Sulfated polysaccharides ensure a carbohydrate-based mechanism for species recognition during sea urchin fertilization

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Comparison between the sulfated fucans from invertebrates and algae

a) Sulfated fucans from marine algae
   → Homogeneous structure.
   → Repetitive units.
   → Glycosylation and sulfation sites vary among the different species.

b) Sulfated fucans from marine invertebrates
   → Heterogeneous structure.
I) Structures of the sulfated polysaccharides from marine invertebrates
Approach used to determine the structure of a sulfated polysaccharide (an example from the sulfated fucan of *A. lixula*)

<table>
<thead>
<tr>
<th>Methylation Analysis</th>
<th>native</th>
<th>desulfated</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3-\text{Me}_2\text{Fuc}</td>
<td>49%</td>
<td>71%</td>
</tr>
<tr>
<td>3-\text{MeFuc}</td>
<td>53%</td>
<td>29%</td>
</tr>
</tbody>
</table>
Approach used to determine the structure of a sulfated polysaccharide (an example from the sulfated fucan of *A. lixula*)
II) What is the biological function of sulfated polysaccharides in sea urchins?

1947: Jean C. Dan described the acrosome reaction.
Keller and Vacquier (Dev. Biol. 162:304-312, 1994): “...sulfated fucans had no significant acrosome reaction-inducing activity. Instead, acrosome reaction inducing activity was associated only with two glycoproteins.”

A skepticism that sulfated polysaccharides could induce such a specific reaction
Sulfated polysaccharides are species-specific inducers of acrosomal reaction in sperm of sea urchins.
III) Structure vs. biological activity of the sea urchin polysaccharides
Effect of sulfation pattern

S. franciscanus fucan

S. purpuratus fucan

% of acrosome reaction

Sulfated fucan
Position of the glycosidic linkage

% of acrosome reaction

Sperm from: S. droebachiensis, S. pallidus, S. purpuratus, S. franciscanus, S. droebachiensis, S. pallidus, S. purpuratus

Sulfated fucans from: S. droebachiensis, S. franciscanus, S. pallidus, S. purpuratus

Structures:
Sulfated $\alpha$-fucan vs. sulfated $\alpha$-galactan

$S.\ franciscanus$

$E.\ lucunter$

$S.\ purpuratus$

▲ $E.\ lucunter$

● $S.\ franciscanus$

△ $S.\ purpuratus$

Graph:

- $\%\ AR$ vs. Sulfated Polysaccharide (µg/ml)

- $S.\ franciscanus$

- $E.\ lucunter$

- $S.\ purpuratus$
Sulfated $\alpha$-galactan vs. sulfated $\beta$-galactan
IV) Two mechanisms of sperm – egg recognition in sea urchins

- Homologous polysaccharide
- Bindin mechanism
- Fertilization envelop
- Heterologous polysaccharide
Species-specificity in the fertilization based on the bindin mechanism

A) Diluted in sea water

B) Pre-reacted sperm

Eggs:
- L. variegatus
- A. Ixula
- E. lucunter

Sperm:
- L. variegatus
- E. lucunter

% of fertilized eggs
Fertilization and induction of acrosome reaction in sea urchins of the genus *Strongylocentrotus*

**A)** Diluted in sea water

**B)** Pre-reacted sperm

**Eggs from:** *S. droebachiensis*  *S. pallidus*  *S. purpuratus*
Two mechanisms of sperm-egg recognition in sea urchins

a) Bindin-protein mechanism
Prevents *S. purpuratus* fertilization by *S. pallidus* and *S. droebachiensis*

b) Sulfated polysaccharide-based mechanism
Prevents *S. droebachiensis* and *S. pallidus* intercrosses
Sulfated fucans: another avenue for speciation?

Myr = Million years of evolutionary divergence

Carbohydrate mechanism

Bindin mechanism

3 Myr

10-20 Myr

30 Myr

> 200 Myr

Structure of the sulfated polysaccharides

Species

Sugar

Glycosidic linkage

Sulfation

1→4

2S 2S

1→4

2S

1→4

2S 2S 4S 4S

1→3

2S 4S (80%)

1→3

2S

1→3

2S 4S 2,4diS

1→3

2S 4S 2,4diS

1→4

2S 2S

1→4

2S 2S

1→3

2S

1→3

2S

1→3

2S

1→4

2S 2S

1→3

2S 4S 2,4diS
V) Isoforms of sulfated fucans
Physiological irrelevant isoforms of sulfated fucans

**a) S. purpuratus**

71 Individual females, 40 had eggs with sulfated fucans II, 22 had eggs with sulfated fucan I and 9 had eggs with both fucans.

**b) S. droebachiensis**

Pacific (USA): 13 individual females had sulfated fucan II, 9 had eggs with sulfated fucan I.

Atlantic (Norway): 9 females contains only sulfated fucan II.
The two isoforms of sulfated fucans induce the acrosome reaction with similar potency in homologous sperm.

These isoforms of sulfated fucans could represent differentiation within the species that might be a predecessor of incipient sympatric speciation.

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**Table 2. Comparison of acrosome reaction–inducing activities of the two isotypes of sulfated fucans (I and II) from* Strongylocentrotus droebachiensis* egg jelly**

<table>
<thead>
<tr>
<th>Sperm from</th>
<th>% AR ± SE&lt;sup&gt;1&lt;/sup&gt; Sulfated Fucan I&lt;sup&gt;2&lt;/sup&gt;</th>
<th>% AR ± SE&lt;sup&gt;1&lt;/sup&gt; Sulfated Fucan II&lt;sup&gt;2&lt;/sup&gt;</th>
<th>Paired t-Test for Comparison of Means</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pacific</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. droebachiensis</td>
<td>45.3 ± 5.8</td>
<td>38.3 ± 9.2</td>
<td>t = 0.712</td>
<td>0.503</td>
</tr>
<tr>
<td>Atlantic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. droebachiensis</td>
<td>40.6 ± 6.5</td>
<td>44.2 ± 10.1</td>
<td>t = -0.577</td>
<td>0.580</td>
</tr>
<tr>
<td>S. pallidus</td>
<td>1.7 ± 0.3</td>
<td>1.0 ± 1.0</td>
<td>t = 1.77</td>
<td>0.305</td>
</tr>
<tr>
<td>S. purpuratus</td>
<td>0.67 ± 0.3</td>
<td>2.3 ± 1.5</td>
<td>t = -1.387</td>
<td>0.300</td>
</tr>
</tbody>
</table>

<sup>1</sup> % AR = percent of sperm having undergone the acrosome reaction (AR) in response to 100 μg hexose/ml of purified sulfated fucan, ± standard error (SE). The sperm’s responses to the two isotypes did not differ significantly.

<sup>2</sup> The structures of sulfated fucan I and sulfated fucan II are shown in Fig. 5 and Fig. 4, respectively.
Physiological relevant isoforms of sulfated fucans in *Lytechinus variegatus*

*Summer:* 70 females collected, all shown exclusively sulfated fucan P1.

*Winter:* 45 females collected, 28 expressed predominantly isotype P1 and 17 secreted isotype P2.
Induction of the acrosome reaction by the two sulfated fucan isoforms

The two isotypes of sulfated fucans in the egg jelly of *L. variegatus*, which differ in their biological activity, maybe involved in the periodicity of the reproductive cycle of the invertebrate.
VI) Preparation of oligosaccharides from the sulfated fucans
A selective 2-desulfation reaction
Maldi-MS suggests that oligosaccharides IV has four tetrasaccharides units composed of 4-fucose and 4 sulfated ester-each
Mild acid hydrolysis of sulfated fucans: a selective 2-desulfation reaction and an alternative for preparing tailored sulfated oligosaccharides

**A** S. pallidus

First stage: 2-desulfation of both residues.

Second stage: cleavage of the glycosidic linkage of the unsulfated unit.

**B** L. variegatus

First stage: 2-desulfation of the 2-sulfated residue linked to a 4-sulfated unit.

Second stage: cleavage of the glycosidic linkage of the unsulfated unit.

**C** S. franciscanus

No 2-sulfate removal.

Unspecific cleavage of the glycosidic linkage.
Decrease of molecular size reduces the effect of the sulfated fucan as inducer of acrosome reaction.
VII) Biosynthesis of the sea urchin polysaccharides
Biosynthesis of the sulfated galactan

Accessory cells

Oocyte

Ovary

Egg jelly
VIII) Medical applications
Interaction of sulfated polysaccharides with serpin (antithrombin)

- Heparin
- Sulfated galactan from *E. lucunter*
- Sulfated fucan from *S. franciscanus*
Interaction of sulfated polysaccharides with P-selectin. Effect on tumorigenesis
Conclusions

1. The jelly coat surrounding sea urchin eggs is not a simple accessory structure.

2. It contains sulfated polysaccharides which modulate cell-cell recognition and species specificity leading to exocytose of the acrosome vesicle, the acrosome reaction.

3. The sulfated polysaccharide-mediated mechanism co-exists with that of bindin and its receptor in the egg.
Conclusions

4. The invertebrate polysaccharides can also be assayed as alternative anticoagulant and antitumoral agents and represent a new source of therapeutic agents.

5. The biological actions of sulfated polysaccharides do not simply depend on their negative charge density, but are also influenced by their structural features (sugar type, specific positons of sulfation and glycosilation...).
Challenges

1. Test of oligosaccharides as inducers of the acrosome reaction and as therapeutic agents.

2. Identification of receptor for the sulfated polysaccharides in the sperm membrane.

3. Identification of the metabolic pathways involved in the biosynthesis of the egg jelly polysaccharides.
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