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PRETREATMENT EFFECTS OF JELLY COMPONENTS ON THE SPERM ACROSOME REACTION AND HISTONE DEGRADATION IN THE STARFISH, *ASTERINA PECTINIFERA*

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SUMMARY: Acrosome reaction (AR) and histone degradation (HD) of *Asterina pectinifera* sperm are induced by co-operation of ARIS and a diffusible fraction (M8) of egg jelly. Once sperm are treated with ARIS or M8 separately for several minutes, they do not undergo the AR in response to the egg jelly. Preincubation of sperm with M8 at 0°C is not effective to block the jelly-induced AR whereas inhibitory effects of ARIS remain at 0°C. Jelly-induced HD is inhibited by pretreatment of sperm with ARIS but is not affected by the incubation with M8. The blockage of the jelly-induced reactions, both AR and HD, by ARIS- or M8-pretreatment can be bypassed by ionophores, A23187 and monensin.

Egg jelly induces the degradation of sperm histones as well as the AR in the starfish, *Asterina pectinifera* (1). In the preceding paper, we showed that the HD is induced by a co-operative action of ARIS and a diffusible fraction (M8), which contains Co-ARIS and sperm-activating peptides (SAP), of the egg jelly in an analogous fashion to the induction of the AR (2-6). In another starfish, *Asterias amurensis*, it is known that neither ARIS nor M8 is effective to induce the AR but they make sperm non-responsive to the egg jelly for the AR within several minutes

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Abbreviations used are: AR, acrosome reaction; CFSW, Ca^{2+}-free seawater; DMSO, dimethylsulfoxide; HD, histone degradation; SAP, sperm-activating peptides.

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(6,7). Preincubation of sperm with pronase-digested M8 or purified Co-ARIS does not affect the competence of sperm to respond to the egg jelly (7). Certain ionophores can bypass the blockages of the jelly-induced AR by ARIS or M8 (7). These results suggest that actions of ARIS and oligopeptides are transient and irreversible, and the partially activated sperm by ARIS or M8 lose the ability of initiating the AR in respond to the egg jelly. In the present study, we examine the effects of jelly components on the jelly-induced AR and HD in the starfish, *A. pectinifera.*

Materials and Methods

All the methods used except those described below were reported in the preceding paper. ASW consisted of 450 mM NaCl, 10 mM KCl, 10 mM $CaCl_2$, 30 mM $MgCl_2$, 20 mM $MgSO_4$ and 15 mM N-(2-hydroxyethl)-piperadine-N'-3-propanesulfonic acid, pH 8.2. Calcium-free ASW (CFSW) was prepared as above except for omitting $CaCl_2$. Ionophores A23187 (dissolved in DMSO) and monensin (in DMSO:ethanol=1:1 v/v), either separately or together, were directly diluted 100-fold into sperm suspensions. For the control, 1% solvents were used. Pronase digestion of M8 was performed as described in Matsui *et al.* (7).

Results and Discussion

Egg jelly absolutely requires external Ca^{2+} in the induction of the AR of echinoderm sperm (8, 9). It is known that sperm incubated with egg jelly in CFSW lose the ability to undergo the AR by subsequent addition of sufficient Ca^{2+} in *A. amurensis* (7). In sea urchins, however, sperm treated with egg jelly in CFSW undergo the AR if Ca^{2+} is enough fortified (10). Therefore we examined first whether the pretreatment of sperm with the egg jelly in CFSW makes them incompetent in *A. pectinifera.* When sperm were treated with egg jelly in CFSW, they became within 2 min non-responsive to the egg jelly in ASW (Fig.1A). Because the AR is induced by a co-operative action of homologous ARIS and M8 in *A. pectinifera* as well as *A. amurensis*, we examined whether ARIS and M8 show a similar pretreatment effect on the sperm also in *A. pectinifera.* As shown in Fig.1B, pretreatments of sperm with either ARIS or M8 made sperm non-responsive to egg jelly. A slightly longer incubation with ARIS was necessary to

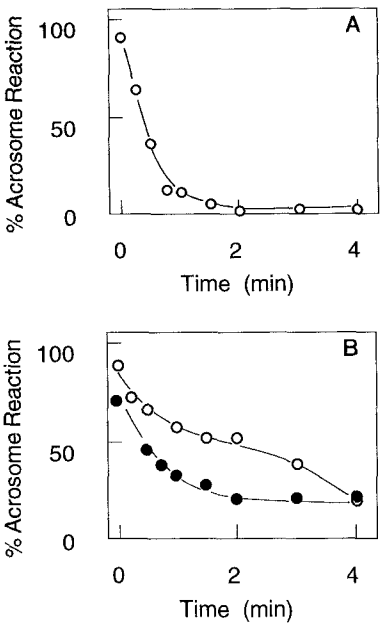

Fig.1. Effects of sequential treatments of sperm with jelly components on the AR.

A. Sperm were preincubated with egg jelly (167 μg fucose/ml) in CFSW for indicated periods on the abscissa and then fortified with Ca^{2+} (10 mM). The mixture was incubated for another 3 min and fixed.

B. Similarly, sperm were preincubated with ARIS (167 μg/ml)(○) or M8 (62.5 μg/ml)(●) for indicated periods followed by the addition of egg jelly (125 μg fucose/ml).

block the jelly-induced AR (4 min) compared to M8 (2 min) and jelly treatment in CFSW. Incubation with the egg jelly of *A. amurensis* did not block the AR of *A. pectinifera* sperm induced by homologous jelly (Table 1), indicating that pretreatment effect is also species-specific. Pronase digest of M8 did not show any pretreatment effects (Table 1), suggesting that oligopeptides in M8 are responsible for the pretreatment effects. At present, it is not clear whether the effective peptides are SAP or not, because we have not yet purified SAP from the egg jelly. Incubation of sperm with ARIS at 0 °C was effective to make them incompetent, however, sperm pretreated with M8 at 0 °C significantly underwent the AR by egg jelly (Table 2). These results suggest that the blockage by M8 of the jelly-induced AR is due to metabolic and/or enzymatic events induced by oligopeptides of M8 rather than to binding of oligopeptides to their receptors. A

Table 1. Induction of the AR in the sperm pretreated with ARIS or M8 by ionophores

Additions		Acrosome Reaction
1st	2nd	(% control)
—	Jelly	100
—	AaJelly	0
—	ARIS	18
—	M8	4
ARIS	Jelly	34
ARIS	A23187 + monensin	91
M8	Jelly	30
M8	A23187 + monensin	109
p-M8	Jelly	96
AaJelly	Jelly	93

Sperm were preincubated with ARIS (125 μg/ml), M8 (62.5 μg/ml) or jelly (250 μg fucose/ml) of *A. amurensis* (AaJelly) for 4 min. After recovery by centrifugation, they were assayed for their capacity to undergo the AR in response to homologous egg jelly (125 μg fucose/ml) or a mixture of A23187 (25 μM) and monensin (10 μM).

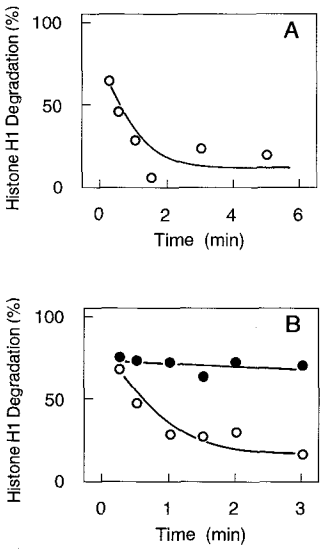

Fig.2. Effects of sequential treatments of sperm with jelly components on the HD.

A. Sperm were preincubated with egg jelly (125 μg fucose/ml) in CFSW for indicated periods and then fortified with Ca^{2+} (10 mM). The mixtures were incubated for another 60 min at 20°C and the incubation was stopped by addition of an equal volume of sample buffer for SDS-polyacrylamide gel electrophoresis.

B. Similarly, sperm were pretreated with ARIS (25 μg/ml)(○)or M8 (50 μg/ml)(●) followed by the addition of egg jelly (125 μg fucose/ml).

Table 2. Induction of the AR in sperm pretreated with ARIS or M8 at 0°C

Additions			Acrosome Reaction
1st		2nd	(% control)
—		Jelly	100
—		ARIS	7
—		M8	0
ARIS	(20°C)	Jelly	27
ARIS	(0°C)	Jelly	26
M8	(20°C)	Jelly	28
M8	(0°C)	Jelly	67

Sperm were preincubated with 100 μg/ml of ARIS or 50 μg/ml of M8 for 4 min at 20°C or 0°C. After recovery by centrifugation, they were assayed for their capacity to undergo the AR in response to 100 μg fucose/ml jelly.

mixture of A23187 and monensin could bypass the blockage of the jelly-induced AR in ARIS- or M8-pretreated sperm (Table 1).

Because HD is induced by co-operation of ARIS and M8 similar to the AR, we then examined whether the jelly-induced HD was also susceptible to the pretreatment effects. Pretreatment of sperm with egg jelly in CFSW (Fig.2A) or with ARIS (Fig.2B) made them incompetent in terms of the jelly-induced HD. In both cases, preincubation for 2 min was necessary for making them incompetent. In contrast to the AR, M8 did not show the pretreatment effect upon the jelly-induced HD (Fig.2B). Therefore, changes in sperm physiology induced possibly by oligopeptides in M8 block the jelly-induced AR but not the HD. A mixture of A23187 and monensin bypassed the blockage of the jelly-induced HD in the sperm pretreated with egg jelly in CFSW (Data not shown). This blockage was bypassed also by elevating Ca^{2+} concentration of seawater or by adding A23187 alone, both of which did not induce the HD, suggesting that pretreatment of sperm with egg jelly in CFSW inhibits the jelly-induced Ca^{2+} uptake.

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References

(1) Amano, T., Y. Okita & M. Hoshi. (1992) Develop., Growth and Differ. **34**, 99-106.
(2) Ikadai. H. & M. Hoshi. (1981) Develop. Growth and Differ. **23**, 73-80.
(3) Matsui, T., I. Nishiyama, A. Hino & M. Hoshi. (1986) Develop., Growth and Differ. **28**, 339-348.
(4) Matsui, T., I. Nishiyama, A. Hino & M. Hoshi. (1986) Develop., Growth and Differ. **28**, 359-368.
(5) Hoshi, M., T. Matsui, I. Nishiyama, T. Amano & Y. Okita. (1988) Cell Differ. and Develop. **25** (suppl.), 19-24.
(6) Hoshi, M., T. Amano, Y. Okita, T. Okinaga & T. Matsui. (1990) in "Mechanism of Fertilization", (ed. B. Dale), 239-251. NATO AST Series Vol. H45, Springer-Verlog, Berlin, Heidelbelg.
(7) Matsui, T., I. Nishiyama, A. Hino & M. Hoshi. (1986) Develop., Growth and Differ. **28**, 349-357.
(8) Dan, J.C. (1954) Biol. Bull. **107**, 335-349.
(9) Schackmann, R.W., E.M. Eddy & B.M. Shapiro. (1978) Dev. Biol. **65**, 483-495.
(10) Schackmann, R.W. & B.M. Shapiro. (1981) Dev. Biol. **81**, 145-154.