Vol. 80, No. 4, 1978 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
February 28, 1978 Pages 917-922

A SPERM FACTOR AS THE COUNTERPART TO THE SPERM-BINDING FACTOR OF THE HOMOLOGOUS EGGS

Kenji Aketa*, Sadako Miyazaki*, Motonobu Yoshida* and Hideko Tsuzuki**

*Biological Institute, Faculty of Science, Nagoya University, Nagoya 464, and
**Kanagawa Dental College, Yokosuka 238, Japan

Received December 27, 1977

SUMMARY : A substance was isolated from sperm of the sea urchin, Hemicentrotus pulcherrimus. When unfertilized eggs of homologous species were pre-treated with this substance, they rapidly lost the fertilizability due to loss of the sperm-binding capacity. Such an effect was not exerted upon eggs either in Ca-Mg free sea water or of heterologous species. This substance caused neither iso-agglutination of eggs nor precipitation of jelly coat. When it was pre-incubated with the sperm-binding factor purified from eggs of homologous species, it lost the fertilization-inhibiting effect on eggs. It seems very likely that a complementary relationship exists between the present substance and the sperm-binding factor.

It is our purpose to describe the interaction between sperm and egg at fertilization in molecular terms. To begin with, we obtained a substance from the unfertilized eggs of the sea urchins. It derived homologous sperm of their fertilizing capacity (1), and the antiserum against this substance blocked fertilization of homologous eggs (2). This substance, the sperm-binding factor, was purified recently from the sea urchin, Hemicentrotus pulcherrimus (3).

In the present report, we describe a substance isolated from the sperm of the sea urchin, H. pulcherrimus. It deprives homologous eggs of their fertilizability, but does not affect heterologous eggs. It appears to be the counterpart to the sperm-binding factor of homologous eggs.

The gametes of the sea urchins, H. pulcherrimus and Anthocidaris crassispina, were obtained and handled as ordinary procedures (3). The sperm of the former species were used for the preparation of the present substance. After being carefully washed in isotonic (0.56 M) NaCl to remove the contaminating seminal plasma, the sperm were resuspended in isotonic NaCl and an equal volume of 10 % trichloroacetic acid was added and stirred for 5-10 min. The suspension was centrifuged at 12,000

0006-291X/78/0804-0917$01.00/0

Table 1 Effect of HpSpCaS (see the text) on the fertilizability of homo- and heterologous eggs.

eggs from;	exposure time (min)	sperm-binding	fertilizability(%) exposed	fertilizability(%) control
Hemicentrotus pulcherrimus	5	—	6.5	100
	10	—	13.5	100
1000-2000 eggs/ml	5	—	11.0	100
	5	—	12.0	100
	5	—	13.0	100
Anthocidaris crassispina				
1250 eggs/ml	30	+	100	100
750 eggs/ml	30	+	99	100
500 eggs/ml	10	+	95	100
500 eggs/ml	20	+	100	100
250 eggs/ml	10	+	99	100
250 eggs/ml	20	+	100	99

Dejellied eggs were exposed to HpSpCaS (0.5 mg/ml) for period indicated, and, after being washed in normal sea water, were inseminated. + and — are indicative of occurrence and unoccurrence, respectively, of the sperm-binding on eggs detected under a light microscope. Only qualitative estimations were made.

g for 20 min and the supernatant was millipore-filtered. After the pH of the filtrate was adjusted to 6 by dropwise addition of 10 N NaOH, 3 volumes of 100 % ethanol was added and kept in cold (4° C) overnight. The precipitates formed were collected by centrifugation at 4,000 g for 20 min, redissolved in distilled water, into which 1 M calcium acetate was added to the final concentration of 50 mM. Purple precipitates appeared, which were removed by centrifugation at 4,000 g for 20 min. Three volumes of 100 % ethanol were then added to the supernatant and the white precipitates formed were collected. They were washed in 75 % ethanol, 100 % ethanol twice and were dried with ether. Alternatively, they were frozen-dried after being redissolved in distilled water. This fraction was tentatively abbreviated as HpSpCaS (calcium-soluble fraction of *H. pulcherrimus* sperm).

As shown in Table 1, HpSpCaS remarkably reduced the fertilizability of dejellied homologous eggs. Sperm could not bind to the surface of the treated eggs. Any sign of cortical damage was observed. It appears that the sperm-binding sites on the vitelline layer (4,5) were "saturated" by the treatment. It did not affect the fertilizability of heterologous eggs. It was also found that the fertilization-inhibiting effect was not exerted on eggs in Ca-Mg free sea water.

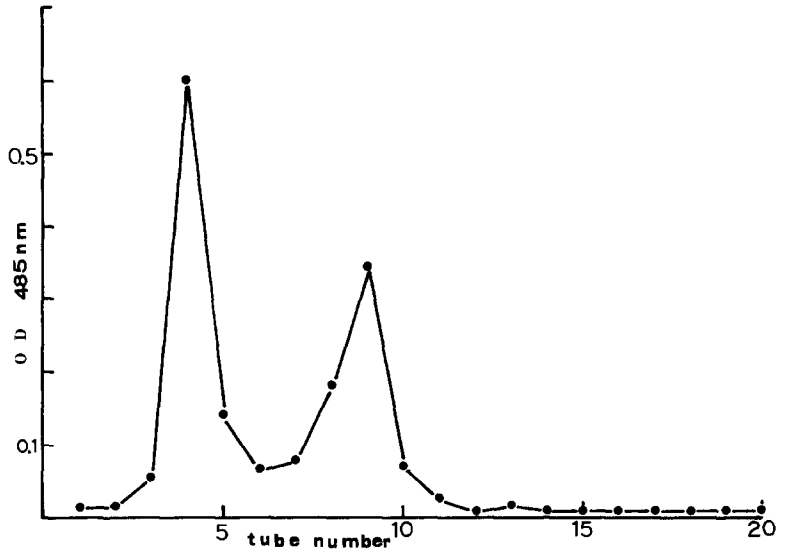

Fig.1 Gel-filtration of HpSpCaS (see the text) through a Sephadex G 200 column (7 x 240 mm). Elution by distilled water. Absorbance at 485 nm after phenol-sulfuric acid reaction according to Dubois et al.(9).

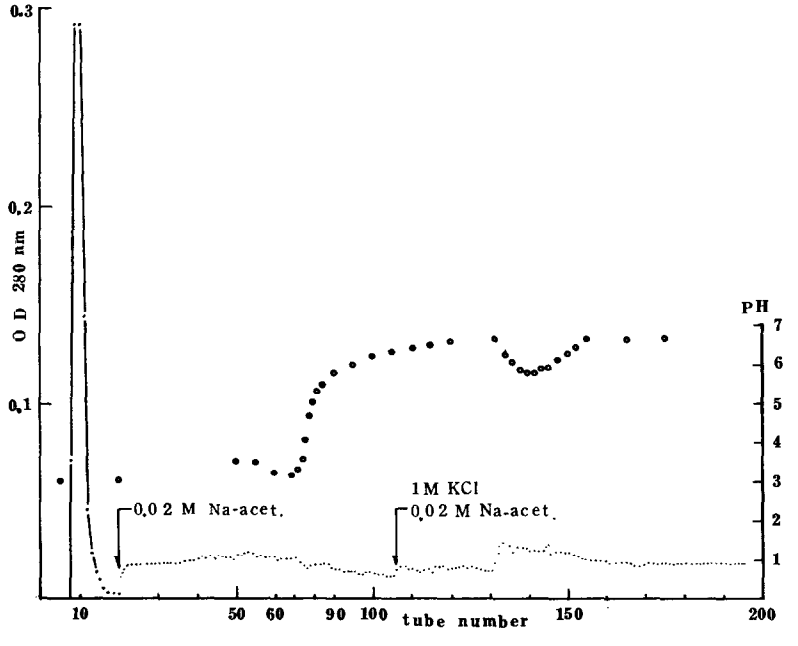

Fig.2 CM cellulose column chromatography of HpSpCaS-A (HpSpCaS fraction emerging in the void volume of the Sephadex G 200 column). CM 23 was equilibrated with 0.02 M acetic acid (pH 2.9). Open circles show pH of each fraction.

When HpSpCaS was gel-filtered through Sephadex G 200(Pharmacia,Sweden) or Ultrogel ACA 34(LKB,Sweden), it was divided into two fractions (Fig.1). The fast emerging fraction was found to be responsible for the observed reduction in the egg fertilizability. The late emerging one was ineffective though it caused iso-agglutination of dejellied homologous eggs. This seems to show that the iso-agglutinability can not always be an index of the participation of the substance concerned in fertilization. The first one, HpSpCaS-A , was further applied to a CM-cellulose (CM 23,Whatman,England) column equlibrated with 0.02 M acetic acid (pH 2.9). The majority of HpSpCaS-A emerged in the exclusion volume of the column, eliminating a small fraction which emerged only after the addition of 1 M KCl in 0.02 M Na-acetate (pH 6.9)(fig.2). The former active fraction was abbreviated as HpSpCaS-A-α .

The purification procedure could be monitored by the electrophoresis on cellulose-acetate membrane (Sepraphore III, German,Michigan,U.S.A.). Three bands appeared in HpSpCaS, one of which remained in HpSpCaS-A-α (Fig.3). Although SDS polyacrylamide gel electrophoresis was examined according to Weber and Osborn (6), the preparation could not enter the gel.

If HpSpCaS-A-α is the counterpart to the sperm-binding factor of homologous eggs, HpCaS20-A, purified according to the method described in the preceding paper (3), it may interact with the latter in vitro and lose the effect on eggs. This really took place.

One ml of HpSpCaS-A-α (1 mg/ml sea water) was mixed with 0.15 ml of HpCaS20-A (1 mg/ml sea water) and left for 10 min at room temp (approx.20° C). Dejellied eggs were then exposed to the mixture for 15 min, and they were inseminated after being washed in normal sea water.

While the eggs exposed to HpSpCaS-A-α alone were deprived of their fertilizability, those exposed to the mixture were exempted from the fertilization-inhibiting effect of this substance (Table 2).

The present substance seems not to be the antifertilizin (7), because it caused neither precipitation of the jelly nor iso-agglutination of the eggs. It appears to interact with the sperm-binding factor located on the vitelline layer of unfertilized homologous eggs (4,8), representing the molecular basis of species-specific gamete bonding at sea urchin fertilization.

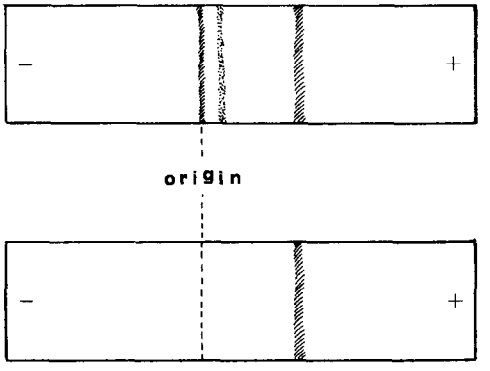

Fig.3 Electrophoresis of HpSpCaS and HpSpCaS-A-α (HpSpCaS-A fraction excluded from CM cellulose column) on cellulose-acetate membrane (Sepraphore III). Upper ; HpSpCaS, Lower ; HpSpCaS-A-α ; Buffer ; acetic acid:pyridine:water (pH 3.5), Current ; 0.8 mA/cm width for 20 min, Stain ; Toluidine blue (0.5 %) in 3 % acetic acid.

Table 2 Cancellation of the fertilization-inhibiting effect of HpSpCaS-A-α (see the text) by the sperm-binding factor (HpCaS20-A) of the egg.

incubated in ;		
HpSpCaS-A-α	HpSpCaS-A-α HpCaS20-A	normal sea water
fertilization (%)		
31.5	92	100
10	69	96.5
23.5	99	99
20	98	100

HpSpCaS-A-α (1 mg/ml, 0.1 ml) was mixed with HpCaS20-A (1 mg/ml, 0.15 ml) for 10 min, and dejellied 200-300 eggs (0.1 ml) were treated with the mixture for 15 min. After being washed in normal sea water, the eggs were inseminated. In case of the treatment with HpSpCaS-A-α alone, normal sea water (0.15 ml) was added in place of HpCaS20-A solution.

According to our preliminary characterization, peptide or protein residue being less than 5 %, more than 95 % of this substance is composed of carbohydrates.

REFERENCES

1. Aketa,K.(1973) Exptl.Cell Res.,80,439-441.
2. Aketa,K. and Onitake,K.(1969) Exptl.Cell Res.,56,84-86.
3. Tsuzuki,H.,Yoshida,M.,Onitake,K. and Aketa,K.(1977) Biochem.Biophys.Res.Commun.,76,502-511.
4. Aketa,K.,Onitake,K. and Tsuzuki,H.(1972) Exptl.Cell Res., 71,27-32.
5. Summers,R.G. and Hylander,B.L.(1974) Cell Tiss.Res.,150, 343-368.
6. Weber,K. and Osborn,M.(1969) J.Biol.Chem.,244,4406-4412.
7. Tyler,A. and O'Melveny,K.(1941) Biol.Bull.,81,364-374.
8. Yoshida,M.,Tsuzuki,H. and Aketa,K.(1976) Zool.Mag.,85,305.
9. Dubois,M.,Gilles,K.A.,Hamilton,J.K.,Rebers,P.A. and Smith,F. Anal.Chem.,350-356.