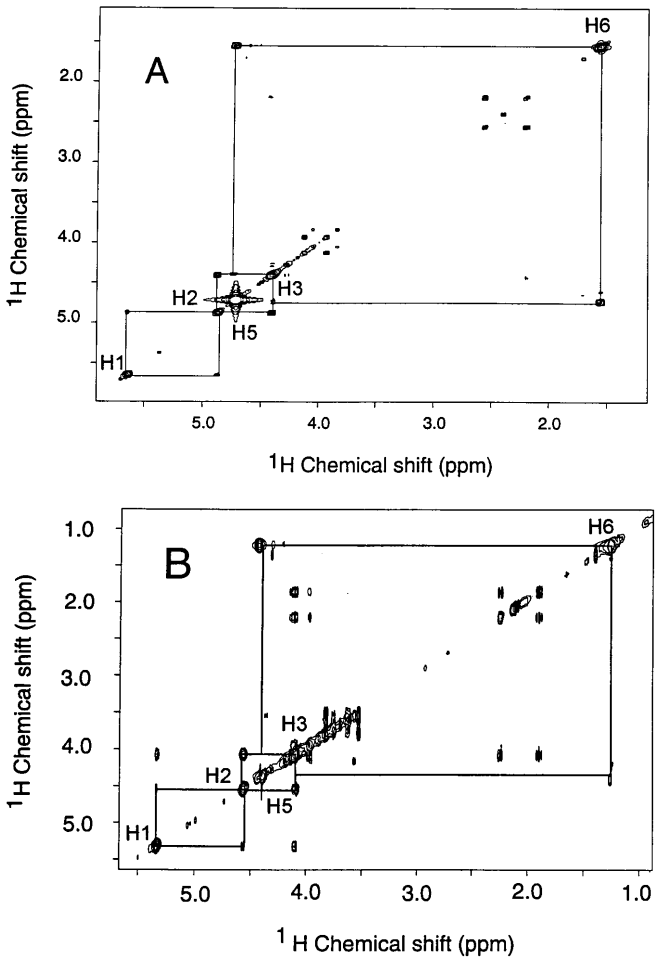


Fig. 4. COSY (**A**) and TOCSY (**B**) spectra of the sulfated α-L-fucan from *S.franciscanus*, at 600 MHz, 60°C, in D_2O. The spin system for α-L-fucopyranose is traced through both spectra.

Table IV. Carbon chemical shifts (p.p.m.) for residues of α-L-fucose in the sulfated α-L-fucan from *S.franciscanus* and in standard compounds

Compound	Chemical shifts (p.p.m.)[a]					
	C1	C2	C3	C4	C5	C6
Present work	96.77	75.52	**75.94**	71.16	68.55	17.32
-3-α-L-Fuc*p*-1-[b]	98.29	69.00	**77.60**	71.10	69.00	18.00
-4-α-L-Fuc*p*-1-[c]	103.10	nr	nr	**82.50**	nr	18.20
-α-L-Fuc*p*-*O*-Me[d]	100.50	69.00	70.60	72.90	67.50	16.50

[a]^{13}C chemical shifts relative to trimethylsilylpropionic acid. These values were obtained from the ^{1}H/^{13}C HMQC spectrum (see Figure 5). Values in boldface indicate positions involved in the glycosidic linkage.
[b]Data from Ribeiro *et al*. (1994).
[c]Data from Alves *et al*. (1997).
[d]Data from Gorin and Mazurek (1975).
nr, Not reported.

The carbon chemical shifts were obtained using the HMQC spectrum. The ^{1}H/^{13}C HMQC spectrum shows six major peaks although some contaminants are also seen. The HMQC was interpreted using the information obtained in the COSY and TOCSY spectra. The H4 overlaps with H3 in the proton dimension but they have well resolved carbon chemical shifts

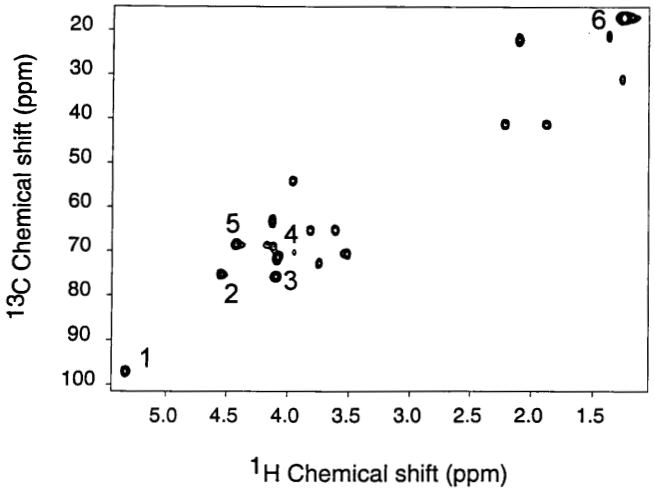


Fig. 5. ^{1}H/^{13}C HMQC spectrum of the sulfated α-L-fucan from *S.franciscanus* at 60°C, in D_2O. Starting from the proton chemical shifts, it was possible to obtain the values of carbon chemical shifts of the α-L-fucopyranosyl residues.

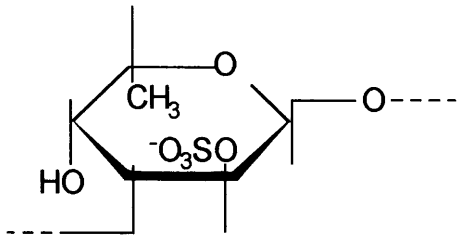


Fig. 6. Deduced structure of the sulfated α-L-fucan from the egg jelly of *S.franciscanus*. This fucan is a 1→3 linked linear polysaccharide with a regular sulfation at *O*-2 position. The structure was deduced from the methylation (Table II) and NMR spectroscopic (Figures 3–5, Tables III and IV) analysis. It is not possible to rule out a small proportions of 4-*O*- or 2,4-*O*-sulfated units, but NMR analysis show no sign of these types of residues.

(Figure 5). The other components were also assigned and their chemical shifts are presented in Table IV.

Comparing the ^{1}H and ^{13}C chemical shifts with the values in the literature show they are near to those reported for 2-*O*-sulfated and 3-linked α-fucose residues. The position of sulfation was deduced from the strong (0.6–0.7 p.p.m.) downfield shift in the position of H2 (4.55 p.p.m.). The position of the glycosidic linkage is not easily deduced from the ^{1}H chemical shifts, but from the ^{13}C chemical shifts, based on the interpretation of the HMQC spectrum (Figure 5), as the carbon 3 shows the characteristic shift of a 3-linked α-fucan (Table IV).

NMR analysis shows no sign of 4-*O*-sulfation or of 2,4-di-*O*-sulfation, although one cannot rule out a small proportion of these residues. Overall, the combination of methylation and NMR spectroscopic analysis confirm the structure of the sulfated α-L-fucan from the egg jelly of *S.franciscanus*, as shown in Figure 6.

Sulfated α-L-fucan induces the acrosome reaction

After we had isolated, purified and characterized the structure of the sulfated α-L-fucan, we tested its ability to induce the acrosome reaction in conspecific sperm. As shown in Figure 7A (open circles), the purified, homologous sulfated α-L-fucan from *S.franciscanus* induces the acrosome reaction in a dose-dependent fashion at concentrations above 0.1 μg hexose/ml. Purified, sulfated α-L-fucans from two other sea urchin species, having a similar backbone structure, were also compared using sperm of *S.franciscanus*. The sulfated α-L-fucan from *L.variegatus*, enriched in the proportion of 2-*O*-sulfated fucose units (Figure 7C), induces the acrosome reaction with approximately the same potency as the homologous fucan (Figure 7A, solid circles). In contrast, the mixture of sulfated α-L-fucans I and II from *S.purpuratus*, which contains a preponderance of 4-*O*-sulfated fucose, induces the acrosome reaction only at concentrations above 10 μg hexose/ml (Figure 7A, solid squares).

In order to evaluate further the species-specificity of sulfated fucans to induce the acrosome reaction, sperm of *S.purpuratus* were also investigated. In this sea urchin, the potency of sulfated fucans to induce the acrosome reaction decreased in the order: *S.purpuratus* > *L.variegatus* > *S.franciscanus* (Figure 7B). This is in agreement with a decrease in the proportion of 4-*O*-sulfated units in the polysaccharide chain (Figure 7C). We have previously demonstrated that purified sulfated fucans I and II are approximately equal in potency in induction of acrosome reaction in sperm of *S.purpuratus* (Alves *et al*., 1998).

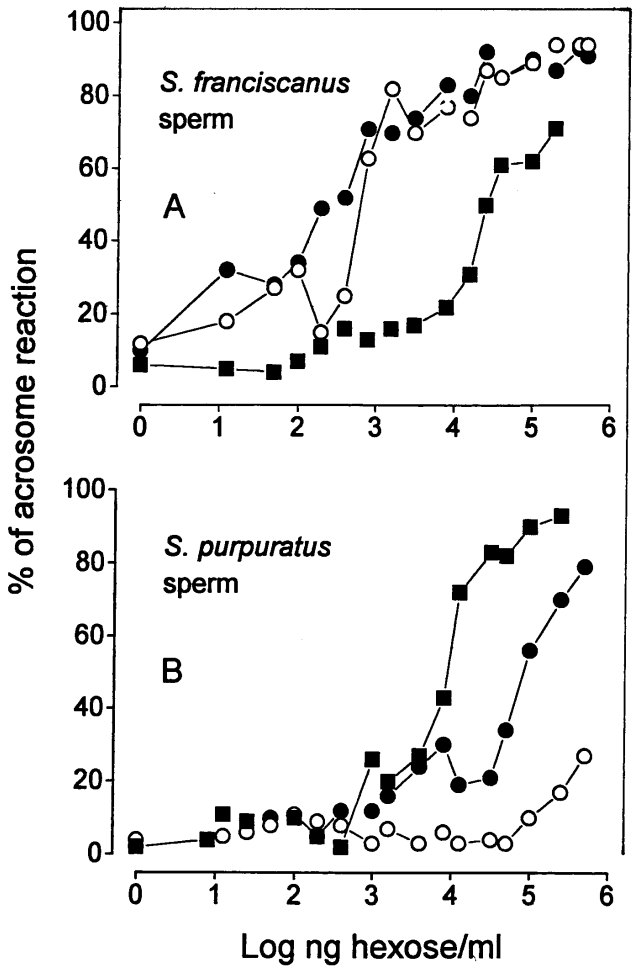
The presumable small proportion (~5%) of 2,4-di-*O*-sulfated fucose residues is unlike to be an important acrosome reaction inducer in sperms of *S.franciscanus*. This unit accounts for 25% of the total residues in the fucan from *L.variegatus* (Figure 7C), but the polysaccharide has a weak potency to induce acrosome reaction in sperms of *S.franciscanus* (Figure 7A).

Sulfated polysaccharides as species-specific inducers of the acrosome reaction in sea urchin spermatozoa

We previously demonstrated the species-specificity of sulfated polysaccharides as inducers of the acrosome reaction in sea urchin sperm (Alves *et al*., 1997). However, in this previous study, sulfated polysaccharides expressing marked interspecific structural variations were used.

In order to evaluate the finer specificity of recognition in the acrosome reaction, we have now compared egg jelly sulfated fucans containing the same backbone of 3-linked α-L-fucopyranosyl units, but with different proportions of 2-*O*- and 4-*O*-sulfation. Although we observed a less strict species-specificity in sperm recognition of sulfated polysaccharides, the potency of acrosome reaction induction clearly depended on the extent of 2-*O*- and 4-*O*-sulfation (Figure 7). Thus, sperm from *S.franciscanus* are sensitive to either homologous or heterologous sulfated fucans enriched in 2-*O*-sulfated units, similar to their own egg jelly, but not to 4-*O*-sulfated fucans (Figure 7A). Alternatively, sperm of *S.purpuratus* have a more strict specificity to the polysaccharide with the closest structural similarity found in their own (Figure 7B). Notably, the reactivity of the sperm from the two species to the various sulfated α-L-fucans has no correlation with the charge density. However, there is a correlation with the specific positions of the sulfate esters on the polysaccharide chain (Figure 7).

Studies of membrane proteins of sperm of *S.purpuratus* indicate that glycoprotein binds to the egg jelly sulfated fucan; this is sufficient to induce the sperm acrosome reaction (Moy *et al*., 1996; Vacquier and Moy, 1997). This unique membrane protein was termed “REJ” (receptor for egg jelly). REJ has the structure of a carbohydrate binding protein. If similar receptors are also found in the sperm of other species of sea urchins, we expect that the sulfated polysaccharides are the ligands for the receptor. Possibly, the structure of the polysaccharide and its sulfation pattern are important structural features for recognition of



C

		Sulfate ester per residue		
		2-*O*	4-*O*	Total
S. franciscanus:	[3-α-L-Fuc*p*- 1]n $2OSO_3^-$	1.00	0	1.00
L. variegatus:	$4OSO_3^-$ $4OSO_3^-$ [3-α-L-Fuc*p*-1→3-α-L-Fuc*p*-1→3-α-L-Fuc*p*-1→3-α-L-Fuc*p*-1]n $2OSO_3^-$ $2OSO_3^-$ $2OSO_3^-$	0.75	0.50	1.25
S. purpuratus:	$4OSO_3^-$ $4OSO_3^-$ $4OSO_3^-$ [3-α-L-Fuc*p*-1→3-α-L-Fuc*p*-1→3-α-L-Fuc*p*-1]n $2OSO_3^-$ Sulfated fucan II	0.33	1.00	1.33
	+ 4R [3-α-L-Fuc*p*-1]n R = H or OSO_3^- $2OSO_3^-$ ~36% ~64% Sulfated fucan I	1.00	~ 0.64	1.64

Fig. 7. Induction of acrosome reaction in the sperm of *S.franciscanus* (**A**) and *S.purpuratus* (**B**) by purified sulfated fucans from the egg jelly of three species of sea urchins (**C**). (**A**) and (**B**) percent of acrosome reaction is plotted against log ng hexose/ml. After lyophilization, the sulfated α-L-fucans from *S.franciscanus* (open circles), *L.variegatus* (solid circles), and *S.purpuratus* (solid squares) were dialyzed into sea water and assayed for acrosome reaction at 18°C (Vacquier, 1986b; Vacquier and Moy, 1997). Between 200–300 spermatozoa were scored per data point. (**C**) Structures of the sulfated α-L-fucans used as inducers of the acrosome reaction. The structure of the sulfated fucan from *L.variegatus* was determined in previous work (Alves *et al*., 1997). Sulfated fucans prepared from a pool of egg jelly from different females of *S.purpuratus* contains a mixture of two different polysaccharides as shown in the panel (see Alves *et al*., 1998). These two sulfated fucans are approximately equal in acrososome reaction inducing potency in sperm of *S.purpuratus*.

these molecules by the receptor in the sperm membrane. Variations in the structure of the egg jelly sulfated polysaccharide may represent one of the barriers which limit interspecific cross fertilization. In addition, our results with sperm of *S.franciscanus* strongly suggest that, even though recognition and binding to sulfated polysaccharides provide an important interspecific discrimination step in fertilization, additional receptor structures must be able to discriminate between homologous and heterologous polysaccharides with similar content of 2-*O*-sulfated units, as happens with egg jelly fucans from *S.franciscanus* and *L.variegatus*. Further studies with isolated sperm membrane receptors for sulfated α-L-fucans should provide additional insights into the mechanism of interaction of these "pattern recognition" receptors in fertilization.

Materials and methods

Sulfated fucan from sea urchin egg jelly

Extraction. Adults of *S.franciscanus* and *S.purpuratus* were collected at La Jolla, CA, and *L.variegatus* was collected at Rio de Janeiro, Brazil. Eggs were spawned into sea water by intracelomic injection of 0.5 M KCl. The crude egg jelly was isolated by the pH 5.0 method and prepared as 30,000 × *g* supernatant and stored at –20°C, or lyophilized after dialysis against distilled water (Vacquier and Moy, 1997). The acidic polysaccharides were extracted from the jelly coat by papain digestion and partially purified by ethanol precipitation, as described previously (Albano and Mourão, 1986).

Purification. The crude polysaccharides (50 mg) were applied to a DEAE-cellulose column (10 × 1 cm), equilibrated with 50 mM sodium acetate (pH 5.0), and washed in 50 ml of the same buffer. The column was then eluted by a linear gradient prepared by mixing 100 ml of 50 mM sodium acetate buffer (pH 5.0) with 100 ml of 3.0 M NaCl in the same buffer. The flow rate of the column was 10 ml/h, and fractions of 2.0 ml were collected. Fractions were checked for fucose and sialic acid by the Dubois *et al*. reaction (1956) and by the Ehrlich assay (Kabat and Mayer, 1971), respectively, and also by their metachromasia (Farndale *et al*., 1986). The NaCl concentration was estimated by conductivity. Fractions containing the sulfated α-L-fucan and the sialic acid-glycoconjugate were pooled, dialyzed against distilled water, and lyophilized.

Chemical analyses

Total fucose was measured by the method of Dische and Shettles (1948). After acid hydrolysis of the polysaccharide (5.0 trifluoroacetic acid for 5 h at 100°C), sulfate was measured by the $BaCl_2$/gelatin method (Saito *et al*., 1968). The presence of hexoses and 6-deoxyhexoses in the acid hydrolysates was estimated by paper chromatography in n-butanol:pyridine:water (3:2:1,v/v) for 48 h and by gas-liquid chromatography of derived alditols (Kircher, 1960). Optical rotation was measured using a digital polarimeter (Perkin-Elmer model 243-B).

Agarose gel electrophoresis

Sulfated polysaccharides were analyzed by agarose gel electrophoresis as described previously (Vieira *et al*., 1991; Alves *et al*., 1997). The sample (~15 μg) was applied to a 0.5% agarose gel and run for 1 h at 110 V in 0.05 M 1,3-diaminopropane:acetate buffer (pH 9.0). The sulfated polysaccharides in the gel were fixed with 0.1% *N*-cetyl-*N*,*N*,*N*-trimethylammonium bromide solution. After 12 h, the gel was dried and stained with 0.1% toluidine blue in acetic acid:ethanol:water (0.1:5:5,v/v).

Desulfation and methylation of fucans

Desulfation of the sulfated α-L-fucan from *S.franciscanus* was performed by solvolysis in dimethylsulfoxide as described previously for desulfation of other types of polysaccharides (Mourão and Perlin, 1987; Vieira *et al*., 1991). The chemical analysis of the desulfated polysaccharide revealed <0.1 sulfate ester groups per fucose residue while the native fucan contains 1.14 sulfate groups per residue (Table I). The native and desulfated polysaccharides (5 mg) were subjected to three rounds of methylation as described previously (Ciucanu *et al*., 1984), with the modifications suggested by Patankar *et al*. (1993). The methylated polysaccharides were hydrolyzed in 6 M trifluoroacetic acid for 5 h at 100°C, reduced with borohydride and the alditols were acetylated with acetic anhydride:pyridine (1:1, v/v) (Kircher, 1960). The alditols acetates of the methylated sugars were dissolved in chloroform and analyzed in a gas chromatography/mass spectrometer.

NMR experiments

^1H and ^{13}C spectra were recorded using a Bruker DRX 600 triple resonance 5 mm probe. About 5 mg of the sulfated α-L-fucan was dissolved in 0.7 ml of 99.9% D_2O (NMR grade from Cambridge Isotope Laboratories) and the spectra were recorded at 60°C, with HOD suppression by presaturation. TOCSY, COSY, and $^1H/^{13}C$ heteronuclear correlation (HMQC) spectra were recorded using states time proportional phase incrementation for quadrature detection in the indirect dimension. COSY spectrum was run with 2048 and 200 points. TOCSY spectrum was run with 4096 × 200 points with a spin-lock field of about 10 kHz and a mixing time of 80 ms, which was previously determined to give optimum results for these samples. HMQC spectrum was run with 1024 ×128 points, and globally optimized alternating phase rectangular pulses was used during acquisition for ^{13}C decoupling. All chemical shifts are relative to internal or external trimethylsilylpropionic acid and methanol.

Sulfated fucans as inducers of the sperm acrosome reaction

Sperm of *S.franciscanus* and *S.purpuratus* were collected as undiluted semen and stored for up to 6 h on ice before dilution and testing for acrosome reaction induction. Assays for acrosome reaction by sulfated polysaccharides were done as described previously (Vacquier, 1986b; Vacquier and Moy, 1997).

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