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Purification and Partial Characterization of a Lectin-like Protein from the Sea Algae *Laminaria diabolica* that Induces Fertilization Envelope Formation in Sea Urchin Eggs

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ABSTRACT—When unfertilized eggs of the sea urchin *Hemicentrotus pulcherrimus* were treated with the extracts from the sea algae *Laminaria diabolica*, fertilization envelope formation (FEF) was observed in 1.5 to 2 min without subsequent cleavage. The molecular mass of the FEF-inducing protein purified from the *Laminaria* extracts was estimated by gel-filtration to be 120 kDa, and it induced a 100% FEF at a concentration of 525 ng/ml (4.4 nM). The FEF activity of the purified protein was completely inhibited by GlcNAc, less effectively by GlcN, and not at all by various other sugars and amino-sugars, suggesting its nature as a lectin. Normal FEF and cleavage were observed when eggs were inseminated in the presence of GlcNAc. Immunohistochemical observations using antibodies against purified protein revealed its specific localization in the cells lining the wall of mucilaginous lacunae and in secretory products in the lumen. The purified protein did not activate the pretreated with verapamil or diltiazem, while ionomycin, a Ca^{2+}-ionophore, did induce activation of the similarly pretreated eggs. These results indicate that the *Laminaria* lectin triggers a very initial step in the cascade of the egg cortical reactions leading to FEF, independently of those induced by sperm.

INTRODUCTION

Current hypothesis on the mechanism of the cascade of cortical reaction at fertilization, particularly for the sea urchins, describes that the G-protein, activated by sperm bound to the egg receptor, activates phospholipase C which increases the cytosolic IP_3 levels, resulting in the release of intracellularly stored Ca^{2+} to induce cortical reaction (for review, Trimmer and Vacquier, 1986). However, the question how sperm triggers this series of egg activation events remains unanswered despite a long list of various kinds of inorganic and organic agents that exhibited induction of egg activation in sea urchins (for review, Harvey, 1956). Among other aspects concerning the relationship between the sperm reception and the egg activation is the classical study by Runnström and Kriszat (1960) who proposed that there are dual systems comprising a trypsin sensitive reception system and a periodate sensitive activation system in the egg. This hypothesis predicts the involvement of a lectin with a role in the initial step of induction of egg activation.

During the search for macromolecules which induce the activation of sea urchin eggs, we found that fertilization envelope formation (FEF) was induced efficiently in *Hemicentrotus pulcherrimus* by tissue extracts from the sea alga, *Laminaria diabolica*. The present paper reports the purification of the FEF-active lectin-like protein from the sea alga and the analysis of its mode of action in the cascade of cortical reaction.

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MATERIALS AND METHODS

Preparation of Gametes

The sea urchins *H. pulcherrimus* were collected in the vicinity of Marine Biological Station of Asamushi, Tohoku University. Other species of sea urchins *Strongylocentrotus intermedius* and *S. nudus* were collected at Akkeshi Marine Biological Station, Hokkaido University. Sea urchins were induced to shed eggs by addition of 0.5 M KCl into the body cavity, and washed with artificial sea water (ASW) before use. Particular care was taken to avoid contamination by sperm. The composition of ASW was follows: NaCl, 460 mM; KCl, 10.1 mM; $CaCl_2$, 9.2 mM; $MgCl_2$, 35.9 mM; $MgSO_4$, 17.5 mM; Tris, 10 mM (pH 8.2). Sperm were obtained by dissecting ripe testes from males. This was used as the original semen (dry sperm). Eggs were inseminated by 8×10^4 dilution of dry sperm, and the batches of eggs showing over 98% fertility were used in all experiments.

Preparation of crude extracts of sea algae

All procedures were carried out at 2–4°C. The sea algae *L. diabolica* were collected from early January to late March. The sea

algae weighing 150 g were homogenized for 2 min using a blender in 100 ml ice-cold buffer containing 30 mM NaCl, 10 mM $CaCl_2$, and 10 mM MOPS-NaOH (pH 7.2) (SCM buffer). The homogenate was centrifuged at 7,500 × g for 10 min, and the supernatant was collected for further centrifugation at 18,000 × g for 10 min. To the supernatant was added ammonium sulfate to 80% saturation. A layer of gelatinous substances near the surface was removed, and the rest of the solution was centrifuged at 25,000 × g for 15 min. The precipitates were dissolved in the SCM buffer, and were dialyzed against the same buffer. The dialyzate was centrifuged at 21,000 × g for 15 min to remove precipitates comprising sticky substances, and the volume of resultant supernatants was adjusted to 10 ml. This was referred to as the *Laminaria* extract, and stored at –70°C until use. Aliquot of the extract was diluted to 50-fold by ASW at the time of bioassays described below.

Assays of fertilization envelope formation (FEF)

To study the induction of egg activation, 5 μl of unfertilized eggs (about 800 eggs) was added to 0.5 ml of various concentrations of *Laminaria* extracts in ASW. After incubation for 5 min, the number of eggs with elevated fertilization envelopes was counted light microscopically. The percentage of eggs with FEF was defined as the rates of activation induced by the *Laminaria* extracts.

For studying the effects of N-acetyl-D-glucosamine (GlcNAc), glucose and other sugars, stock solutions of GlcNAc (5 mM), D-glucosamine hydrochloride (500 mM), and D-glucose (500 mM) were diluted with ASW at pH 8.2. To 0.5 ml of various concentrations of these sugars, 10 μl of purified Laminaria protein (0.21 mg/ml) was added for 5 min preincubation, followed by addition of 5 μl of eggs. Five min later, the rates of induced egg activation were determined as described above.

Ca^{2+}-channel inhibitors, verapamil·HCl and diltiazem·HCl, were dissolved in ASW. The Ca^{2+}-ionophore, ionomycin, was dissolved at 5 mM in ethanol, and was diluted by ASW to 10 μM. Eggs were treated for 5 min with various concentrations of verapamil or diltiazem, washed with ASW, and 5 μl of these inhibitor-treated eggs was added to 0.5 ml of purified *Laminaria* protein (4.2 μg/ml) or 10 μM ionomycin. After 5 min, the number of eggs with elevated fertilization envelopes was counted.

Chromatographies

Chromatographic procedures were performed at 2–4°C throughout. Before applying to column chromatographies, crude *Laminaria* extract was filtrated by AP-25 type (Millipore, 8 μm), followed by durapore membrane (Millipore, 0.65 μm). To a DEAE-Toyopearl 650 M column (2 × 15 cm) equilibrated with SCM buffer, 180 ml of *Lamnaria* extracts (derived from 2.7 kg sea algae) was applied. The absorbed materials were eluted by a linear gradient of NaCl in 200 ml SCM buffer from 0 to 970 mM at a flow rate of 15 ml/hr. The eluate was monitored by the absorbance at 280 nm and fractions of 4 ml were collected. Aliquots (10 μl) of each fraction were diluted 1:50 for determination of FEF activity.

The FEF-active fractions from DEAE-Toyopearl were pooled, and dialyzed against the buffer containing 0.2 M NaCl, 10 mM $CaCl_2$, and 10 mM MOPS-NaOH, pH 7.2. The dialyzate was concentrated to 4.5 ml by ultrafiltration with Millipore Immersible CX-30, and applied on a column (2 × 95 cm) of TSK gel Toyopearl HW-55F equilibrated with the same buffer, for gel-filtration at a flow rate of 15 ml/hr. Fractions of 2 ml were collected, and the aliquots were diluted to 1:50 for the assay of FEF activity. The fractions with activity were pooled, and it was dialyzed against SCM buffer, and was concentrated to 5 ml by Millipore Immersible CX-30 for application to a 15 ml column of TSK gel AF-Blue Toyopearl 650ML. The column was eluted with SCM buffer, and then with a linear gradient of KCl in 100 ml SCM buffer from 0 to 2 M at a flow rate of 15 ml/hr. Fractions of 2 ml were collected, and the FEF-active fractions were combined, concentrated and dialyzed against SCM buffer for storage at –70°C until use.

Preparation of antibodies against purified *Laminaria* protein

Purified *Laminaria* protein (0.42 mg/ 2 ml) was emulsified in an equal volume of Freund's complete adjuvant, and 4 ml of purified *Lamnaria* protein/adjuvant mixture was injected into a rabbit *via* subscapular route. A booster shot of 0.33 mg protein was given one month later. The anti-serum and preimmune serum were precipitated by 33% saturation of ammonium sulfate, and were purified by protein A column (Ampure PA Kit, Tosoh Inc.). The eluate Ig was dialyzed against phosphate buffered saline (PBS; 150 mM NaCl, 8 mM Na_2HPO_4, 1.5 mM KH_2PO_4, pH 7.4), and stored at –70°C until use.

Histology of sea algae

For immunohistological studies on *L. diabolica*, about 3 mm square pieces in the area above 10 cm from the lowest part of thallus were dissected, and fixed in a solution of 2.5% glutaraldehyde and 1% paraformaldehyde in 0.1 M cacodylate buffer containing 0.35 M sucrose (pH 7.2), for 4 hr at room temperature (18–20°C). After fixation, specimens were dehydrated in ethanol series, embedded in paraffin, and sectioned at 8 μm. The sections were stained with 1:100 dilutions of antiserum and anti-rabbit IgG-Rhodamine B (1:100 dilution) (AL13406: BIOSOURCE International, Camarillo, CA., USA) for observations with fluorescence microscope. Some sections were stained with hematoxylin and eosin.

Protein determination and electrophoresis

Protein concentration was determined by the method of Bradford (1976), using bovine serum albumin as a standard. Polyacrylamide gel electrophoresis under nondenaturing conditions was carried out with a 7.5% slab gel at pH 8.9, by the method of Davis (1964). Gels were stained with Coomassie brilliant blue.

Chemicals

The sources of the reagents and gels used in this study were as follows: N-acetyl-D-glucosamine (GlcNAc), D-galactose (Gal), N-acetyl-D-galactosamine (GalNAc), diltiazem hydrochloride, verapamil hydrochloride and molecular weight marker proteins for gel-filtration, from Sigma Chemical Co., USA; D-glucose (Glc), D-glucosamin hydrochloride (GlcN), L-glucose (L-Glc), D-mannose (Man), L-fucose (Fuc), N-acetylneuraminic acid (NANA), maltose, sucrose, and ammonium sulfate, from Nacalai Tesque, Inc., Japan; DEAE-Toyopearl 650M, Toyopearl HW-55F, and AF-Blue Toyopearl 650ML, from Tosoh Inc., Japan; 3-morpholinopropansulfonic acid (MOPS) from Dojindo Laboratories Co., Japan; ionomycin from Hoechst, La Jolla, CA, USA; molecular weight marker "DAIICHI" (for Davis' method) from Daiichi Pure Chemnicals Co., LtD., Japan.

RESULTS

Laminaria extract elicits fertilization envelope formation (FEF)

When unfertilized eggs of *H. pulcherrimus* were immersed in 1:50 (21 μg/ml)–1:100 (10.5 μg/ml) dilutions of *Laminaria* extracts, fertilization envelope was formed in more than 99% of eggs in 1.5 to 2 min (Fig. 1). Electron microscopic observation revealed that the cortical glanules were broken down in these eggs as in normally fertilized eggs (data not shown). No cytolysis was discernible in the eggs exposed to the extracts up to 5 hr. The number of the eggs with fully elevated fertilization envelope sigmoidally decreased at concentrations lower than 1:200 dilutions (5.25 μg/ml) (Fig. 2). No FEF was observed when the eggs of other species, *S. intermedius* or *S. nudus*, was treated with the extracts in 1:50 dilutions.

The ability of *Laminaria* extracts to induce FEF was com-

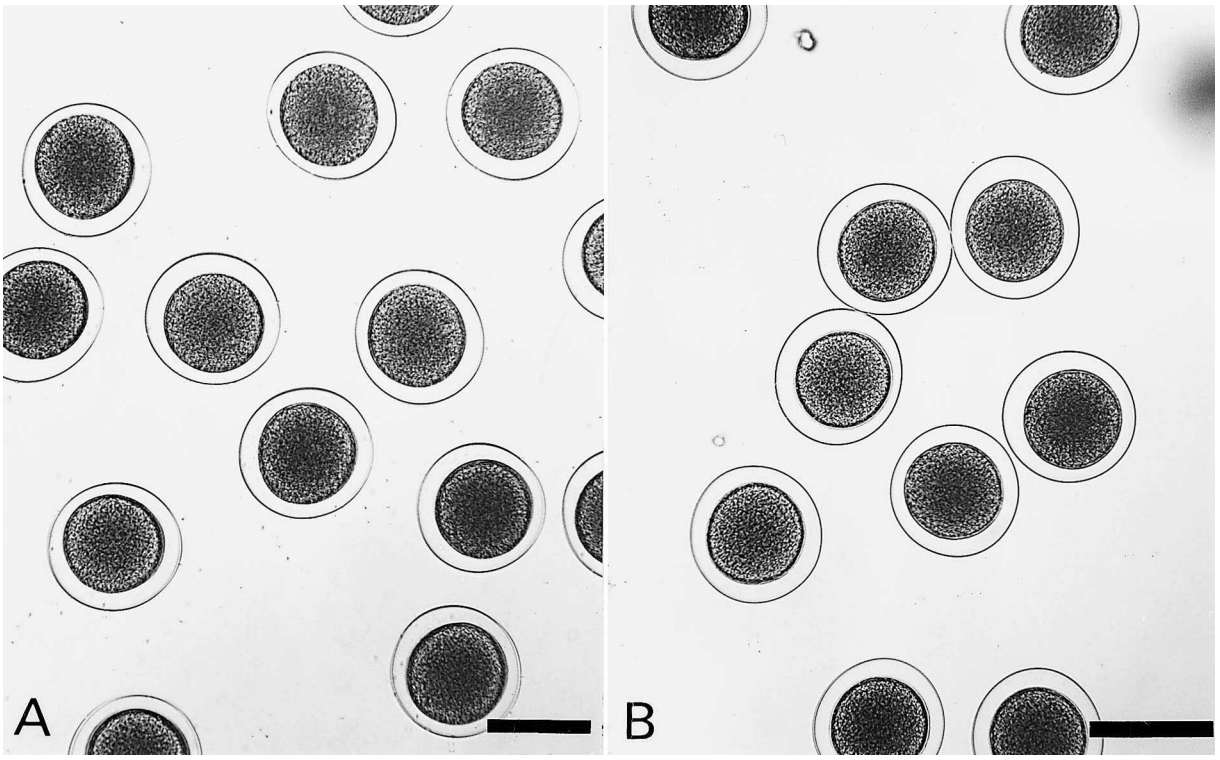

Fig. 1. The eggs of *H. pulcherrimus* showing formation of fertilization envelope 2 min after insemination (**A**) or treatment with 50-fold dilutions of *Laminaria* extract (**B**), at 15°C. Scale bars indicate 100 μm.

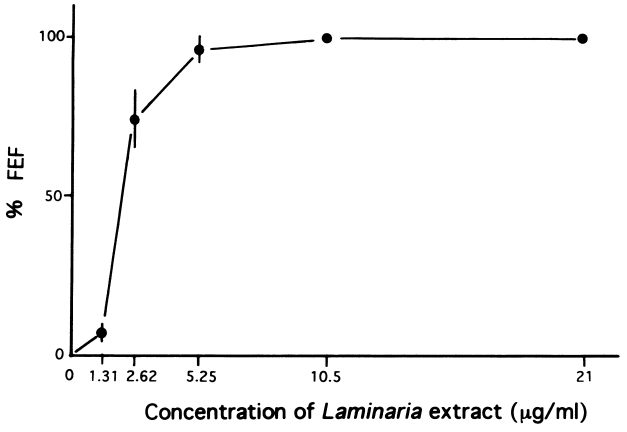

Fig. 2. Activation of *H. pulcherrimus* eggs induced by crude extracts from *L. diabolica*. Eggs were incubated in various concentrations of the extracts at 15°C, and the percentage of eggs with elevated fertilization envelope was scored 5 min after incubation. Values are means ± standard error of 3 separate experiments using different batches of eggs. Undiluted extracts contained 1.05 mg/ml proteins.

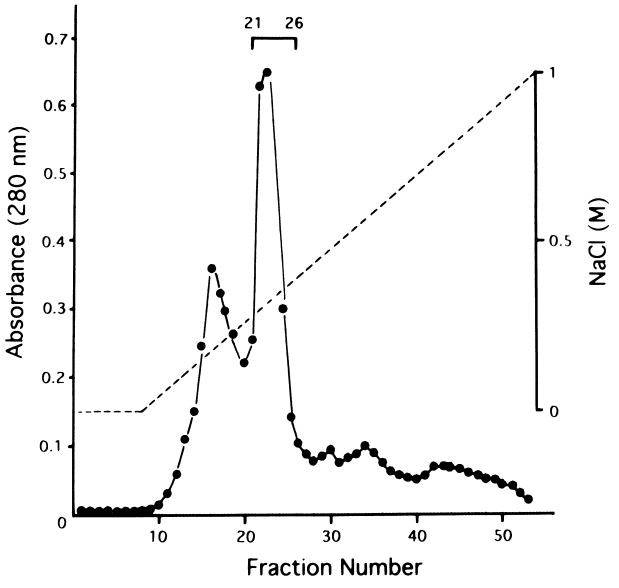

Fig. 3. DEAE-Toyopearl chromatography of the crude extracts from sea algae. The extracts derived from 2.7 kg algae was chromatographed as described in MATERIALS AND METHODS, and each fraction was monitored by the absorbance at 280 nm. A horizontal bar on top indicates the fractions exhibiting 100% fertilization envelope formation (FEF) upon dilution at 1:50, and these fractions were pooled for further gel-filtration shown in Fig. 4.

pletely lost after heating at 60°C for 5 min. In preliminary attempts to characterize the active components contained in the extracts, it was found that the extracts cause aggregation of Sephadex-G gels, suggesting its lectin-like nature.

Purification of FEF-inducing activity from *Laminaria* extracts

The extracts derived from 2.7 kg sea algae (180 ml) were applied to DEAE-Toyopearl column (Fig. 3). The fractions of the highest protein peak (fractions 21–26) eluted with about 0.3 M NaCl had the FEF activity upon dilution to 1:50.

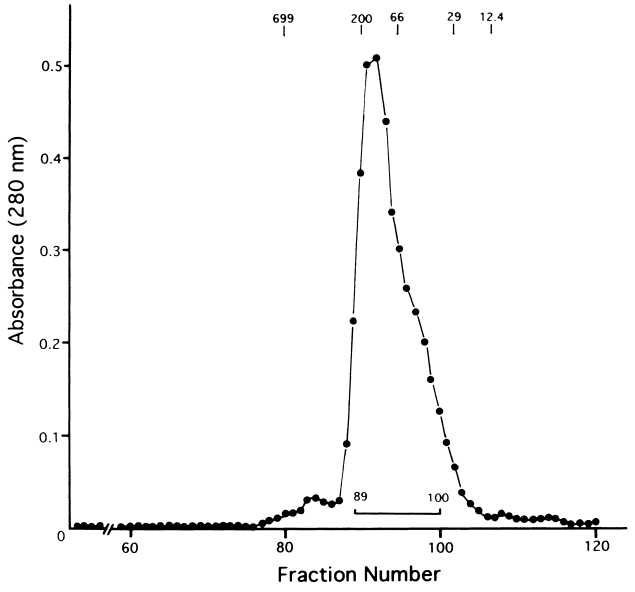

Fig. 4. Gel-filtration on Toyopearl HW-55F of FEF-active fractions from DEAE-Toyopearl chromatographed extracts shown in Fig. 3. The fractions 21-26 in Fig. 3 were gel-filtrated under the conditions given in MATERIALS AND METHODS, and the absorbance at 280 nm and the FEF activity of each fraction were measured. A horizontal bar at the bottom indicates fractions exhibiting 100% FEF activity upon dilution at 1:50. Numbers on the top indicate the elution positions of molecular mass markers in kDa: 669 (thyroglobulin); 200 (β-amylase); 66 (bovine serum albumin); 29 (carbonic anhydrase); 12.4 (cytochrome ***c***).

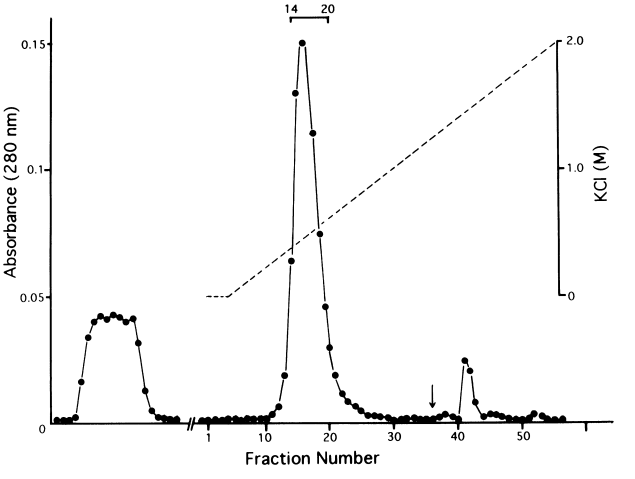

Fig. 5. Chromatography on AF-Blue Toyopearl of FEF active fractions from Toyopearl HW-55F gel-filtration shown in Fig. 4. The active fractions 89–100 in Fig. 4 were chromatographed under the conditions given in MATERIALS AND METHODS, and the bar at the top shows the fractions exhibiting 100% FEF activity upon dilution at 1:50.

The FEF-active fractions (fractions 21–26) from DEAE-Toyopearl column were pooled, dialyzed against the buffer which is basically similar to SCM but differs in containing 0.2 M NaCl, and were applied to gel filtration on Toyopearl HW-55F (Fig. 4). The main peak fractions (fractions 89–100) with the FEF-activity upon dilution to 1:50 were pooled, and replaced by SCM buffer for group affinity-chromatography on AF-Blue Toyopearl 650ML (Fig. 5). The fractions (fractions 14–20) eluted by 0.4 M KCl contained the FEF-activity. These fractions were pooled and concentrated to 3.5 ml. Assays for its FEF activity revealed that a 100% FEF was induced upon dilution at 1:400, *i.e.*, at the concentration of 525 ng/ml. Native PAGE of FEF-active fractions after these fractionation steps gave a single band (Fig. 6). The molecular mass of active component in *Laminaria* extract was estimated to be about 120 kDa, based on gel filtration on Toyopearl HW-55F as shown in Fig. 4. Thus a half maximal concentration of a FEF-active substance extrapolated from Fig. 2 was 2.5 μg/ml.

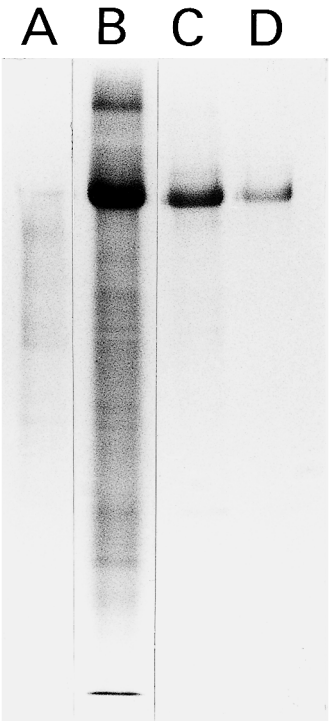

Fig. 6. Electrophoresis under non-denaturing conditions of FEF-active fraction at various steps of purification shown in Fig. 3–5. Each lane was loaded with 14 μl sample. A, crude extract; B, active fraction of DEAE Toyopearl chromatography; C, active fraction of gel-filtration on Toyopearl HW-55F; D, active fraction of group affinity chromatography on AF-Blue Toyopearl.

The mode of action of FEF-active substance

As mentioned in the preceding section, the aggregation of Sephadex-G gels induced by the extracts suggests its lectin-like nature. To verify this assumption, effects of various sugars on FEF-activity were examined. When eggs were treated with the medium containing 4.2 μg/ml purified *Laminaria* protein and various concentrations of GlcNAc, the number of eggs with fully elevated fertilization envelope reciprocally decreased as the concentrations of GlcNAc increased, with a 100% inhibition of FEF at 24 μM GlcNAc (Table 1). When eggs were inseminated in the presence of 3-24 μM GlcNAc, however, fertilization envelopes were formed by normal cleavage.

Table 1. Effects of co-incubation with GlcNAc, GlcN, and Glc on the fertilization envelope formation (FEF) activity of purified *Laminaria* protein. Purified *Laminaria* protein (4.2 μg/ml) was preincubated for 5 min with various concentrations of amino-sugars or sugars, to which unfertilized eggs were added 5 min later for determination of FEF. Results from 2 experiments on distinct females (Expt. 1 and 2) are shown.

Sugars	Concentration	%FEF	
		Expt. 1	Expt. 2
	(Control)	100	100
GlcNAc	24 (μM)	0	0
	12	3	43
	6	73	100
	3	95	100
GlcN	96 (mM)	0	0
	48	2	7
	24	60	98
	12	90	100
	6	88	100
	3	90	100
Glc	96 (mM)	23	99
	48	74	99
	24	92	100
	12	94	100

GlcN also inhibited the FEF-activity of purified *Laminaria* protein, but it required more than 2,000 times higher concentration than GlcNAc to supress the FEF. The inhibitory effect of Glc was much more weaker than GlcN (Table 1). No inhibitory effect on FEF was observed when other sugars, galactose, N-acetyl-D-galactosamine, L-glucose, D-mannose, L-fucose, N-acetylneuraminic acid, maltose, and sucrose were applied to the eggs along with the purified protein. These results indicate that the FEF-active protein in *Laminaria* extracts is a lectin specifically reactive to GlcNAc.

In an attempt to elucidate the mechanism of *Laminaria* protein-induced FEF in the cascade of egg activation, the effects of Ca^{2+}-channel inhibitors on FEF were studied. Unfertilized eggs were pretreated with various concentrations of verapamil or diltiazem, and then were treated with purified *Laminaria* protein (4.2 μg/ml) or the Ca^{2+}-ionophore, ionomycin (10 μM). The results depicted in Fig. 7 revealed that only about 14% of the eggs pretreated with 200 μM diltiazem were activated by the *Laminaria* protein while more than 98% of eggs pretreated was activated by 10 μM ionomycin. Similarly, pretreatment with varapamil at 200 μM reduced the *Laminaria* protein-induced FEF to about 27%. Treatment of eggs with 500 μM diltiazem or verapamil completely inhibited egg activation induced by purified *Laminaria* protein (Fig. 7). In contrast, ionomycin-mediated FEF was not affected at all by treatment with Ca^{2+}-channel inhibitors of any concentration, indicating that the inhibitors used did not cause abortive effects on the cascade of cortical reaction.

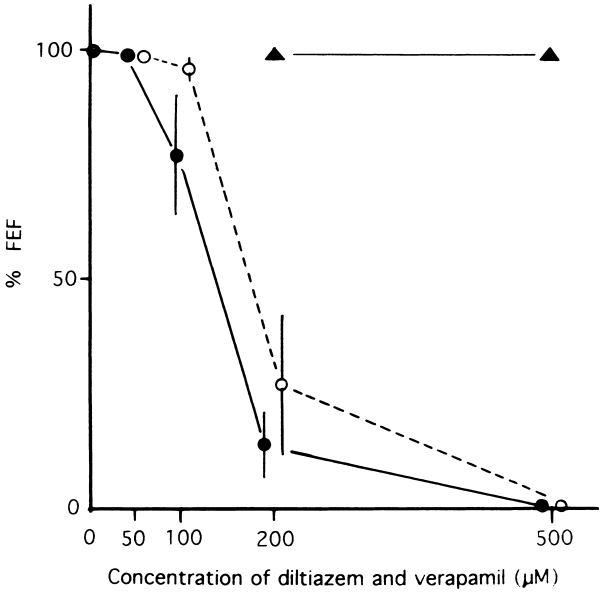

Fig. 7. Effects of Ca^{2+}-channel inhibitors on FEF-inducing activity of *Laminaria* protein and ionomycin. Eggs were preincubated for 5 min with various concentrations of diltiazem (●) or verapamil (○), washed twice, and were treated with purified *Laminaria* protein (4.2 μg/ml). ▲, treated with diltiazem or verapamil and activated with 10 μM ionomycin. The percentage of the eggs with fertilization envelope was scored 5 min after addition of *Laminaria* protein or ionomycin. Values shown are means ± standard errors of the results from 3 experiments with different females.

Immunohistological localization of FEF-active protein in sea algae

Ten μl of solution containing 0.21 mg/ml of purified *Laminaria* protein was added to 0.5 ml of ASW containing various concentrations (1–4%) of anti-*Laminaria* lectin protein IgG. After incubation of eggs in these solutions for 10 min, the number of eggs with FEF decreased to 12.5% and 0% in Ig solutions at 3% and 4%, respectively. There was no inhibition of FEF in eggs incubated in the *Laminaria* protein with 3%–4% Ig from normal serum.

Immunohistological staining of *L. diabolica* tissues demonstrated an array of strongly fluorescent masses located 350 μm deep from and parallel to the surface of thallus (Fig. 8A and 8B). Control staining comprising treatments with Ig from normal rabbit serum or anti-rabbit Ig without pretreatment with anti-*Laminaria* protein antibodies revealed a weak fluorescence in the outermost layer of cells (Fig. 8C), indicating that the strongly fluorescent masses shown in Fig. 8A represent specific reaction to *Laminaria* protein. Hematoxylin stained sections revealed that strongly fluorescent masses represent the mucilaginous lacunae which comprise secretory cells and deposition of secretory products (Fig. 8D). These observations clearly indicate that the purified *Laminaria* protein is produced and secreted from cells lining the wall of mucilaginous lacunae.

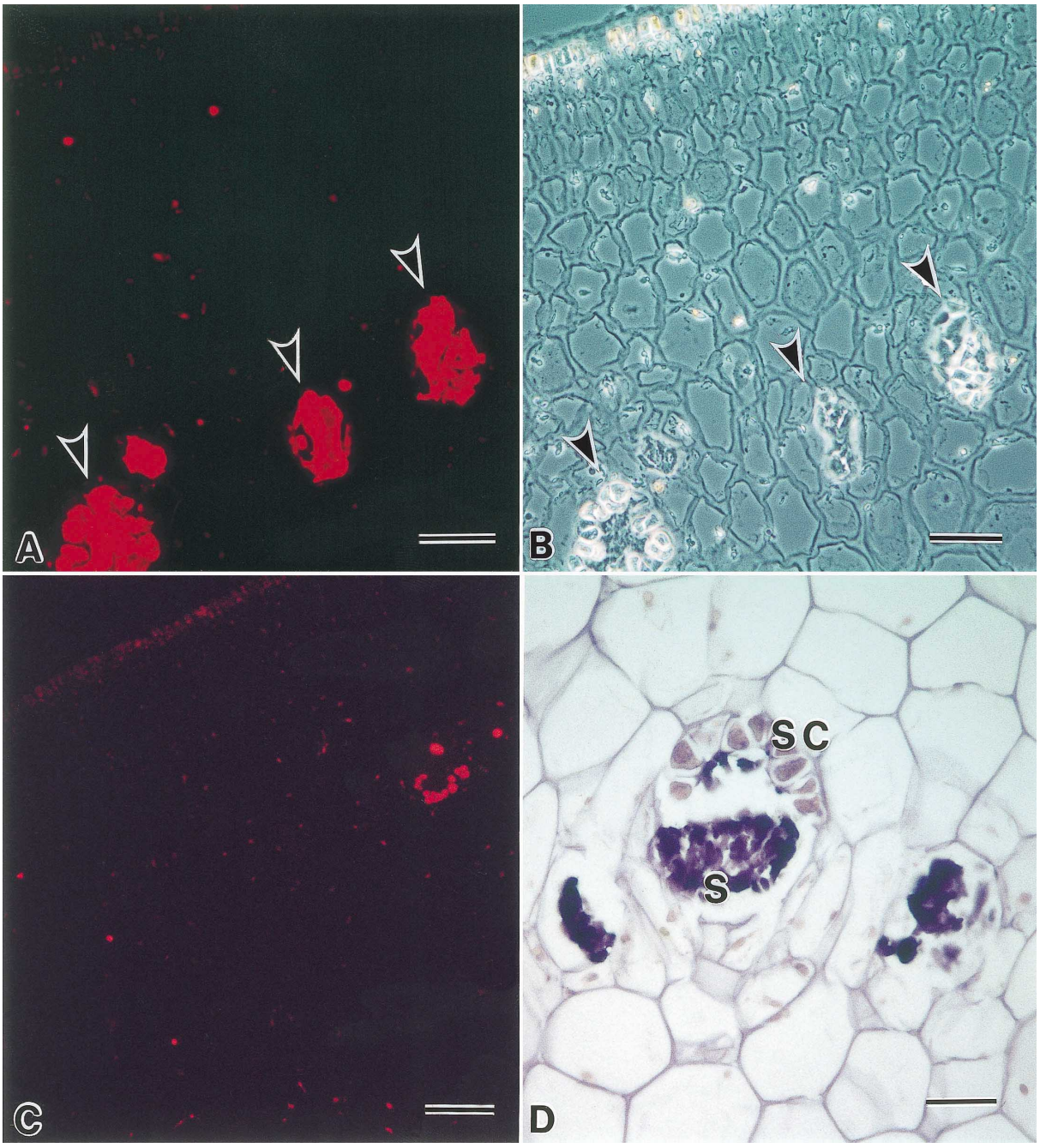

Fig. 8. Transverse sections through the thallus of *L. diabolica*, showing immunohistochemical localization of *Laminaria* protein in the mucilaginous lacunae (arrow heads in Fig. 8A and 8B). **A**, stained with anti-*Laminaria* protein and Rhodamine B-labeled anti-rabbit IgG; **B**, phase contrast view of the section shown in A; **C**, control stained with Ig from normal rabbit serum; **D**, stained with hematoxylin and eosin showing mucilaginous lacunae with secretory cells (SC) and secretory materials (S). Scales in A–C: 50 μm; D: 100 μm.

DISCUSSION

We have shown in this study that the protein purified from the sea algae with a highly efficient activity of fertilization envelope formation (FEF) in *H. pulcherrimus* eggs is a lectin-like protein with reactivity specific to GlcNAc. No FEF-activity has so far been observed in other sea urchin species tested, *i.e.*, *S. intermedius* and *S. nudus*. Preliminary attempts in our laboratory to collect this lectin-like protein have indicated that the amount of this material contained in the algal tissue is highly variable depending on the seasons of a year: the highest during January-March period and the lowest during April-July period, reflecting the difference in growth phase of this plant. Our immunohistochemical observations have revealed that this protein is produced in and secreted from the cells lining the wall of mucilaginous lacunae, although its function in the

life of *Laminaria* algae is not known. It is of interest that this specific algae do not inhabit in the areas where the sea urchin *H. pulcherrimus* inhabit, thus naturally avoiding the possibility of spontaneous FEF in this species of sea urchin.

It has not been successful to locate the receptors for the *Laminaria* lectin in the *H. pulcherrimus* eggs. Surprisingly low concentration of GlcNAc that effectively inhibits FEF activity of the lectin (Table 1) suggests that an extremely small number of the receptor is sufficient for eliciting the FEF reactions in the egg. Although the outer surface of the (extracellular) vitelline envelope could be the primary site of the contact with this lectin, it is not conceivable that our lectin can induce FEF without binding to the receptor on the egg surface (plasma membrane) or egg cortical cytoplasm. Perhaps it is relevant to mention that *Laminaria* lectin failed to elicit FEF in the eggs pretreated with Ca^{2+} channel inhibitors, under the conditions that Ca^{2+} ionophore did successfully induce FEF. In view of the well-documented facts that Ca^{2+} ionophores affect directly to release intracellularly stored Ca^{2+} to induce the cortical reaction in the egg (Chambers *et al*., 1974; Steinhardt and Epel, 1974), the results presented in Fig. 7 indicate that *Laminaria* lectin acts on the initial steps of the cascade of egg cortical reactions, probably at the step well before the formation of IP_3. Thus, the GlcNAc-containing receptors reactive with *Laminaria* lectin conceivably be localized in the egg plasma membrane.

The occurrence of an entirely normal fertilization in the presence of GlcNAc that perfectly inhibits the lectin-induced FEF implies that *Laminaria* lectin does not imitate the function of a fertilizing sperm. There may be a system on the egg surface which mediates the sperm reception toward the G-protein activation in the egg *via* bypassing the GlcNAc and counteracting lectin interactions demonstrated in the present study. Regarding the sperm reception and the egg activation for the sea urchins, Runnström and Kriszat (1960) have proposed the participation of dual systems, *i.e*, a trypsin-sensitive reception system and a periodate-sensitive activation system. Of these, the latter was proposed based on the observed activating effect of periodate which was predicted to remove inhibitory mucopolysaccharides present on the egg surface. Our finding of lectin-carbohydrate interactions as an inducer of an egg activation is compatible with this classical hypothesis, further suggesting that it is distinct from a trypsin-sensitive sperm reception system. There has been a wealth of evidence showing that bindin exposed on the surface of sperm acrosomal process reacts with its receptor on the egg surface in a manner of lectin-carbohydrate interactions (Vacquier and Moy, 1977; Glabe *et al*., 1982). Bindin, however, does not activate unfertilized eggs (Glabe and Vacquier, 1977; Glabe and Lennarz, 1979; Minor *et al*., 1989), probably because its receptor may be localized not on the egg plasma membrane but on the surface of vitelline envelope. Currently there is a gap in the information as to the molecular mechanisms mediating the initial sperm binding and the initiation of signal transduction which leads to egg activation. A candidate for the egg receptor molecule responsible for mediating sperm bindin and egg activation has been found in the eggs of *S. purpuratus* (Foltz and Lennarz, 1990, 1992), but its exact function in the process of egg activation awaits further studies. Thus, under the current paucity of information on the mechanisms of sperm-induced egg activation, the *Laminaria* protein purified in the present study could be a useful tool for analyzing the initial steps of signal transduction process that leads to G-protein activation in the egg cytoplasm, although it does not mimic the function of a fertilizing sperm.

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