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EGG JELLY COMPONENTS RESPONSIBLE FOR HISTONE DEGRADATION AND ACROSOME REACTION IN THE STARFISH, *ASTERINA PECTINIFERA*

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SUMMARY: In the starfish, *Asterina pectinifera*, egg jelly induces the degradation of sperm histones as well as the acrosome reaction. We have isolated histone degradation-inducing components from the egg jelly. The histone degradation and the acrosome reaction are induced by a co-operative action of ARIS, which is an extremely large, sulfated glycoprotein with diffusible substance(s) in the jelly. Co-ARIS I, a steroidal saponin of the jelly, is effective to induce both reactions in the presence of ARIS.

The jelly coat of echinoderm eggs plays important roles in fertilization (1). It induces the acrosome reaction (AR) that is essential for fertilization (2,3) and triggers the modifications of sperm histones (4-6). Our previous study shows that the egg jelly induces the degradation of sperm histones besides the AR in the starfish, *A. pectinifera* (6). Because egg jelly consists of several components, each of which has distinct effects on sperm physiology (7-10), it is important to identify the egg jelly component(s) responsible to initiate the histone degradation (HD). In this study, we isolate egg jelly substances that induce the HD.

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Abbreviations used are: AR, acrosome reaction; ASW, artificial seawater; DW, distilled water; HD, histone degradation; HDIS, histone degradation-inducing substance; P-ARIS, pronase-digested ARIS; Tris, tris(hydroxymethyl)amino-methane.

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Materials and Methods

Artificial Seawater (ASW): ASW consisted of 450 mM NaCl, 10 mM KCl, 10 mM $CaCl_2$, 30 mM $MgCl_2$, and 20 mM $MgSO_4$ and 15 mM N-(2-hydroxyethyl)-piperadine-N'-3-propanesulfonic acid, pH 8.2.

Gametes, Egg Jelly Solution and Related Substances: The starfish *A. pectinifera* was used. Sperm and egg jelly solution were obtained as previously described (6). ARIS, pronase-digested ARIS (P-ARIS), and Co-ARIS I of *Asterias amurensis* were obtained as previously described (10-12).

Bioassays: AR was assayed as described in Matsui *et al*. (11). Because spermatozoa of *A. pectinifera* were strongly aggregated by incubation with jelly or ARIS, fixed sperm suspensions were sonicated in a sonic bath for 3 sec immediately before microscopic observation. Assay of the HD was performed as previously described (6).

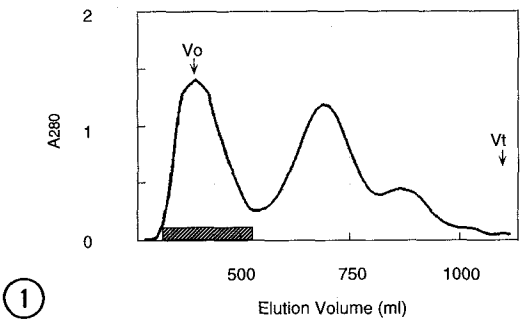
Purification of Egg Jelly Substances: All the procedures except for the evaporation and reversed-phase chromatography were performed at 4°C. Egg jelly solutions (100 ml, 0.8-2.0 mg fucose/ml) were dialyzed twice against 10 volumes of DW. Diffusible fractions were collected and evaporated. The residue was dissolved into 25 ml of DW and applied on a Sep-Pak (C18) cartridge (Waters Assoc.) for desalinization. The activity was recovered by 80% methanol elution. The eluent (M8) was dried and used for bioassays.

Dialyzed jelly (50-100 ml) was centrifuged to remove a small quantity of insoluble materials, and then applied on a Sepharose CL-4B (Pharmacia LKB) column (3.8x80 cm) equilibrated with 0.05 M Tris-HCl buffer pH 8.0, containing 0.1 M NaCl. The column was eluted with the same buffer. Each fraction was monitored for absorbance at 280 nm and for sugar (13), and used for bioassays after dialysis against ASW. Active fractions obtained by gel filtration were applied on a DEAE-Toyopearl 650M (Tosoh) column (2.8x16 cm), equilibrated with 0.1 M NaCl in 0.05 M Tris-HCl buffer, pH 8.0. The column was washed with the same buffer and then eluted with a linear gradient from 0.1 to 1 M NaCl in 0.05 M Tris-HCl buffer, pH 8.0. Each fraction was dialyzed against ASW and used for bioassays. Active fractions (HDIS) were collected and lyophilized after dialysis against DW.

Cellulose Acetate Electrophoresis: Cellulose acetate electrophoresis was performed as described in Ikadai and Hoshi (10).

Results and Discussion

Fig.1 shows a typical elution profile of dialyzed jelly on a Sepharose CL-4B column. Histone degradation-inducing substance (HDIS) was recovered in the glycoprotein fraction that passed through the column. In this step, a neutral glycoprotein and low molecular weight substances were eliminated. As shown in Fig.2, HDIS was well separated from the major glycoprotein on a DEAE-Toyopearl 650M column. HDIS gave a single peak with gel filtration by a GLYCERYL-CPG



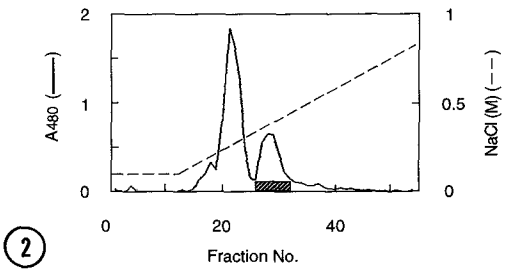


Fig.1. Gel filtration on Sepharose CL-4B of dialyzed jelly. Fractions in the shaded area induced the HD and were pooled for ion-exchange chromatography.

Fig.2. DEAE-Toyopearl 650M column chromatography of the active fraction in Fig.1. The elution was monitored for sugar contents by the phenol-sulfuric acid method. Fractions in the shaded area were combined and used as purified HDIS.

(pore size 3000 Å, CPG inc.) column. It hardly migrated on cellulose acetate electrophoresis at pH 3.5 and 5.0 (Data not shown). The activity of HDIS was significantly decreased after the gel filtration. HDIS induced the full HD only in a few batches of spermatozoa, in most cases, it induced the degradation of about

Table 1. Induction of the HD and the AR by HDIS

Treatments	Acrosome Reaction (% control)	Histone Degradation*
ApJelly	100	++
HDIS	30	+/-
HDIS (50 mM Ca^{2+})**	74	ND ***
HDIS (pH 9.5)**	86	ND ***
ApM8	0	-
HDIS + ApM8	82	++
+ Co-ARIS I	83	++
AaJelly	0	-
AaP-ARIS + ApM8	0	-

Asterina pectinifera sperm were treated with the egg jelly or its components at the following concentrations: 100 μg fucose/ml for the homologous jelly (ApJelly), *Asterias amurensis* jelly (AaJelly); 50 μg/ml for HDIS and P-ARIS of *A. amurensis* (AaP-ARIS); 62.5 μg/ml for M8 from *A. pectinifera* jelly (ApM8); 100 μg/ml for Co-ARIS I from *A. amurensis* jelly. *Degradation of sperm histones was represented as follows; fully degraded (++), 20-30% of histone H1 was degraded (+/-, see text), and the HD did not occur (-). ** AR hardly occurred in high Ca^{2+} or high pH seawater without HDIS. *** Not determined.

20-30% of histone H1. These results suggest that HDIS requires a low molecular weight component to induce the HD. M8 hardly induced the HD but it greatly enhanced the activity of HDIS (Table 1). Thus, we conclude that HDIS and M8 are necessary for the induction of HD.

These results hint us the possibility that the HD is induced by ARIS and Co-ARIS, both of which are essential for inducing the AR in another starfish, *A. amurensis* (11,12,14-17). We examined whether the purified HDIS also induces the AR. In normal seawater, the AR was slightly induced (10-20%) by HDIS alone, whereas it was considerably induced when spermatozoa were treated with HDIS and M8 (Table 1). In high Ca^{2+} or high pH seawater, HDIS alone induced the AR considerably (Table 1). These features are quite similar to ARIS of *A. amurensis* (11,14). Furthermore, no other fractions of egg jelly affected the AR. Thus, we conclude that HDIS is ARIS itself. Inductions of the HD and the AR required ARIS and M8 simultaneously.

Although there were the same properties in inducing the AR between ARIS of *A. pectinifera* (ApARIS) and of *A. amurensis* (AaARIS), and they were extremely large glycoproteins containing a significant amount of sulfate residues, they differed in the sugar composition. ApARIS contained fucose, arabinose and galactose as major sugar components, whereas AaARIS contained fucose, galactose, xylose and hexosamines (10, 18, unpublished results).

As shown in Table 1, ARIS species-specificially induced the HD and the AR, whereas M8 did not have species-specificity. It is known that M8 contains Co-ARIS and sperm-activating peptides (SAP); Co-ARIS is essential for the AR and SAP stimulates the reaction induced by ARIS and Co-ARIS (11,12,14,17,19). We have not yet purified Co-ARIS in *A. pectinifera*. However, the combination of ApARIS and purified Co-ARIS I from *A. amurensis* did induce both the AR and the HD in *A. pectinifera* sperm (Table 1). Preliminary results showed that egg jelly of *A. pectinifera* contained saponins including Co-ARIS I judged by high performance reversed-phase liquid chromatography and thin layer chromatography on a silica gel plate. Thus, we think that one of the diffusible substances required for both reactions may be Co-ARIS.

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