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Effect of Zinc Ion on Formation of the Fertilization Membrane in Sea Urchin Eggs

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ABSTRACT—The effect of Zn^{2+} on the formation of the fertilization membrane was observed in eggs of the sea urchin, *Anthocidaris crassispina*. When the eggs were transferred into sea water containing more than 5 μM Zn^{2+} within 10 sec after insemination, formation of the fertilization membrane was inhibited. This inhibition was irreversible. Although the time of the first cleavage was delayed in a dose-dependent fashion, cleavage occurred normally in the presence of up to 1 mM Zn^{2+}. Electron microscopic examinations revealed that the exocytosis of cortical granules progressed. However, the elevation and the hardening of the vitelline layer were blocked by more 5 μM Zn^{2+}.

INTRODUCTION

When sea urchin eggs are fertilized or artificially activated, cortical granules, which are located beneath the plasma membrane, are exocytotically discharged and cortical granule components are released into the perivitelline space. Some of the components hydrate and elevate the fertilization membrane, while others are incorporated into the fertilization membrane to make it thick and tough. Thus the fertilization membrane is formed around the eggs [see references for review 1, 6].

It is well known that Zn^{2+} inhibits the formation of the fertilization membrane [2, 4, 7]. However, the mechanism of the inhibition by Zn^{2+} is unknown. Moreover, not much is known about the mechanism of the fertilization membrane formation itself. We consider that clarifying the inhibition mechanism by Zn^{2+} may provide information concerning the mechanism of formation of the fertilization membrane. As our first step, we observed in detail the effect of Zn^{2+} on formation of the fertilization membrane. The results suggest that Zn^{2+} inhibits the elevation and hardening of the fertilization membrane occurring after the cortical reaction in the process of the fertilization membrane formation.

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MATERIALS AND METHODS

Gametes from the sea urchin, *Anthocidaris crassispina,* were collected by intracoelomic stimulation with 0.5 M KCl. Sperm was kept 'dry' in a refrigerator until use; eggs were spawned into filtered sea water, and washed three times by sedimentation. Experiments were done at 25–29°C.

Small amounts of Zn^{2+} from a stock solution (100 mM $ZnCl_2$) in deionized water were added to the experimental sea water. The eggs were transferred to the sea water containing Zn^{2+} 10–60 sec after insemination.

Formation of the fertilization membrane was observed with an inverted microscope (Olympus CK-2). Micrographs were taken on Fuji Neopan F film.

Eggs for electron microscopy were fixed in 2% glutaraldehyde in 80% sea water (pH 7.0), and post-fixed in 1% OsO_4 in 50 mM sodium cacodylate (pH 7.2). After dehydration through an ethanol series, they were infiltrated and embedded in epoxy resin (Epok 812, Oken Shoji Co., Ltd., Tokyo). Ultrathin sections were stained with uranyl acetate and Reynold's lead citrate and examined with a JEOL 100C electron microscope operated at 80 kV.

RESULTS AND DISCUSSION

The eggs of *Anthocidaris crassispina* were treated with various concentrations of Zn^{2+} from 10, 30 and 60 sec after insemination. Figure 1 shows the

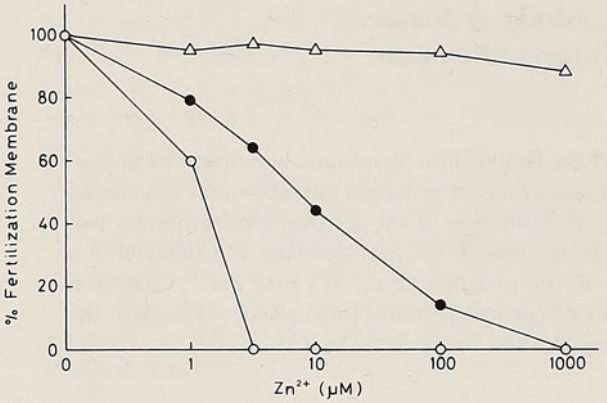


FIG. 1. Effect of Zn^{2+} on formation of the fertilization membrane in *Anthocidaris crassispina* eggs. At 10 sec (○), 30 sec (●), or 60 sec (△) after insemination, the eggs were transferred into sea water containing various concentrations of Zn^{2+}.

effects of increasing Zn^{2+}-concentration on formation of the fertilization membrane. Although the rate of inhibition varied from experiment to experiment, formation of the fertilization membrane was completely prevented when more than 5 μM Zn^{2+} was applied within 10 sec after insemination (Figs. 1 and 2b). This inhibition was irreversible. When eggs without a fertilization membrane in 5 μM Zn^{2+} -containing sea water were removed and placed in fresh sea water, they could not form a fertilization membrane. The surfaces of the eggs in Zn^{2+}-containing sea water were rougher than those of unfertilized eggs in normal sea water (Fig. 2b, c).

To confirm that fertilization occurred in the eggs without a fertilization membrane in Zn^{2+}-containing sea water, we measured percentage of cleavage in those eggs (Fig. 3). The time of the first cleavage was delayed by Zn^{2+} in a dose-dependent manner, but cleavage occurred normally in the presence of up to 1 mM Zn^{2+}. For

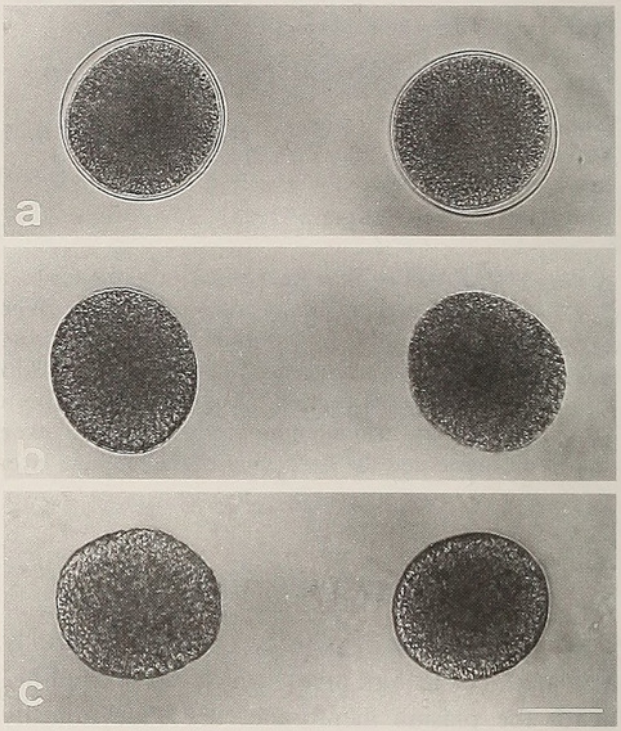


FIG. 2. Formation of the fertilization membrane in eggs treated or untreated with Zn^{2+}. (a) Control, (b) 5 μM, and (c) 1 mM Zn^{2+}. Bar, 50 μm.

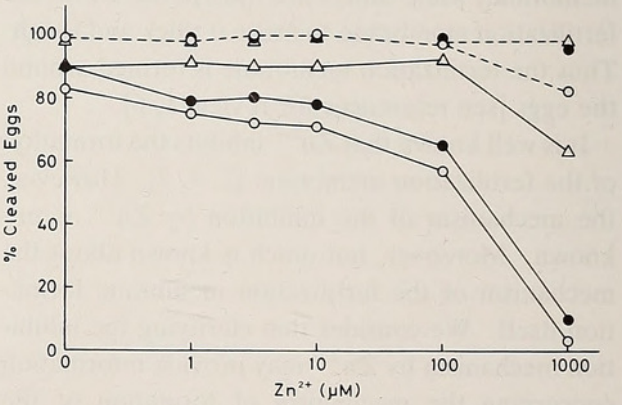
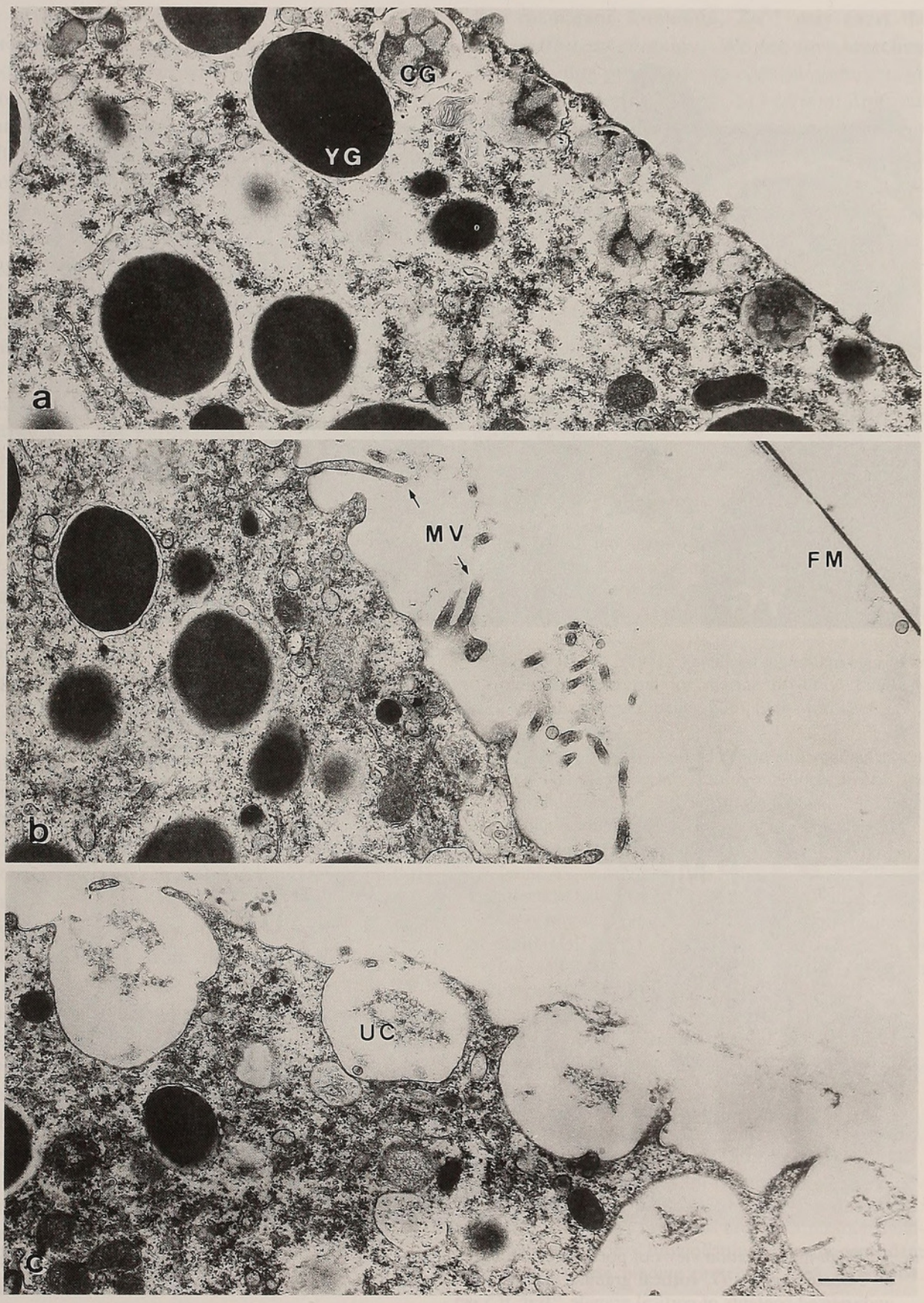


FIG. 3. Percentage of cleaved eggs in the presence of various concentrations of Zn^{2+}. At 10 sec (○), 30 sec (●), or 60 sec (△) after insemination, the eggs were transferred into sea water containing Zn^{2+}. The percentage was measured at 55 min (solid line) and 65 min (dotted line) after insemination.

FIG. 4. Electron micrographs of sea urchin eggs. The eggs were fixed at 10 min after insemination. An unfertilized egg (a), a fertilized egg (b), and a fertilized egg treated with 5 μM Zn^{2+} from 10 sec after insemination (c). CG, cortical granule; FM, fertilization membrane; MV, microvillus; YG, yolk granule; UC, undispersed contents of cortical granule. Bar, 1 μm.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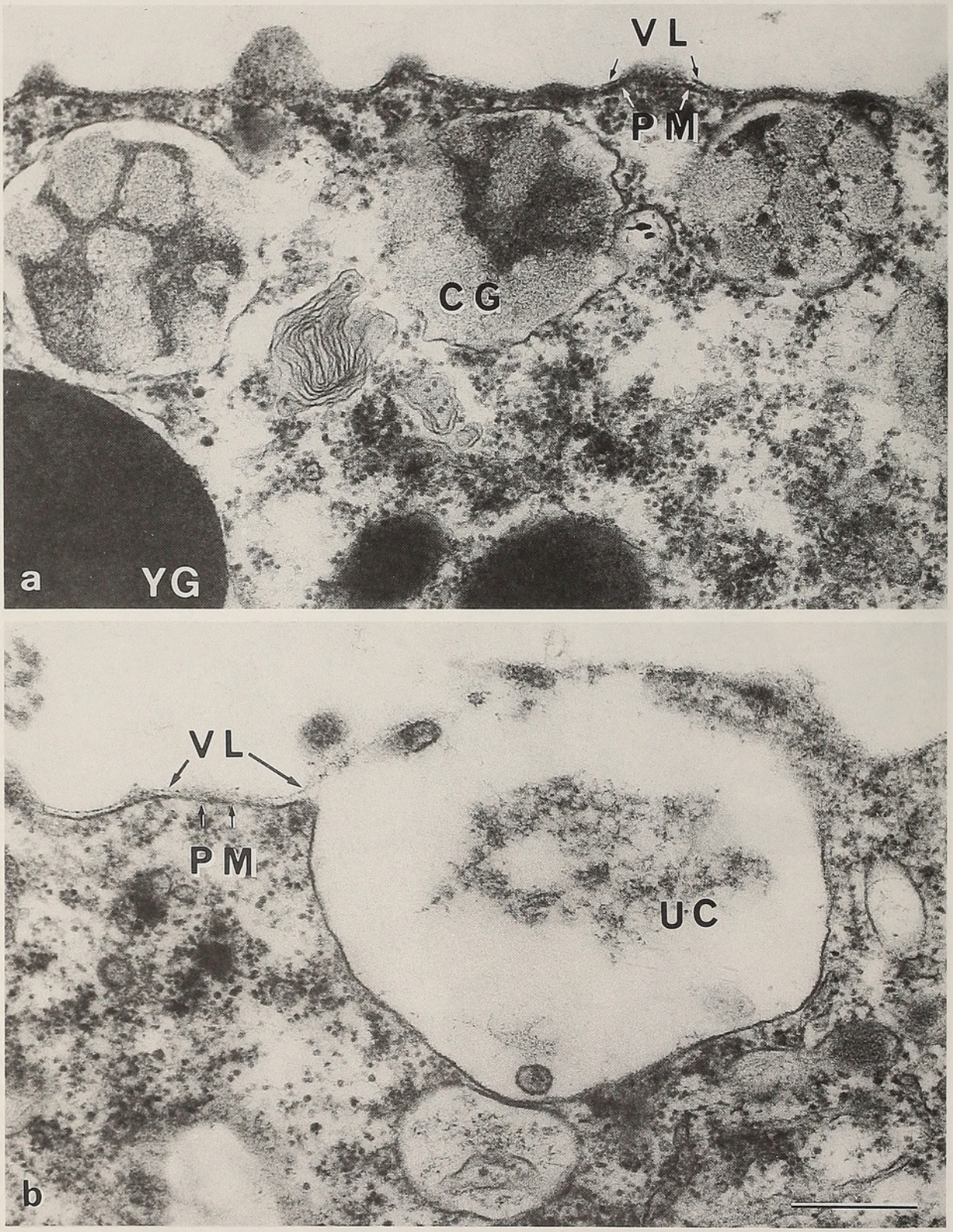

FIG. 5. High-magnification views of portions of the eggs shown in Fig. 4 (a) and (c). (a) Unfertilized egg, and (b) Z^{2+}-treated egg. CG, cortical granule; VL, vitelline layer; PM, plasma membrane; YG, yolk granule; UC, undispersed contents of cortical granule. Bar, 0.5 μm.

ly in the presence of up to 1 mM Zn^{2+}. For example, 3% of eggs transferred into 1 mM Zn^{2+} -containing sea water within 10 sec after insemination cleaved by 55 min after insemination, but the rate of cleavage had recovered to 82% by 65 min after insemination. This result shows that fertilization occurred normally in Zn^{2+}-treated eggs.

Next, we examined by electron microscopy whether the cortical granules react normally in eggs treated with Zn^{2+} (Figs. 4 and 5). Exocytosis of cortical granules occurred in Zn^{2+}-containing sea water (Figs. 4c and 5b), i.e., the membrane of cortical granules fused with the plasma membrane of the egg and the contents were released out of the egg. However, dispersion of the contents was incomplete, and part of them were undispersed by 10 min after insemination. Moreover, detachment of the vitelline layer from the plasma membrane and hardening of the vitelline layer were blocked by more than 5 μM Zn^{2+}. This resulted in rupture of the vitelline layer by the pressure from influx of water into the perivitelline space or swelling of the cortical granule components. The undispersed contents may relate to the detachment and hardening of the vitelline layer.

In normal formation of the fertilization membrane, the following processes occur [1, 6]. (a) Intracellular concentration of free Ca^{2+} is increased by fertilization or artificial activation. (b) This Ca^{2+} triggers the cortical granule exocytosis. (c) Proteases in the cortical granules break the attachment between the vitelline layer and the plasma membrane of the eggs. (d) The vitelline layer is elevated by hydration and/or osmotic effects resulting from the secretion of cortical granule contents. (e) The vitelline layer is hardened through cross-linkage and structuralization with cortical granule-derived proteins, such as ovoperoxidase [1, 6] and proteoliaisin [8]. The results we obtained in this study suggest that Zn^{2+} inhibits steps (c), (d) and (e) in the process of formation of the fertilization membrane.

Zn^{2+} is a potent animalizing agent in sea urchin embryos. However, we do not know how animalization is induced by Zn^{2+}. Lallier [3] suggested that when Zn^{2+} binds to proteins, their structure and biochemical properties may become altered, so that abnormal development occurs. In fertilization membrane formation, Zn^{2+} may exert the same effect on proteins. We are now searching Zn^{2+}-binding protein in the cortical granule components of unfertilized eggs. In addition, Zn^{2+} has an affinity for -SH groups, and action of Zn^{2+} on the enzymes whose activity depends on -SH groups has also been suggested [5]. It should be noted that -SH groups may play an important role in the elevation and the hardening of the vitelline layer.

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