Sulfated Polysaccharides from the Egg Jelly Layer Are Species-specific Inducers of Acrosomal Reaction in Sperms of Sea Urchins*

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We have characterized the fine structure of sulfated polysaccharides from the egg jelly layer of three species of sea urchins and tested the ability of these purified polysaccharides to induce the acrosome reaction in spermatozoa. The sea urchin *Echinometra lucunter* contains a homopolymer of 2-sulfated, 3-linked α-L-galactan. The species *Arbacia lixula* and *Lytechinus variegatus* contain linear sulfated α-L-fucans with regular tetrasaccharide repeating units. Each of these sulfated polysaccharides induces the acrosome reaction in conspecific but not in heterospecific spermatozoa. These results demonstrate that species specificity of fertilization in sea urchins depends in part on the fine structure of egg jelly sulfated polysaccharide.

Successful fertilization by free-spawning organisms such as sea urchins can occur only if a series of constraints are overcome before the sperm ever makes contact with the egg (1). First, males and females must synchronize the time of release of their gametes (2). Once spawned, the sperm must find and interact with an egg of the correct species. A further event necessary for successful fertilization is induction of the acrosome reaction in the sperm (3, 4), which involves fusion of the acrosomal vesicle membrane with the plasma membrane. This results in exocytosis of the vesicle contents, which include proteases and bindin. Concomitantly, actin in the subacrosomal region of the sperm polymerizes and causes extension of the tip of the sperm. As a consequence of these two events, bindin is localized to the outside of the tip of the process where it can then interact with an egg protein (3, 4).

The acrosome reaction is induced when the sperms contact the egg jelly layer. The sea urchin egg is surrounded by a transparent gelatinous layer composed mainly of sulfated fucan (6, 7). In addition, these authors suggested that a glycoprotein or peptide, tightly associated with the sulfated fucan, was also involved in acrosome reaction induction (8–11). A further event that fully described carbohydrate structures have been shown to regulate part of the process at such a specific level.

**EXPERIMENTAL PROCEDURES**

**Sulfated Polysaccharides from the Jelly Coat of Sea Urchin**

*Extraction*—Mature species of sea urchins were collected in Guanabara Bay (Rio de Janeiro, Brazil) and gametes were isolated by intracestral injection of 0.5 M KCl (5 ml/specimen). Eggs were collected in a solution containing 450 mM NaCl, 9 mM KCl, 48 mM MgSO₄, 7H₂O, 10 mM CaCl₂, and 6 mM NaHCO₃. The egg jelly was separated by pH shock, as described previously (6). The acidic polysaccharides were extracted from the jelly coat by papain digestion and partially purified by esterlymphidium chloride and ethanol precipitation as described (12).

*Purification*—The crude polysaccharides (~100 mg) from the jelly coat of the sea urchins were applied to a DEAE-cellulose column (15 × 2 cm), equilibrated with 50 mM sodium acetate buffer (pH 5.0), and washed with 250 ml of the same buffer. The column was eluted in three different steps. Initially, the column was eluted by a linear gradient prepared by mixing 50 ml of 50 mM sodium acetate buffer (pH 5.0) with 50 ml of 1.0 M NaCl in the same buffer. Then the column was washed with 100 ml of the sodium acetate buffer containing 1.0 M NaCl. Finally, the column was eluted by a linear gradient prepared by mixing 100 ml of 1.0 M NaCl with 100 ml of 5.0 M NaCl, both in the same sodium acetate buffer. The flow rate of the column was 15 ml/h, and fractions of 3.5 ml were collected in the different elution steps. Fractions were checked for fucose (or galactose) and sialic acid by the Dubois et al. reaction (13) and by the Ehrlich assay (14), respectively, and by their metachromasia (15). The NaCl concentration was estimated by conductivity. Fractions containing the sulfated α-L-fucan or the sulfated α-L-galactan were pooled, dialyzed against distilled water, and lyophilized.

The DEAE-cellulose-purified sulfated polysaccharide (~40 mg) was applied to a Sephacryl S-400 column (90 × 1.5 cm) and eluted with 50 mM sodium acetate buffer (pH 5.0) at a flow rate of 8 ml/h. Fractions of 1.5 ml were collected and assayed for activity of the Dubois et al. reaction (13) and by the Ehrlich assay (14), respectively, and by their metachromasia (15). The column was calibrated using blue dextran and cresol red as markers of $Vₐ$ and $Vₕ$, respectively.

*Chemical Analyses*—Total galactose was measured by the method of

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Purification of the sulfated polysaccharides from the sea urchin jelly coat (A–C) and analysis of the purified polysaccharides on agarose gel electrophoresis (D). The crude polysaccharides (∼100 mg) from the jelly coat of the species *L. variegatus* (A), *E. lucunter* (B), and *A. rixula* (C) were purified on a DEAE-cellulose column as described under “Experimental Procedures.” Fractions containing the sulfated α-L-fucan or the sulfated α-L-galactan, as indicated by the Dubois and metachromatic positive tests (horizontal bars) were pooled, dialyzed against distilled water, and lyophilized. SG indicates sialic acid-glycoconjugate. D, purified sulfated α-L-fucan or sulfated α-N-galactan (∼15 mg) were 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% acetate buffer (pH 9.0). 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).

Dubois *et al.* (13) and total fucose measured by the method of Dubois and Shettles (16). After acid hydrolysis of the polysaccharides (5.0 M trifluoroacetic acid for 5 h at 100 °C), sulfate was measured by the BaCl2/gelatin method (17). The percentages of hexoses and 6-deoxyhexoses in the acid hydrolysates were estimated by paper chromatography of derived alditol acetates (18). Optical rotations were measured with a digital polarimeter (Perkin-Elmer model 243-B).

**Oxidation with α-L-Fucose Dehydrogenase**—Fucose obtained by acid hydrolysis of the sulfated fucans (5.0 M trifluoroacetic acid for 5 h at 100 °C), and authentic samples of α- or ω-L-fucose (20 µg of each) were incubated with 0.2 units of porcine liver ω-L-fucose dehydrogenase, as described (19).

**Oxidation with ω-Galactose Oxidase**—Galactose obtained by acid hydrolysis of the sulfated galactan (see above) and authentic samples of ω- or ω-L-galactose (20 µg of each) were incubated with 1 unit of *Dactylium dendroides* ω-galactose oxidase, as described (20).

**Agarose Gel Electrophoresis**—Sulfated polysaccharides were analyzed by agarose gel electrophoresis as described (12). Purified sulfated α-L-fucan or sulfated α-L-galactan (∼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 the Polysaccharides**—Desulfation of the sulfated polysaccharides was performed by solvolysis in dimethyl sulfoxide, as described previously for desulfation of other types of polysaccharides (12, 20). The native and desulfated polysaccharides (∼5 mg) were subjected to three rounds of methylation as described (21), with the modifications suggested by Patankar *et al.* (22). The methylated polysaccharides were hydrolyzed with 6 N trifluoroacetic acid for 5 h at 100 °C, reduced with borohydride, and the alditoles were acetylated with acetic anhydride:pyridine (1:1, v/v) (18). The alditoles of the methylated sugars were dissolved in chloroform and analyzed in a gas chromatography/mass spectrometry unit.

**NMR Spectroscopy**—1H spectra were recorded at 500 MHz and 13C spectra at 125 MHz using a Varian Unity 500 spectrometer. The polysaccharide sample (∼15 mg) was converted to the sodium salt by passage through a column 10 × 1 cm of DOWEX 50-X8 Na+ form, and all samples were dissolved in approximately 0.7 ml of 99.8% D2O. The spectra were recorded at 60 °C with suppression of the HOD signal by presaturation. 13C-spectra were recorded with full proton decoupling. Two-dimensional double-quantum filtered COSY, TOCSY,1 and NOESY experiments were performed using pulse sequences supplied by Varian. TOCSY spectra were run with a spin-lock field of about 10 kHz and a mixing time of 80 ms; NOESY spectra were run with a mixing time of 100 ms. All chemical shifts were relative to internal or external trimethylsilylpropionic acid.

**Effects of the Sulfated Polysaccharides as Inducers of the Acrosome Reaction**—The effects of the various sulfated polysaccharides as inducers of the acrosome reaction in conspecific and heterospecific spermatozoa were assayed essentially as described (6). Spermatozoa were prepared by intracelomic injection of 0.5 x KCl (12). The reaction mixtures contain ∼106 sperm and ∼100 µg/ml (as galactose or fucose content) various sulfated polysaccharides in 200 µl of filtered sea water. After incubation at 20 °C for 3 min, an equal volume of cold 6% glutaraldehyde in sea water was added, and the acrosome reaction was monitored by direct counting, using transmission electron microscopy to identify the distinct morphological changes characteristic of the acrosome reaction, of at least 100 sperms for each point.

**RESULTS AND DISCUSSION**

**Sulfated α-L-Fucans or Sulfated α-L-Galactans Are Found in the Egg Jelly Coat of Sea Urchins**—Sulfated polysaccharides were purified from the jelly coat of three species of sea urchins. Purification was achieved by anion exchange chromatography on a DEAE-cellulose column (Fig. 1, A–C). A peak rich in sialic acid was completely eluted by 1.0 M NaCl. A second peak, A, was eluted by 0.5 M KCl (12). The reaction mixtures contain ∼105 sperm and ∼100 µg/ml (as galactose or fucose content) various sulfated polysaccharides in 200 µl of filtered sea water. After incubation at 20 °C for 3 min, an equal volume of cold 6% glutaraldehyde in sea water was added, and the acrosome reaction was monitored by direct counting, using transmission electron microscopy to identify the distinct morphological changes characteristic of the acrosome reaction, of at least 100 sperms for each point.

**1** The abbreviations used are: TOCSY, totally correlated spectroscopy; NOESY, nuclear Overhauser effect spectroscopy.
Sulfated Polysaccharides Induce Acrosome Reaction

TABLE I

Chemical composition and specific optical rotation of the sulfated polysaccharides from the egg jelly coat of different species of sea urchins

| Species    | Chemical composition | [α]D(20)°C | molar ratios |
|------------|----------------------|------------|-------------|
| A. lixula  | L-Fucose             | 0.00       | <0.01       |
| L. variegatus | L-Galactose          | 1.00       | <0.01       |
| E. lucunter | Sulfate              | 0.42       | 0.30        |

Fucose occurs entirely in the L-enantiomeric form since this sugar is totally oxidized by L-fucose dehydrogenase.

The enantiomeric form of L-galactose was determined by the resistance of this sugar to oxidation by L-galactose oxidase. In addition, its specific rotation of ~81° is similar to that recorded for a mutarotated solution of authentic L-galactose (20). The methodology used to characterize L-galactose is described in Refs. 20 and 23.

We found that egg jelly coats of the species Lytechinus variegatus and Arbacia lixula contain sulfated L-fucans as has also been reported for other sea urchin species (6, 7), but surprisingly, the jelly coat of the sea urchin Echinometra lucunter contains a sulfated L-galactan (Table I). These sulfated polysaccharides have a similar molecular mass, as indicated by their migration on polyacrylamide gel electrophoresis (not shown). The variation in electrophoretic mobilities observed among sulfated polysaccharides from different species of sea urchins (Fig. ID) may be accounted for in part by slight differences in the sulfate:sugar molar ratio, but this variation may also reflect other important structural differences (24), as discussed below.

The Egg Jelly of the Sea Urchin E. lucunter Contains a Homopolymer of 2-Sulfated, 3-Linked α-L-Galactan—Methylation of the native sulfated L-galactan from E. lucunter yields 4,6-di-O-methylgalactose, whereas 2,4,6-tri-O-methylgalactose is the predominant methyl ether derivative from the desulfated L-galactan (Table II). This indicates a linear polysaccharide composed of 3-linked and 2-sulfated L-galactopyranoside residues, due to structure confirmed by the 1H and 13C NMR spectra. The 1H spectrum (Fig. 2) contains 6 resonances for COSY and TOCSY spectra (not shown). On desulfation of the L-galactan alterations in chemical shifts of proton signals are consistent with 2-sulfation: -0.57 ppm for H2, -0.09 for H3, and -0.03 for H4 (Fig. 2, A-C), confirming that C2 is the position of sulfation. The 13C spectrum of the native polysaccharide (Fig. 2D) contains 6 resonances (indicating that the sample is a homopolymer): an anomeric signal at 97.16 ppm, unsubstituted C-6 at 63.8 ppm, glycosylated or sulfated carbon at 76.1 and 75.9 ppm, and two other ring carbons at 73.9 and 69.1 ppm. In the 13C spectrum of the desulfated L-galactan (Fig. 2E), a single substituted carbon at 77.25 ppm is observed, as expected. Tentative assignments indicated in Fig. 2E are based on comparison with literature values (25).

The Egg Jelly of the Sea Urchins A. lixula and L. variegatus Contain Linear Sulfated α-L-Fucans with Regular Tetrasaccharide Repeating Units—The structure of the sulfated α-L-fucan from the egg jelly coat of L. variegatus was described in our previous study (24). This sulfated α-L-fucan is essentially a linear polymer, composed of a regular repeating sequence of 4 residues, as follows: [3-α-L-Fucp-(OSO3)1-3-α-L-Fucp-(OSO3)1-3-α-L-Fucp-(OSO3)1-3-α-L-Fucp-(OSO3)1]n (24).

The structure of the sulfated α-L-fucan from the sea urchin A. lixina has not previously been investigated. As for the polysaccharide from L. variegatus, we observed that the high-field 1H NMR spectrum of the sulfated α-L-fucan from A. lixina contains four anionic residues in equal proportions by integration (Fig. 3A). Double quantum filtered COSY and TOCSY spectra (not shown) confirm that the four anomic residues correspond to four spin systems, each consistent with α-fucose. The spin systems can be traced, giving the values of Table III. Strong downfield shifts of H2 of residues A and B relative to H2 of C and D indicate that two of the residues are sulfated at C2. Thus, the sulfated α-L-fucan from A. lixina also has a tetrasaccharide repeating unit but consists of two residues sulfated at the O-2 position and two unsulfated residues.

The order of the four residues was determined by the NOESY spectrum (Fig. 4). As in the NOESY spectra of the other fucans from the sea urchin L. variegatus (24) or from the sea cucumber (24, 26), cross-peaks can be seen from H1 of each residue to protons on one and only one of the other residues (besides, of course, NOes to other protons in the same residue). This suggests that the four residues make up a linear tetrasaccharide repeating unit, as in the case for the other echinoderm fucans we have studied (24, 26). The pattern of the NOes is, however, different. In the fucans from the sea urchin L. variegatus (24) and from a sea cucumber (26) studied previously, the major inter-residue NOes were between H1 and H3 of the next residue. In the sulfated α-L-fucan from A. lixina, NOes can be seen from each H1 to one of the H6 signals, from H1 of residues A and C to the envelope containing signals from H4 of residues A and B (4.01 ppm), and from H1 of residues B and D to the overlapping H4 signals of residues C and D (3.92 ppm) (Fig. 4). This pattern of NOes is indicative of 1→4 linkages (27) rather than the 1→3 linkages previously seen in other similar compounds (24, 26) and indicates the repeating 1→4-linked structure -B-D-C-A- in which two consecutive 2-sulfated residues are followed by two unsulfated residues to give the structure shown in Fig. 5A.

Methylation analysis (Table II) confirms the occurrence of 1→4 linkages in the sulfated L-fucan from A. lixina; 62% of 2,3-di-O-methylfucose and 38% of 3-methylfucose were formed from the native polysaccharide. Although the proportions of the methylated derivatives are not exactly as expected, they are consistent with a polysaccharide composed of 4-linked fucose residues, half of them sulfated at the O-2 position and half unsulfated units (23).

The 1H spectrum of the polysaccharide resulting from successive desulfation processes (Fig. 3, B and C) shows a reduc-
The 13C spectrum of the native α-L-fucan from A. lixula shows 4 signals of anomic carbons (Fig. 3D). Most of the ring carbons resonate at about 69–71 ppm and overlap heavily. The 13C spectrum of the desulfated polysaccharide (Fig. 3E) contains one major anomic signal at 103.1 ppm; again, most of the ring carbons overlap heavily. The 13C spectra corroborate our proposition that the sulfated polysaccharide from A. lixula is composed of α-L-fucose units with a single type of linkage and a tetrasaccharide repeat unit defined by a specific pattern of sulfation.
Overall, the combination of chemical analysis, specific optical rotation, methylation experiments and NMR spectroscopy has allowed us to determine the fine structure of the sulfated polysaccharides isolated from the egg jelly coat of three species of sea urchins. The sea urchin *E. lucunter* contains a homopolymer of sulfated α-L-galactose, composed of 2-sulfated and 4-linked α-L-galactopyranosyl units (No. 2 in Fig. 5A). The sulfated α-L-fucans from *A. lixula* and *L. variegatus* are essentially linear polymers, composed of a regular tetrasaccharide repeat unit defined by the pattern of O-sulfation. The specific pattern of sulfation varies in the two species. The α-L-fucan from *L. variegatus* consists of two residues sulfated at the O-2 position, one sulfated at both O-2 and -4 positions, and one residue sulfated at the O-4 position (No. 3 in Fig. 5A). The sulfated α-L-fucan from *A. lixula* consists of two residues sulfated at the O-2 position and two unsulfated residues (No. 1 in

| Table III | Proton chemical shifts (ppm) for residues of α-L-fucose in the sulfated L-fucan from *A. lixula* |
|-----------|--------------------------------------------------------------------------------------------------|
| Proton    | Residues | A | B | C | D |
|-----------|-----------|---|---|---|---|
| H1        | 5.31      | 5.25 | 5.05 | 5.01 |
| H2        | **4.56**  | 4.51 | 3.89 | 3.84 |
| H3        | 4.23      | 4.16 | 4.07 | 4.01 |
| H4        | 4.01      | 4.01 | 3.92 | 3.92 |
| H5        | 4.52      | 4.56 | 4.54 | 4.59 |
| H6        | 1.35      | 1.38 | 1.30 | 1.35 |

The 1H spectrum was recorded at 500 MHz, 60 °C in 99.8% D2O. Chemical shifts are referenced to internal trimethylsilylpropionic acid. Boldface indicates positions bearing sulfate groups. Assignments for H5 of residue A and H5 of residue D may be reversed.

Fig. 4. Detail of the NOESY spectrum of the fucan from *A. lixula*. Interresidue NOE cross-peaks from anomeric protons are labeled, as are some H1-H2 intraresidue cross-peaks. H1 of residue A shows cross-peaks to H4 and H6 of residue B; H1 of B shows cross-peaks to H4 and H6 of residue D; H1 of D to H4 and H6 of C and H1 of C to H4 and H6 of A. This evidence indicates the sequence and linkage 2-(1→4)-A-(1→4)-B-(1→4)-D-(1→4)-C-(1→).

Fig. 5. Structures of several sulfated polysaccharides isolated from marine invertebrates (A) and their effects as inducers of the acrosome reaction in sperms of three species of sea urchins (B). A, the structures of the sulfated L-fucan and sulfated L-galactan from the sea urchins *A. lixula* and *E. lucunter*, respectively, were determined in this study. The structures of the sulfated L-fucan from the sea urchin *L. variegatus* (24), of the sulfated L-fucan from the sea cucumber *L. grisea* (24, 26), and of the sulfated L-galactan from the ascidian *H. monus* (30) were determined in previous studies. B, the effects of the various sulfated polysaccharides as inducers of the acrosome reaction in conspecific and heterospecific spermatozoa were assayed as described under “Experimental Procedures.” The acrosome reaction was monitored by direct counting on a transmission electron microscopy of at least 100 sperms for each point. The values in panel B are the average and S.E.

Sperms from: (1) *A. lixula* (2) *E. lucunter* (3) *L. variegatus*

Sulfated polysaccharide from:
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Fig. 6. Sepharose CL-4B chromatography of the sulfated α-L-fucan from L. variegatus. Sulfated polysaccharide (25 mg) purified on a DEAE-cellulose column (see Fig. 1A) was applied to a 84 × 1.8 cm column of Sepharose CL-4B eluted at a flow rate of 10 ml/h with 0.5 M sodium acetate buffer (pH 5.0). Fractions of 2.5 ml were collected and assayed by their metachromasia (15). Fractions indicated by horizontal bar were pooled, dialyzed against distilled water, and lyophilized.

Fig. 5A). The fucose residues are 3-linked in L. variegatus and 4-linked in A. lixula (compare Nos. 1 and 3 in Fig. 5A).

Purified Sulfated Polysaccharides from the Jelly Coat Induce the Acrosome Reaction in Conspecific but Not in Heterospecific Spermatzoa—Once we had isolated, purified, and characterized the fine structure of the sulfated polysaccharides from the egg jelly layer of three species of sea urchins, we were in a position to test the ability of these polysaccharides to induce the acrosome reaction in conspecific and heterospecific spermatzoa. Transmission electron microscopy can be used to differentiate sperms that have undergone the acrosome reaction from those that have not, by clear and unambiguous morphological differences, so that the extent of the acrosome reaction among a sample of sperm may be monitored by direct counting. Effectively, we observed a species-specific induction of the acrosome reaction in the sperms of the three sea urchin species (Fig. 5B). Thus, the sulfated α-L-galactan from the egg jelly coat of E. lucunter induces the acrosome reaction exclusively in sperm of this species (No. 2 in Fig. 5B). Even the similar sulfated α-L-galactan from the ascidian Herdmania monus, which is also linear but composed of 4-linked and 3-sulfated α-L-galactose units (28), does not induce the acrosome reaction in this species of sea urchin.

The acrosome reaction in sperms of L. variegatus is induced exclusively by the sulfated α-L-fucan from the egg jelly coat of this species (No. 3 in Fig. 5B). Sulfated α-L-fucans from A. lixula or from the sea cucumber Ludwigothurea grisea, although composed of regular repeating tetrasaccharide units but with a different sulfation pattern (compare Nos. 1, 3, and 4 in Fig. 5A), do not induce acrosome reaction in sperms of L. variegatus (No. 3 in Fig. 3B). We also observed a conspecific but not heterospecific induction of the acrosome reaction by the sulfated α-L-fucan from A. lixula (No. 1 in Fig. 5B).

Further purification of the sulfated α-L-fucan from L. variegatus on a gel filtration column yields a single and narrow peak (Fig. 6), which induces the acrosome reaction in sperms of this species of sea urchin at the same extent reported in Fig. 5B. This experiment indicates that a sulfated polysaccharide itself, and not a contaminant present in the preparation, is in fact the inducer of acrosome reaction.

CONCLUSION

Specificity in fertilization is the result of a series of interactions between molecules located on the surfaces of the egg and of the sperm. This is especially relevant in free-spawning or-

organisms and constitutes a barrier to prevent interspecific crosses, and consequently the formation of hybrids. It may be that the induction of acrosome reaction by sulfated polysaccharides from the egg jelly coat is the first level of recognition during fertilization in sea urchin species while the more specific interaction of egg receptor with sperm serves as a second level of recognition (4).

Our results indicate that the acrosome reaction in sea urchin is mediated by sulfated polysaccharide and in fact is regulated by the structure of the saccharide chain and its sulfation pattern. This constitutes an unusually clear-cut example of a biological event regulated by sulfated polysaccharides. Variations in the structure of these polymers among the species of sea urchins may represent one of the barriers which prevent interspecific crosses.

Approximately 900 species of sea urchins have been described (29). To act as a recognition molecule for the acrosome reaction in such a large number of species, the sulfated polysaccharides from the egg jelly must have a wide capacity for structural variation. In fact, an array of sulfated esters in the eight possible sulfation positions of the tetrasaccharide repeat units of a linear sulfated L-fucan (as those reported in Fig. 5) allows 256 combinations. Different positions of the glycosidic linkages (2-, 3-, or 4-linked), as we reported for the sulfated L-fucans from the sea urchins L. variegatus and A. lixula, increase the possible combinations to 765. In addition, the presence of sulfated L-galactan in some other species, for example E. lucunter, expands the variation possibilities for sulfated polysaccharides from the egg jelly of sea urchins.

Recent studies have suggested that glycoproteins or peptides, tightly associated with the sulfated polysaccharides of the egg jelly coat, are in fact the inducers of the acrosome reaction in sea urchins (8–11). It is unlikely that glycoproteins or peptides would resist the drastic protease digestion conditions we have used to extract sulfated polysaccharides from the egg jelly or would remain associated with the sulfated polysaccharides at the NaCl concentration used to elute these polymers from the DEAE-cellulose column (Fig. 1A-C). But, if this is the case, and the sulfated polysaccharides are not the inducers of the acrosome reaction but the “carriers” of glycoproteins or peptides, which directly induce the process, our proposition is still valid. That is, there is a species-specific variation in the structure of the sulfated polysaccharide from the sea urchin egg jelly coat, and these polymers are either directly involved in the induction of the acrosome reaction or are specific “carriers” of acrosome reaction-inducing molecules. We believe the relative importance of these glycoproteins or peptides and of the sulfated polysaccharides as inducers of the acrosome reaction in sea urchins requires further investigation.

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