Isolation and Partial Characterization of the Plasma Membrane of the Sea Urchin Egg

WILLIAM H. KINSEY, GLENN L. DECKER, and WILLIAM J. LENNARZ
Department of Physiological Chemistry, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

ABSTRACT The cell surface complex (Detering et al., 1977, J. Cell Biol. 75, 899-914) of the sea urchin egg consists of two subcellular organelles: the plasma membrane, containing associated peripheral proteins and the vitelline layer, and the cortical vesicles. We have now developed a method of isolating the plasma membrane from this complex and have undertaken its biochemical characterization. Enzymatic assays of the cell surface complex revealed the presence of a plasma membrane marker enzyme, ouabain-sensitive Na+/K+ ATPase, as well as two cortical granule markers, proteoesterase and ovoperoxidase. After separation from the cortical vesicles and purification on a sucrose gradient, the purified plasma membranes are recovered as large sheets devoid of cortical vesicles. The purified plasma membranes are highly enriched in the Na+/K+ ATPase but contain only very low levels of the proteoesterase and ovoperoxidase. Ultrastructurally, the purified plasma membrane is characterized as large sheets containing a "fluffy" proteinaceous layer on the external surface, which probably represent peripheral proteins, including remnants of the vitelline layer. Extraction of these membranes with KI removes these peripheral proteins and causes the membrane sheets to vesiculate. Polyacrylamide gel electrophoresis of the cell surface complex, plasma membranes, and KI-extracted membranes indicates that the plasma membrane contains five to six major proteins species, as well as a large number of minor species, that are not extractable with KI. The vitelline layer and other peripheral membrane components account for a large proportion of the membrane-associated protein and are represented by at least six to seven polypeptide components. The phospholipid composition of the KI-extracted membranes is unique, being very rich in phosphatidylethanolamine and phosphatidylinositol. Cholesterol was found to be a major component of the plasma membrane. Before KI extraction, the purified plasma membranes retain the same species-specific sperm binding property that is found in the intact egg. This observation indicates that the sperm receptor mechanisms remain functional in the isolated, cortical vesicle-free membrane preparation.

The sea urchin egg has been the object of much study in the areas of molecular and developmental biology during the last several decades. Recently, this cell type has gained increasing importance in studies in cell-cell adhesion, exocytosis, and other membrane-associated events. It is clear that the sea urchin egg provides an excellent model for these studies of more general biological interest, as well as for studies related to developmental and reproductive biology per se.

Despite the interest in this cell type, the cell surface of the echinoderm egg has received little attention and is poorly understood at the biochemical level. The available information indicates that the egg is surrounded by a morphologically "typical" plasma membrane, coated on the outer surface with a highly developed array of external peripheral proteins collectively termed the vitelline layer. The vitelline layer has been studied in some detail (9, 10, 22, 31, 32). It is known to contain glycoproteins, one or more of which may be involved in sperm-egg adhesion (9, 23, 32), and is involved in formation of the fertilization envelope after the egg is fertilized. The cytoplasmic face of the plasma membrane has associated with it arrays of specialized secretory granules, the cortical vesicles, which undergo exocytosis upon fertilization.

Earlier work in this laboratory resulted in the isolation of a cell surface complex consisting of the plasma membrane, with
was isolated, ultracentrifugation of diisopropylfluorophosphate (0.5 M in isopropanol) was used for concentration of 10 kallikrein inactivator units/ml. After the cellsurface complex was prepared as described (6) except that a 40-ml homogenizing buffer, containing 5 mM HEPES buffer, pH 7.5, mM EGTA, pH 7.5, and Trasylol, 10 U/ml. The procedure for the preparation of plasma membranes was as described above, except that the membrane sheets were present as a single band, whereas the residual cortical vesicles were found in the pellet.

Preparation of Plasma Membranes

A typical preparation began with 0.5-0.8 ml of packed cell surface complex. This was suspended in 10 vol of 1.0 M sucrose adjusted to pH 8.0 with KOH and containing 10 U/μl of Trasylol. The cell surface complex was pelleted in a clinical centrifuge and then resuspended in 100 vol of this sucrose solution. The suspension was gently homogenized in a glass homogenizer with a Teflon pestle (VWR Scientific Inc., San Francisco, Calif.) until the cortical vesicles were completely detached, as determined by phase-contrast microscopy. This procedure produced large sheets of plasma membrane, as well as smaller membrane fragments and free cortical vesicles. The membrane sheets were separated from the cortical vesicles and small membrane fragments by centrifugation in a Sorvall SS34 fixed angle rotor (DuPont Instruments-Sorvall, DuPont Co., Newton, Conn.) at 5,000 rpm for 20 min at 5°C. The supernate was discarded, and the pellet, consisting largely of the membrane sheets, was resuspended in 100 vol of the sucrose solution by gentle homogenization. This suspension was again centrifuged at 5,000 rpm for 20 min. The resulting pellet, largely depleted of cortical vesicles, was then resuspended in the sucrose solution and layered on a discontinuous sucrose gradient containing 6 ml of 40%, 6 ml of 60%, 8 ml of 70%, and 5 ml of 78% sucrose. After centrifugation in a Beckman SW-27 rotor (Beckman Instruments, Inc., Fullerton, Calif.) for 12-16 h at 72,000 g, the large membrane sheets were present as a single band, whereas the residual cortical vesicles were found in the pellet.

Extraction of Plasma Membranes with KI

To determine which components of the plasma membrane were actually integral membrane proteins, we attempted to extract only peripheral proteins associated with the plasma membrane. The membrane sheets obtained as described above were adjusted to 30% sucrose and pelleted by centrifugation at 100,000 g for 30 min. The pellet was resuspended in 10 ml of 0.5 M KI containing 5 mM HEPES buffer, pH 7.5, mM EGTA, pH 7.5, and Trasylol. 10 U/μl. The membranes were extracted with this buffer for 3 h with gentle homogenization every 30 min. The suspension was then layered on a discontinuous gradient of 7 ml of 20%, 8 ml of 40%, 8 ml of 60%, and 6 ml of 70% sucrose. The purified membranes recovered from the sucrose gradient were diluted fivefold with 0.9% saline and centrifuged at 72,000 g for 1 h in a SW-27 rotor to pellet the membranes.

Analytical Procedures

Before analysis, membrane-containing fractions were diluted fivefold with 0.9% NaCl and centrifuged at 100,000 g for 60 min. Protein was determined on lipids extracted or TCA-precipitated samples by two independent methods. Samples were neutralized, if necessary, with 0.1 M NaOH, then adjusted to 100 μl of 0.1% w/v in sodium dodecy sulfosuccinate (SDS), and boiled for 5 min or until all material was solubilized. For protein determinations by fluorescamine assay (30) or by the assay of Lowry et al. (14), samples were adjusted to 1 ml with distilled water (final concentration of SDS, 1%). In both assays, bovine serum albumin standards were made to 1% w/v in SDS before use. Two assays agreed very closely in the determinations of membrane protein.

To determine membrane phospholipids, we washed membranes a second time with 0.9% NaCl before extraction. Phospholipids were extracted by the procedure of Folch et al. (8) and stored in CHCl3, under nitrogen at -20°C. Lipid phosphorus was determined by the method of Bartlett (2). Individual phospholipids were identified by thin-layer chromatography of Silica-G plates (VWR Scientific Inc., San Francisco, Calif.) using three different solvent systems: (A) chloroform:methanol:water (65:25:4); (B) chloroform:methanol:ammonium (65:25:5); and (C) a two-dimensional system using chloroform:methanol:acetic acid:water (30:40:10:5) in the second dimension. Lipids were visualized by iodine vapor, and each spot was scraped from the plate for quantitative analysis of lipid phosphorus after perchloric acid digestion (2). Individual phospholipids were identified by comparison with authentic standards, and recoveries calculated for each phospholipid standard were used to correct for losses during extraction and chromatography.

The identification of phosphatidylinositol was confirmed by gas-liquid chromatographic analysis of the inositol recovered after hydrolysis. After thin-layer chromatography, the spot containing phosphatidylinositol was recovered from the plate, and the silica gel was extracted twice with 2 ml of chloroform:methanol:water (10:10:3). The extract was dried under vacuum and hydrolyzed in 0.5 ml of 2 N HCl under nitrogen at 100°C for 48 h. The hydrolysate was dried under vacuum and acetylated by heating in acetic anhydride:pyridine (1:1) at 100°C for 1 h. The acetylated derivatives were chromatographed on a column containing 3% SP-2340, 0-200 mesh Supelcoport (Supelco, Inc., Bellefonte, Pa.). Recovery of inositol was estimated by carrying a known amount of authentic phosphatidylinositol through the entire procedure; 20% of the inositol was recovered. The ratio of inositol to PO₄ in the isolated membrane lipid was found to be 0.37.

Total cholesterol in the lipid extract was determined after saponification as described by Dittmer and Wells (7).

Enzyme Assays

Ca2+-sensitive sodium-potassium-ATPase activity was determined under the following conditions: 50 mM NaCl, 50 mM HEPES buffer, pH 7.2, 30 mM KCl, 3 mM MgCl₂, 3 mM ATP, with or without 1 mM ouabain, at 25°C. P; was released, determined by the method of Marsh (15). Peroxidase activity was measured by the method of Herzog and Fahimi (10). Protease activity was measured as described earlier (6) using [3H]-n-tosyl-l-arginine methylester ([3H]TAME) (Amersham Corp. Arlington Heights, Ill.) as a substrate. Preliminary experiments were performed to ensure that each enzyme activity was measured under conditions where product formation was linearly dependent on enzyme concentration.

SDS Polyacrylamide Gel Electrophoresis

Washed membranes were precipitated with ice-cold 10% TCA. The pellet was neutralized with 0.1 M NaOH and extracted twice with 3 ml of diethyl ether. The sample was then solubilized and electrophoresed according to the discontinuous system of Laemmli (13). A 3% acrylamide stacking gel was used, and the running gel was poured as a linear gradient from 5 to 20% acrylamide. After electrophoresis, the gel was fixed in 10% TCA and stained with Coomassie Blue or by the periodic acid-Schiff technique.

Electron Microscopy and Sperm-binding Assay

The membrane pellets were prepared for electron microscopy as previously described (6). To test the ability of the plasma membrane sheets and KI-extracted membranes to bind sperm, we prepared membrane sheets as described above except that the treatment with dixisopropyl fluorophosphate was omitted. After purification on a sucrose gradient, half of the sample was extracted with KI as described above. Both the purified plasma membrane and extracted plasma membranes were washed with 40 ml of 0.9% NaCl and resuspended in artificial seawater buffered with 10 mM Tris-HCl at pH 8.0 (TBS).

Sperm (200 μg of sperm protein) were diluted to 0.5 ml with TBS containing homologous egg jelly (200 mM fucose/ml) for 30 s to induce the acrosome reaction (7), or with TBS as a control. Either 20 μg of the control (untreated) sperm or of the egg jelly-treated sperm was then added to a suspension of plasma membranes (7-10 μg protein) and allowed to bind for 30 s. At the end of this
RESULTS

Isolation of the Egg Plasma Membrane

The cell surface complex (Fig. 1a) is composed of two subcellular organelles: the plasma membrane with its associated vitelline layer and the cortical vesicles. Initial attempts to separate the cortical vesicles by exposure to various conditions of pH, high ionic strength, chaotropic agents such as KI or KBr, and urea met with limited success. Whereas some of these conditions could release up to 50% of the cortical vesicles, none were effective enough to be used in isolation of the plasma membrane. However, when the cell surface complex was suspended in 1 M sucrose under conditions resulting in a 1,000-fold dilution of the ions originally present, virtually all of the cortical vesicles were released by gentle homogenization. After this treatment, the plasma membranes were separated from the vesicles by centrifugation. A schematic representation of this purification scheme is presented in Fig. 2.

Ultrastructural analysis of samples at various points during the procedure indicated that the cortical vesicles were ruptured during or soon after the detachment process. However, the lamellar contents remained inside the "opened" vesicles; these vesicles could be recovered from the supernatant at step 3. The plasma membranes obtained at step 3 were in the form of large sheets that could be separated from any residual cortical vesicles by sucrose density gradient centrifugation (step 4). The elution profile from such a gradient is presented in Fig. 3a. Electron microscope examination (Fig. 1b) of the membrane sheets recovered from the gradient indicated the presence of a "fluffy" layer, probably proteinaceous, associated with the external surface of the membranes (i.e., the surface opposite to that to which an occasional cortical granule can be seen still bound; see Fig. 1b, inset). Ultrastructural examination of the cell surface complex after suspension in 1.0 M sucrose suggests
Comparison of the Cell Surface Complex and the Plasma Membrane

To assess the extent of purification of the plasma membranes after removal of cortical vesicles, we assayed the cell surface complex and the purified membranes for marker enzymes for the plasma membrane and the cortical vesicles. The results, shown in Table I, indicate that the plasma membrane enzyme, ouabain-sensitive Na⁺/K⁺ ATPase, and, as previously reported (6), two cortical granule enzymes, proteoesterase and ovoperoxidase, are readily detectable in the cell surface complex. Furthermore, it is apparent that further purification of the plasma membrane per se by removal of the cortical vesicles leads to an almost 10-fold increase in the specific activity of the plasma membrane marker enzyme. Also, it is clear that the purified plasma membrane has minimal contamination with cortical vesicle components, at least as assessed by the presence of the cortical vesicle enzymes, because only very low levels of proteoesterase and ovoperoxidase are detectable. Thus, although 33% of the plasma membrane marker Na⁺/K⁺ ATPase is recovered in the purified plasma membrane, <0.3% of the two cortical granule enzymes are present in the preparation.

Some additional characteristics of the cell surface complex and the plasma membrane are presented in Table II. The cortical vesicles and other components removed from the cell surface complex by treatment in sucrose account for a considerable amount of the protein in the complex because the purified membrane sheets are less dense (1.25 g/cm³) and have a much lower ratio of protein to lipid (2.8). Even so, the egg plasma membrane is unusually dense and protein rich, probably because of the presence of the vitelline layer and other peripheral proteins that compose the fluffy layer seen in Fig. 1b.

To characterize the peripheral and integral proteins, we treated the purified plasma membranes with the chaotropic agent, KI, which would be expected to solubilize peripheral membrane proteins. This could be done either after step 3 (Fig. 2) or after the density gradient centrifugation. This treatment caused the membrane sheets to vesiculate, and when centrifugation through a discontinuous sucrose density gradient was carried out two bands of membranes were resolved (Fig. 3b). The lighter band (buoyant density 1.12), accounting for most of the membranes, consists of vesicles that are essentially free of the fluffy layer (Fig. 1c). The heavier band (buoyant density 1.25) consists of small amounts of membrane in both sheet and vesicle form, as well as membrane fragments bound to residual cortical vesicles. This material was discarded. As seen in Table II, the membranes recovered after extraction with KI are significantly less dense than the native plasma membrane and have a lower ratio of protein to lipid, indicating that substantial amounts of peripheral proteins have been removed.

An analysis of the cell surface complex and the plasma membrane by SDS polyacrylamide gel electrophoresis is shown in Fig. 4. The cell surface complex contains at least 18 major polypeptide species, as well as many minor ones. Six of these major species stain positively with the periodate-Schiff procedure and are probably glycoproteins. The purified plasma membrane sheets obtained upon removal of cortical vesicles are depleted in several polypeptide species of ~450,000, 180,000, 110,000, 100,000, 65,000, and 33,000 daltons that are apparent in the cell surface complex; presumably, these proteins represent cortical vesicle components. Of the proteins remaining associated with the membrane, 14 exist as major components; a large number of minor components are also present.

When the membrane sheets were subsequently extracted with KI, several additional proteins of 55,000, 50,000, and 32,000 daltons, as well as several bands between 26,000 and 10,000 daltons, were removed. Although the number of major proteins in the KI-extracted membranes was considerably reduced (five to six major bands), a large number of minor proteins were now visible. To rule out the possibility that

---

**Table I**

| Preparation          | Na/K ATPase Specific activity | Na/K ATPase Total activity | Peroxidase Specific activity | Peroxidase Total activity | Proteoesterase Specific activity | Proteoesterase Total activity |
|----------------------|-------------------------------|----------------------------|----------------------------|----------------------------|---------------------------------|--------------------------------|
| Cell surface complex | 135 (1)                       | 13,500                     | 0.195 (1)                  | 19.50                      | 5.4 x 10^5 (1)                  | 5.3 x 10^6                     |
| Plasma membranes    | 1,148 (8.5)                   | 4,420                      | 0.014 (0.07)              | 0.54                       | 0.3 x 10^5 (0.06)              | 0.01 x 10^6                    |

The specific activity of the ouabain-sensitive, Na⁺/K⁺ ATPase activity is expressed as nanomoles of released P per milligram of protein per hour. The specific activity of the peroxidase is expressed as the change in absorbance units (at 465 nm) per milligram of protein per minute. The specific activity of the proteoesterase is expressed as counts per minute of [3H]CH₃OH released from [3H]TAME per milligram of protein per hour. Values in parentheses represent the relative specific activities.

---

**Table II**

| Preparation          | Total protein | Total phospholipid | µg Protein/µg phospholipid | Buoyant density |
|----------------------|---------------|--------------------|---------------------------|-----------------|
| Egg homogenate       | 141,000       | 160                | 10.0                      | >1.3            |
| Cell surface complex | 6,520 (100%)  | 728                | 2.8                       | 1.25            |
| Sucrose-treated       | 867 (13%)     | 90                 | 2.8                       | 1.25            |
| KI-extracted membranes| 160 (2.5%)    | 123                | 1.3                       | 1.12            |

These data represent the results of a typical plasma membrane preparation. The cell surface complex is considered to be the starting material, and the numbers in parentheses represent the recovery of lipid and protein relative to it.

---

1 The reason why this ATPase could not be detected in our earlier study (6) is unknown.
proteolysis (27) or ovoperoxidase-mediated cross-linking was responsible for the changes in the relative abundance of certain polypeptides described above, we tested for a time-dependent change in the polypeptide profile of the cell surface complex after homogenization in sucrose. After a 20-h incubation, we found that, except for an increase in the intensity of the 200,000-dalton band, no other changes in the polypeptide profile were detectable. We conclude that proteolysis or cross-linking is not responsible for the change in the abundance of selected proteins observed as the membranes are purified.

Lipid Analysis

A determination of phospholipids and cholesterol was performed on samples of KI-extracted membranes. The results, shown in Table III, indicate that phosphatidylethanolamine was the predominant phospholipid, followed by phosphatidylinositol and phosphatidylcholine. All the other phospholipids listed were detected at only very low levels. Cholesterol was found to be a major component of the plasma membrane.

Sperm Binding

An earlier report demonstrated that the cell surface complex could induce the acrosome reaction in sperm and subsequently bind them species specifically (5). These findings suggest that the cell surface complex contains sufficient residual jelly coat to induce the acrosome reaction, as well as sperm receptors. In view of these findings, it was of interest to examine the purified plasma membranes with respect to these properties of the cell surface complex. As shown in Fig. 5a, homologous sperm, in which the acrosome reaction had not been previously induced, did not bind to the plasma membrane sheets. However, if the acrosome reaction was previously induced in the sperm, extensive binding to the membrane was observed (Fig. 5b). This indicates that the purified membrane sheets retain receptors for sperm but probably have lost the residual jelly coat necessary for inducing the acrosome reaction in the sperm. Heterologous sperm from Arbacia punctulata did not bind to the membrane sheets, even when the acrosome reaction was preinduced by soluble jelly coat from A. punctulata (Fig. 5c and d). Thus, the species-specific nature of the sperm-binding event inherent in the intact egg and the isolated cell surface complex is retained in these purified membranes. Sperm adhesion to eggs is thought

![TABLE III
Lipid Analysis of the Purified and KI-Extracted Plasma Membranes

| Lipid                  | nmol lipid/100 µg protein |
|------------------------|---------------------------|
| Phosphatidylethanolamine | 61.0                      |
| Phosphatidylinositol    | 25.3                      |
| Phosphatidylcholine     | 16.2                      |
| Phosphatidylserine      | <2.5                      |
| Sphingomyelin           | <2.5                      |
| Phosphatic acid         | <2.5                      |
| Cardiolipin             | Not detectable            |
| Cholesterol             | 86.0                      |

Values have been corrected for recovery. The ratio of inositol to P1 in the phosphoinositide is 0.37, but we have not determined the proportions of mono-, di-, and triphosphoinositides by chromatographic means. Several other lipids not containing phosphate were detected by TLC and have not been identified.

![FIGURE 5 Sperm binding to purified plasma membranes. (A) S. purpuratus sperm that have not undergone the acrosome reaction (0% reacted) do not bind to the purified membrane sheets prepared from S. purpuratus eggs. (B) Many of the S. purpuratus sperm previously induced to undergo the acrosome reaction (90% reacted) bind to the membrane sheets. Neither unreacted, heterologous A. punctulata (C) sperm nor (D) reacted (57% reacted) A. punctulata sperm bind to the membranes, indicating that sperm binding to these purified membrane sheets is species specific. X 333.](image)
to be mediated by peripheral membrane components of the vitelline layer (8, 16, 21, 27). Because KI extraction of the isolated egg plasma membrane apparently removed all peripheral components, we tested the ability of S. purpuratus sperm to bind to KI-extracted membranes. We found that this egg plasma membrane preparation was essentially devoid of sperm binding capacity (data not shown). These results indicate that the cell surface complex contains both jelly coat and receptors for sperm, whereas the purified plasma membranes appear to contain the sperm receptor but no jelly coat. Extraction of the purified membranes with KI removes all traces of the vitelline layer and almost totally abolishes the ability of the membranes to bind sperm, presumably by solubilizing or denaturing the sperm receptors.

**DISCUSSION**

This study has extended earlier work from this laboratory in which the cell surface complex was first described (6) and its functional properties examined (5). Our present objective was to isolate and characterize the egg plasma membrane per se. To accomplish this, we devised a method of detaching the cortical vesicles from the cell surface complex and recovering the plasma membrane. The membranes obtained are in the form of large sheets that are devoid of cortical vesicles but contain an amorphous fluffy layer detected ultrastructurally on the external surface. The results of biochemical studies clearly document the efficacy of the procedure used to remove the cortical vesicles from the plasma membrane. Whereas the cell surface complex contains the plasma membrane marker enzyme, ouabain-sensitive Na⁺/K⁺ ATPase, as well as the cortical vesicle enzymes, proteoesterase and ovoperoxidase, the purified plasma membrane has only trace levels of the latter two enzymes. Moreover, the specific activity of the plasma membrane marker enzyme in the isolated plasma membrane preparation is enhanced almost 10-fold over that found in the cell surface complex.

The fluffy layer observed in the purified plasma membrane may represent a disorganized form of the vitelline layer, as well as other peripheral proteins, but it is clear from the marker enzyme studies that there is little contamination with cortical granule contents. The protein hyaline is known to be tightly bound to the surface of fertilized eggs (see reference 26 for a review). In unfertilized eggs, there is evidence suggesting that hyaline is present both in cortical granules (1, 26) and on the cell surface (16). In view of these facts, hyaline is a potential contaminant of the purified plasma membrane. However, the binding of hyaline to the cell surface has an absolute requirement for Ca²⁺ (1, 26). Because the isolation medium used for preparation of the cell surface complex is devoid of Ca²⁺ and contains EDTA, and because all subsequent media used to isolate the purified plasma membrane are devoid of Ca²⁺, it is highly unlikely that hyaline is a significant contaminant in the purified plasma membrane preparation. Further work is now underway to more precisely define the components of the vitelline layer, cortical vesicles, and the fluffy layer of the purified membranes.

Extraction of the purified membranes with the chaotrophic agent, KI, solubilized this fluffy layer and yielded membranes recovered as vesicles that were relatively low in protein content, compared with plasma membranes from most mammalian cells (4, 21). Characterization of the protein components of the cell surface complex, and of the plasma membrane before and after KI extraction by gradient gel electrophoresis gave us much more information than the results obtained earlier with the SDS-urea system (6). The cell surface complex consists of at least 18 major protein components, as well as a large number of minor ones. Removal of the cortical vesicles by homogenization in sucrose results in the loss of several major polypeptide species, including three major periodate-Schiff-positive components that presumably are glycoproteins. Some of these proteins may represent components unique to the cortical vesicles. Schuel et al. (25) have provided histochemical evidence that sea urchin cortical vesicles are rich in acidic glycoproteins.

As mentioned above, extraction of the purified membranes with KI removed all traces of the vitelline layer, and analysis of the extracted membranes revealed that several of the major membrane-associated proteins were removed by this procedure. The picture that emerges from this type of analysis is that the cell surface complex contains a large number of major proteins that are not integral components of the plasma membrane per se. The cortical vesicles seem to account for 5–10 of these, including several high-molecular-weight glycoproteins. The vitelline layer and other peripheral components contain several of the remaining major species. After removal of these by KI extraction, the purified plasma membrane contains five or six major polypeptides, as well as a large number of minor components that were barely detectable in the cell surface complex.

One of the most important functions of the sea urchin egg surface is the recognition of, binding to, and fusion with sperm of the same species. The cell surface complex is known to retain the capability of inducing the acrosome reaction in homologous sperm and of binding these reacted sperm to the vitelline layer (5). The cortical granule-free plasma membranes prepared in the present study are capable of binding sperm only if the sperm are preinduced to undergo the acrosome reaction. This suggests that the jelly coat material that normally induces the acrosome reaction in the intact egg (28) has been removed during the isolation procedure. The fact that these membranes can bind sperm species specifically indicates that the sperm receptors are still intact. The receptor(s) for sperm are thought to reside in the vitelline layer (9, 24, 28), which persists in a modified form in the plasma membrane preparation as an amorphous fluffy layer. KI extraction removes this layer and essentially abolishes the ability of the membranes to bind sperm. Presumably, the receptor(s) have been solubilized by this procedure. The KI and other extraction procedures are currently being investigated as methods of solubilizing the sperm receptor in active form.

Determination of the lipid composition of the sea urchin egg plasma membrane revealed that, for a rather normal cholesterol to phospholipid molar ratio of ~1:1, this cell type has a rather atypical plasma membrane. Phosphatidylethanolamine was the major phospholipid, followed by phosphatidylinositol and phosphatidylcholine. This composition is quite different from that found in other plasma membranes, where phosphatidylcholine is the predominant phospholipid and phosphatidylinositol is only a minor component (19–21). Additionally, sphingomyelin, which is a major component of mammalian plasma membranes, was barely detectable in the egg plasma membrane.

---

2 Another egg plasma membrane marker enzyme, adenyl cyclase (3), was also detected in the cell surface complex, but it proved too labile to be used reliably as a marker.

W. H. Kinsey, G. L. Decker, and W. J. Lennarz *Plasma Membrane of Sea Urchin Egg* 253
Of particular interest is the high content of phosphatidylinositol, which in other types of plasma membranes comprises at most 3–9% of the total phospholipids (19–21, 29). For over 20 years it has been known that in a wide variety of secretory systems stimulation of the secretory event is accompanied by rapid breakdown and resynthesis of phosphatidylinositol (18). Also, it is clear that Ca²⁺ plays a key role in both the secretory process and the breakdown of phosphatidylinositol. The sea urchin egg possesses thousands of secretory vesicles, the cortical vesicles, poised at the plasma membrane and ready to be discharged rapidly upon fertilization. The very high level of phosphatidylinositol in the egg plasma membrane may play an essential role in this massive exocytotic event. Indeed, early work from this laboratory (24) showed that there is rapid incorporation of inositol into phosphatidylinositol immediately upon fertilization of eggs. Clearly, the secretory response observed at fertilization bears striking parallels to the more classical secretory systems that have been studied (see reference 26 for review). Because of the massive, concerted nature of the exocytotic event in both the egg and the isolated cell surface complex, this system may prove most useful in studying the biochemical steps of secretion.

The authors gratefully acknowledge the expert technical assistance of Mrs. Betty Earles. We also wish to express our gratitude to Ms. Ann Fuhr and Ms. Susan DeFrancesco for their help in the preparation and typing of this manuscript.

This work was supported by a grant from the National Institutes of Health (HD 08357) to Dr. Lennarz. Dr. Kinsey was the recipient of a Special Postdoctoral Fellowship from The Rockefeller Foundation.

Received for publication 25 March 1980, and in revised form 9 June 1980.

REFERENCES

1. Asotinu, A ., and D. R. McClay. 1979. Appearance and localization of hyalin during sea urchin development. J. Cell Biol. 83(2), Pt. 2:211 (Abstr.).
2. Bartlett, G. R. 1959. Phosphorus assay in column chromatography. J. Biol. Chem. 234: 466-468.
3. Castaneda, M ., and A. Tyler. 1968. Adenyl cyclase in plasma membrane preparations of sea urchin eggs and its increase in activity after fertilization. Biochim. Biophys. Res. Commun. 33:782-787.
4. Chapman, D. 1976. Physicochemical studies of cellular membranes. In Mammalian Cell Membranes I. G. A. Jamieson and D. M. Robinson, editors. Butterworth Publishing, Inc., Woburn, Mass. 97-137.
5. Decker, G. L ., and W. J. Lennarz. 1979. Sperm binding and fertilization envelope formation in a cell surface complex isolated from sea urchin eggs. J. Cell Biol. 81:92-103.
6. Detert, N. K ., G. L. Decker, E. D. Schnell, and W. J. Lennarz. 1977. Isolation and characterization of the plasma membrane associated cortical granules from sea urchin eggs. J. Cell Biol. 75:899-914.
7. Dittmer, J. C ., and M. A. Wells. 1969. Quantitative and qualitative analysis of lipids and lipid components. Methods Enzymol. 14:482-530.
8. Folch, J., M. Lees, and H. S. Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226:497-508.
9. Glabe, C. G., and Y. D. Vasquez. 1977. Isolation and characterization of the vitelline layer of sea urchin eggs. J. Cell Biol. 75:410-421.
10. Heller, E. M ., and M. A. Raftery. 1976. The vitelline envelope of eggs from the giant keyhole limpet Megathura crenulata. I. Chemical composition and structural studies. Biochemistry. 15:3671-3678.
11. Herzog, V. H ., and H. D. Fahimi. 1973. Peroxidase assay: a new sensitive colorimetric assay for peroxidase using 3,3'-diaminobenzidine as hydrogen donor. Anal. Biochem. 56:344-352.
12. Kinsey, W. H ., J. A. Rubin, and W. J. Lennarz. 1980. Studies on the specificity of sperm binding in echinoderm fertilization. Dev. Biol. 74:242-250.
13. Lennarz, W. J., and U. K. Cleland. 1970. Cleavage of proteins during the assembly of the head of bacteriophage T4. Nature (London). 227:681-685.
14. Lowry, O. H ., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem. 193:265-275.
15. March, B. B. 1959. The estimation of inorganic phosphate in the presence of adenosine triphosphate. Biochim. Biophys. Acta. 23:357-361.
16. McClain, P. J ., and E. J. Carroll. 1980. Sea urchin egg hyaline layer: evidence for the localization of hyalin on the unfertilized egg surface. Dev. Biol. 75:137-147.
17. Metz, C. B. 1978. Sperm and egg receptors involved in fertilization. In Current Topics in Developmental Biology. A. A. Moscona and A. Monroy, editors. Academic Press, Inc., New York. 12:107-138.
18. Michell, R. H ., S. J. Jaffery, and L. M. Jones. 1977. The possible role of phosphatidylinositol breakdown in the mechanism of stimulus response coupling at receptors which control cell-surface Ca²⁺ gates. Adv. Exp. Med. Biol. 83:447-464.
19. Perkins, R. G ., and R. E. Scott. 1978. Plasma membrane phospholipid, cholesterol, and glycolipid composition of differentiated and undifferentiated L6 myoblasts. Lipids. 13:313-382.
20. Renkonen, O ., C. G. Graham, K. Simmons, and L. Kaariainen. 1972. The lipids of the plasma membranes and endoplasmic reticulum from cultured baby hamster kidney cells (BHK-21). Biochim. Biophys. Acta. 255:66-78.
21. Rouser, G ., G. J. Nelson, S. Flescher, and G. Simon. 1968. Lipid composition of animal cell membranes, organelles, and organs. In Biophysical Membranes, Physical Fact and Function. D. Chapman, editor. Academic Press, Inc., New York. 5-64.
22. Runnstrom, J ., 1966. The vitelline membrane and cortical particles in sea urchin eggs and their function in maturation and fertilization. In Advances in Morphogenesis. Academic Press, Inc., New York. 5:321-325.
23. Schnettl, E ., B. J. Earles, C. Braus, and W. J. Lennarz. 1977. Identification of a sperm receptor on the surface of eggs of the sea urchin Arbacia punctulata. J. Cell Biol. 73:35-46.
24. Schnell, E ., and W. J. Lennarz. 1974. Phospholipid metabolism in the eggs and embryos of the sea urchin Arbacia punctulata. Biochemistry. 13:414-4121.
25. Schuel, H ., J. W. Kelly, E. R. Berger, and W. J. Lennarz. 1974. Sulfated acid mucopolysaccharides in the cortical granules of eggs. Exp. Cell Res. 88:24-30.
26. Schuel, H ., 1978. Secretory functions of egg cortical granules in fertilization and development. Gamete Res. 1:299-302.
27. Shapiro, B. M ., 1975. Limited proteolysis of egg surface components is an early event following fertilization of the sea urchin S. purpuratus. Dev. Biol. 46:89-96.
28. Summers, R. G ., B. L. Hylander, L. H. Colwin, and A. L. Colwin. 1975. The functional anatomy of the echinoderm spermatozoon and its interaction with the egg at fertilization. J. Exp. Zool. 193:523-551.
29. Turner, J. D ., and G. Rouser. 1974. Removal of lipid from intact erythrocytes and ghosts by aqueous solutions and its relevance to membrane structure. Lipids. 9:49-54.
30. Udenfriend, S ., S. Stein, P. Bolen, W. Dairman, W. Leimgruber, and M. Weigle. 1972. Application of fluororescine as a new reagent for the assay of amino acids, peptides, proteins and other primary amines in the picomole range. Science (Wash. D. C.). 178:871-874.
31. Versen, M. C., F. Forster, E. M. Eddy, and B. M. Shapiro. 1977. Sequential biochemical and morphological events during assembly of the fertilization membrane of the sea urchin. Cell. 10:321-328.
32. Wolf, D. F ., T. Nishihara, D. M. West, R. E. Wyck, and J. L. Hedrick. 1976. Isolation, physicochemical properties and the macromolecular composition of the vitelline and fertilization envelopes from Xenopus laevis eggs. Biochemistry. 15:3671-3678.