Binding of Concanavalin A to the Surface of Sea Urchin Eggs and Its Alteration upon Fertilization*

(Received for publication, July 2, 1976)

MICHEL VERONA AND BENNETT M. SHAPIRO
From the Department of Biochemistry, University of Washington, Seattle, Washington 98195

The binding of concanavalin A (Con A) to Strongylocentrotus purpuratus eggs has been investigated using 125I-concanavalin A (125I-Con A). The lectin binds specifically to the egg surface and does not produce agglutination of the eggs. High 125I-Con A concentrations are necessary to saturate all of the surface binding sites. Scatchard plots of the binding data are biphasic and may be interpreted as showing two main classes of sites. Unfertilized eggs have $4 \times 10^3$ high affinity sites/egg ($K_a = 8 \times 10^{-7} M$) and $4.5 \times 10^5$ sites of lower affinity ($K_a = 4 \times 10^{-7} M$). The sites may be assigned to different layers of the egg surface by studying the effects of removal of egg coats on the binding reaction. Removal of the jelly coat by washing eggs at pH 4.5 has no effect on binding. However, disruption of the vitelline layer with dithiothreitol leads to a decrease in the number of high affinity sites. After fertilization, the high affinity sites are found upon the fertilization membrane produced from the vitelline layer. Lower affinity sites predominate in the plasma membrane, and are not affected by treatments which alter the vitelline layer. The number of low affinity sites doubles upon fertilization, with the insertion of new membrane into the egg surface, as a result of cortical granule exocytosis. The doubling of sites is not due to hyaline material released from the cortical vesicles at fertilization, and thus these sites appear to reside upon the new membrane that is inserted from the cortical vesicles. If eggs are activated with ammonia, bypassing the cortical reaction, no change in the binding of Con A occurs.

Con A inhibits fertilization at concentrations higher than 0.1 mg/ml, where less than 50% of the high affinity (vitelline layer) binding sites are occupied, and there is little binding to the low affinity (plasma membrane) sites. Thus, the interaction of sperm with vitelline layer components may be an obligatory step in the fertilization process. A fraction of the 125I-Con A binding sites is cleaved from the egg surface upon fertilization or after activation by the calcium ionophore A23187. This release of Con A binding sites occurs during the limited proteolysis of surface components that accompanies the cortical reaction, and does not occur with ammonia activation of the egg, where the cortical reaction does not occur. Thus, the changes in Con A binding at fertilization are caused by the massive cortical granule exocytosis that occurs within minutes of sperm penetration, and not by activation of the egg per se.

The sea urchin egg surface is a complex structure which is extensively modified in structure and function at fertilization (reviewed in Refs. 1-3). The plasma membrane of the unfertilized egg (80 μm in diameter) is raised in numerous microvilli (approximately $2 \times 10^4$/egg) that serve to double the membrane area (4). A vitelline coat (glycocalyx) is tightly apposed to the external surface of this plasma membrane. Upon fertilization the cortical vesicles (approximately $15,000$/egg) that underlie the plasma membrane undergo a wave of exocytosis, which proceeds from the point of attachment of the fertilizing sperm. The cortical vesicle contents interact with the vitelline layer, which elevates to make a fertilization membrane. Proteolytic activity released from the cortical vesicles (1, 5-9) leads to a limited proteolysis of egg surface components (10), resulting in the detachment of the vitelline layer and in the cleavage of sperm binding sites. The effect of this cortical reaction is to produce a fertilized egg that is surrounded by a hard fertilization membrane and resistant to fertilization by additional sperm. After the cortical reaction, the plasma membrane becomes a topographically mosaic structure, composed of cortical vesicle membrane and the original plasma membrane (4). The microvilli are separated by smooth areas which appear to be derived from the cortical vesicle membranes, and the total membrane surface area is doubled (4). This increase of membrane surface is accompanied by microvillar elongation, in order to accomodate the inserted cortical vesicle membrane material (4).

The cortical reaction is followed by the activation of metabolic events and development of the embryo. The egg may be activated by several parthenogenic agents, such as butyric acid (11) and a divalent ionophore (A23187) (12), both of which elicit the cortical reaction. Other agents, such as NH₄Cl (at pH 9), activate the egg, yet bypass the cortical reaction (13). In this case, protein synthesis and other "late" reactions following fertilization are activated, but cortical vesicle exocytosis and fertilization membrane elevation do not occur (13-15).

In order to see what effects fertilization has on the fate of glycoprotein components of the egg surface, we have examined the interaction of the lectin, concanavalin A, with the egg surface. Con A binds stereospecifically to manno-o-o-pyra-
inhibits fertilization of Paracentrotus lividus (25) and induces modification of the cell surface during differentiation in a manner (17, 18), Con A has proved useful in the study of the membrane surface following cortical granule exocytosis (41, 28). Micromeres are agglutinated more readily by Con A than the dissociation constants for binding to the high and low affinity sites, respectively.

Preparation of \(^{125}\text{I}\)-Con A - \(^{125}\text{I}\)-Con A was prepared by a slight modification of the protein iodination procedure of Marchalonis (31) and Arndt-Jovin and Berg (32). Except for lactoperoxidase, which was dissolved in distilled water, all the other solutions were made in sea water adjusted at pH 7.4 with NaOH. The commercial solution of Con A was dialyzed for 2 h against sea water before use. Radioactive labeling was performed in a stopped, sealed, conical vial (Kontes tubing Co.). First, 19 ml of carrier-free \(^{125}\text{I}\) (100 mCi/ml) were mixed with 10 ml of 0.1 mM sodium sulfite to reduce any I to I\(^{-}\) (33). Next, 0.1 ml of 1 mM KI, 0.5 ml of 1 M \(\alpha\)-methyl mannoside, 1.5 ml of sea water, 2 ml of dialyzed Con A (~40 mg/ml), and 0.4 ml lactoperoxidase (2 mg/ml) were sequentially added. The reaction, performed at room temperature, was started by the addition of 10 ml of 0.06% H\(_2\)O\(_2\), the addition of which was repeated every 2 min. After 10 min the reaction was stopped with 0.5 ml of 5 mM dithiothreitol. The reaction mixture was then extensively dialyzed at 4° against sea water containing 0.5 mM NaCl (NaCl/sea water). Active Con A was purified when necessary by affinity chromatography (32) on Sepha-

dex G-100 equilibrated with NaCl/sea water, and eluted by a solution of 0.1 M \(\alpha\)-methyl mannoside in NaCl/sea water, then dialyzed extensively at 4° against NaCl/sea water.

The radioactivity of \(^{125}\text{I}\)-Con A was measured under the same conditions as that of \(^{125}\text{I}\)-Con A egg complexes (see below). Protein concentration was measured by the method of Lowry et al. (34). The specific radioactivity of \(^{125}\text{I}\)-Con A was usually around 1 \(\mu\)Ci/nmol, assuming the molecular weight of Con A to be 102,000 (35) in its tetrameric form. The labeled Con A has the same affinity for the surface receptors of the egg as the native lectin, as shown by competition experiments. \(^{125}\text{I}\)-Con A was very stable when stored as a 10-

mg/ml solution in NaCl/sea water at 0°. Immediately before use, it was centrifuged to remove the slight precipitate which accumulates during storage and appropriate dilutions were made in NaCl/sea water.

**Binding of \(^{125}\text{I}\)-Con A to Eggs** - The binding assays were performed in Corex conical centrifuge tubes (15 ml), by incubating 0.1 ml of a 3 to 5% egg suspension with appropriate dilutions of \(^{125}\text{I}\)-Con A, in a total volume of less than 150 \(\mu\)l. Unless otherwise indicated, the incubation was performed in an ice bath for 15 to 20 min. Control incubations were as above but contained, in addition, 10 \(\mu\)l of 1 M \(\alpha\)-methyl mannoside in sea water. The reaction was always initiated by addition of \(^{125}\text{I}\)-Con A. At the end of the incubation period, the eggs were suspended in 10 ml of ice-cold Millipore-filtered sea water, centrifuged rapidly, and the supernatant solution was aspirated with a Pasteur pipette. This procedure was repeated five times, after which the eggs were resuspended in 6 ml of ice-cold sea water and immediately filtered through glass fiber filter discs (Whatman GF-C). The entire wash procedure took less than 2 min. The incubation was stoppered with a filter funnel which had been prewashed with 4 ml sea water, and the filter was removed from its support. The filters were dried and placed in scintillation vials, and Liquifluor (Beckman) oxintillant was added for counting in a Beckman LS230 liquid scintillation counter. The tedious procedure of successive washing and centrifugation before filtration of the eggs was necessary, since free Con A alone is retained on the filters, even after extensive washing with sea water. We have tried different kinds of filters, none of which gave a background sufficiently low to avoid preliminary washing. This problem was especially severe when very low quantities of eggs were used, as in all the binding assays used to establish saturation curves. We call "specific binding" the difference between the amount of \(^{125}\text{I}\)-Con A found in the absence and in the presence of 0.1 M \(\alpha\)-methyl mannoside. When the binding assay was performed without a control for specific binding being done, we refer to the amount of \(^{125}\text{I}\)-Con A bound as "total binding". The amount of \(^{125}\text{I}\)-Con A bound is expressed as micrograms of lectin bound/50,000 eggs, or as the number of bound nucleotides/egg. The egg concentration was estimated by counting the number of eggs in a 50-\(\mu\)l pipette full of egg suspension; the average of three separate determinations was used to determine the concentration.

**RESULTS**

**Binding of \(^{125}\text{I}\)-Con A to Eggs** - Fig. 1 shows the binding of \(^{125}\text{I}\)-Con A to eggs as a function of time at 0°. There is a rapid binding that occurs within 15 min, followed by a slow increase...
in the amount of bound lectin. This delayed binding is not reduced when the incubation is performed in the presence of 4 mM sodium azide, nor is it sensitive to temperature; binding assays done at 10°C were no different than at 0°C. The inclusion of α-methyl mannoside in the incubation mixture essentially eliminates all 125I-Con A binding; also, addition of 0.1 M α-methyl mannoside to eggs previously saturated with 125I-Con A causes the release of the bound radioactivity. We routinely used a 15- to 30-min incubation to obtain the binding data reported in this manuscript.

The amount of 125I-Con A bound to the eggs is directly proportional to the egg concentration only in dilute egg suspensions, and when no more than 10,000 eggs are present on each filter at the last step of the binding assay (results not shown). Therefore, we used 0.1-ml samples of suspensions (containing less than 10⁶ eggs/ml) routinely in the binding assay. The use of relatively few eggs per assay made extensive washing necessary to reduce the background to a minimum. About 0.5 to 5% of the total radioactivity present in each assay tube was bound to the eggs.

**Determination of Number and Affinity of 125I-Con A Binding Sites of Unfertilized Eggs**—As shown in Fig. 2A, relatively high concentrations of Con A are required to saturate the α-methyl mannoside-sensitive Con A binding sites of unfertilized eggs. The extent of specific binding was calculated for each 125I-Con A concentration and was plotted according to Scatchard (36) as micrograms of bound 125I-Con A expressed for binding to 5 × 10⁴ eggs. Two classes of binding sites are found: n₁ = 4 × 10⁸, K₁ = 8 × 10⁻⁷ M; n₂ = 4.5 × 10⁸, K₂ = 4 × 10⁻⁸ M.

**Effect of Dithiothreitol Treatment on Binding of 125I-Con A to Unfertilized Eggs**—Fig. 3 shows a Scatchard plot for 125I-Con A binding to eggs previously treated with dithiothreitol. This reagent disrupts the vitelline layer and does not permit elevation of a fertilization membrane. Low affinity sites are present in the same number and with the same affinity as found with untreated eggs, but the number and affinity of high affinity sites is reduced. Since binding to low affinity sites masks that on the less numerous high affinity sites, and there are few points with which to estimate the data, the dotted line is used to indicate our uncertainty about the location of the curve for lower Con A concentrations. Also, as mentioned under "Discussion," the classes of sites are likely heterogeneous in molecular species. Moreover, there is a substantial variability in the effect of dithiothreitol treatment on high affinity sites from one experiment to another, which we are not able to control. It is therefore difficult to give a precise estimate of the number of high affinity sites present on dithiothreitol-treated eggs. In the experiment of Fig. 3 it is around 10⁶ sites/egg; in no experiment did it exceed 1.5 × 10⁷ sites/egg.

**125I-Con A Binding to Embryos**—Fig. 4 shows a Scatchard plot for 125I-Con A binding to 20-min embryos, which are surrounded by hardened fertilization membranes. Only high affinity sites are titrated: there are 2 × 10⁹ high affinity sites/egg, Kₐ = 6 × 10⁻⁷ M. No low affinity sites are found in this experiment. At higher Con A concentrations, binding continues to increase in a nonsaturable process; this binding is not competed for by α-methyl mannoside and thus does not correspond to titration of specific Con A receptor sites. Because of the high level of nonspecific binding, number and affinity of high affinity sites are calculated from the total binding, shown in Fig. 4.

Fig. 5 shows the binding data obtained with 20-min embryos from eggs that had the vitelline layer altered with dithiothreitol to inhibit elevation of the fertilization membrane. In sharp contrast to the situation observed with intact embryos surrounded by a fertilization membrane, low affinity sites persist and their number (n₁ = 8 × 10⁸) is roughly doubled, compared with unfertilized eggs (see Figs. 2 and 3). In the experiment of Fig. 5, some high affinity sites are also titrated (n₂ = 2 × 10⁹ sites/egg; K₂ = 3 × 10⁻⁷ M) but they represent no more than...
The increase in the number of $^{125}$I-Con A sites on fertilized dithiothreitol treated eggs was further investigated. Fig. 6 shows an experiment in which $^{125}$I-Con A binding is measured as a function of the time after fertilization. The number of Con A receptor sites rises quickly during the first minute following the mixing of the eggs with sperm and soon reaches a constant level. This rapid appearance of new sites on the egg surface at fertilization occurs concomitantly with the cortical reaction. It is not triggered by ammonia activation of the eggs (Fig. 6, open triangle), a procedure that allows activation of the eggs but bypasses the cortical reaction. The doubling of Con A sites at fertilization is seen even if the eggs are allowed to develop in Ca$^{2+}$-free sea water containing 10 mM EGTA, a procedure that disrupts the hyaline layer arising on embryos after fertilization (data not shown). Thus, the doubling in Con A sites seems dependent upon the occurrence of the cortical reaction but is not due to the addition of hyaline material released from the cortical granules, for the hyaline dissolves in Ca$^{2+}$-free medium (2). In the experiment of Fig. 6, fertilized eggs were fixed to arrest development in order to measure binding at short intervals after fertilization. Control experiments (data not shown) have shown that fixation with paraformaldehyde does not alter the number of $^{125}$I-Con A binding sites on the eggs significantly, although nonspecific binding is enhanced. However, the nonspecific binding was always lower than 20% of the specific binding. In preliminary experiments (data not shown) it was found that fixation with glutaraldehyde altered the binding of Con A to the eggs much more dramatically than did paraformaldehyde.

Effect of Activation of Eggs on $^{125}$I-Con A Previously Bound to Unfertilized Eggs — Unfertilized eggs were saturated with $^{125}$I-Con A, excess $^{125}$I-Con A was removed by washing, and the release of bound lectin was measured as a function of time after activation. As shown in Fig. 7, some radioactivity is spontaneously released from the egg surface when eggs are transferred to fresh sea water. However, the amount of released radioactivity is very small; after 50 min of incubation in Con A-free sea water, the total amount of radioactivity released from the cell surface is less than 10% of the total. Upon activation by ionophore, the release of bound radioactivity is dramatically increased (Fig. 7). This phenomenon is clearly linked to the cortical reaction, since no such increase is observed upon "ammonia activation" of the eggs (Fig. 7). By 20 min, the release of radioactivity induced by ionophore activation is terminated, and the kinetics of further release are similar to those of the control. The total amount of radioactivity specifically released from the cell surface by ionophore activation corresponds to 10 to 15% of the bound radioactivity.

Fertilization of Eggs Previously Exposed to Con A — Unfertilized eggs were incubated with various $^{125}$I-Con A concentrations, thoroughly washed and assayed for the extent of fertilization. As shown in Fig. 8, the fertilizability of the eggs decreased greatly as the Con A concentration increased above 40 $\mu$g/ml and disappeared at Con A concentrations above 100 $\mu$g/ml, when about 50% of the high affinity sites have been titrated with Con A. The block to fertilization was not overcome by massive excess of sperm. In contrast, addition of 0.1 $\mu$-methyl mannoside immediately restored the fertilizability of the eggs, even after prior incubation with high Con A concentrations.

When eggs previously exposed to $^{125}$I-Con A were fertilized, after washing off excess free lectin, a part of the bound $^{125}$I-Con A was released from the egg surface. Table I shows that around 3% of the bound radioactivity is released upon fertilization of eggs previously incubated in various concentrations of $^{125}$I-Con A. It is clear that this applies only to situations where fertilization is not absolutely inhibited. Therefore, no release of bound radioactivity is observed upon sperm addition when the eggs had been previously incubated in more than 0.1 mg/ml of $^{125}$I-Con A.

* A change does occur in the refractive index of the surface of the Con A-treated egg after fertilization although a normal fertilization membrane does not elevate nor do the eggs divide.
1290 Binding of Concanavalin A to Eggs

Fig. 6 (left). The number of $^{125}$I-Con A binding sites as a function of time after fertilization. Dithiothreitol-treated eggs were fertilized at zero time. At the times indicated, 3-ml aliquots were taken and mixed with 1 ml of a freshly prepared 2% paraformaldehyde solution in sea water (the stock solution was 10% w/v in H$_2$O adjusted at 60° to 9.0 with 1 M NaOH) to arrest development. After 15 min of fixation, the eggs were washed three times and the amount of bound $^{125}$I-Con A was measured with 0.1-ml samples incubated in 500 pg/ml of labeled lectin. The data corresponds to total $^{125}$I-Con A binding and is shown for (○) fertilized eggs, (△) control eggs, and (△) eggs activated with ammonia.

Fig. 7 (right). Release of bound $^{125}$I-Con A from the egg surface upon activation by ionophore A23187. Of concentrated egg suspension (~50%), 1 ml was incubated for 30 min with 1 mg/ml of $^{125}$I-Con A at 10°. The eggs were then rinsed ten times with 10 ml of sea water, divided into three 5-ml fractions, and centrifuged. At zero time the fractions were resuspended in either (○) 5 ml of sea water, (△) 5 ml of sea water containing 10 µM ionophore A23187, or (△) 5 ml of NH$_4$Cl, 10 mM in sea water, and gently agitated. At indicated times, 0.5-ml aliquots were quickly hand-centrifuged and 100 µl of the supernatant was counted in 16 ml of Triton/Omnifluor (New England Nuclear) scintillation liquid. Of the eggs, 100% elevated fertilization membranes upon activation. Eggs resuspended in Millipore-filtered sea water containing a 1/500 dilution of pure dimethylsulfoxide gave the same results as the control in sea water alone.

<table>
<thead>
<tr>
<th>Table I</th>
<th>Release of surface bound $^{125}$I-Con A upon fertilization</th>
</tr>
</thead>
</table>
| Samples (1 ml) of a concentrated egg suspension (~50%) were incubated at 10° for 40 min with various amounts of $^{125}$I-Con A. After washing (five times with 8 ml of sea water) the eggs were fertilized, incubated for 5 min, and centrifuged rapidly. To the supernatant (0.8 ml) was added 100 µl of 0.5 mg/ml bovine serum albumin, as a carrier. The protein was precipitated with 10% trichloroacetic acid, filtered through Millipore filters, and the filters were dried and radioactivity was determined as described under "Experimental Procedures."

<table>
<thead>
<tr>
<th>Fertilization membrane</th>
<th>Con A Released</th>
</tr>
</thead>
<tbody>
<tr>
<td>µg/ml $^a$</td>
<td>% $^b$</td>
</tr>
<tr>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>10</td>
<td>90</td>
</tr>
<tr>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

$^a$ Free $^{125}$I-Con A concentration in the incubation mixture preceding fertilization.

$^b$ Expressed as per cent of eggs with an elevated fertilization membrane 5 min after insemination.

$^c$ Counts per min present in supernatant solution 5 min after fertilization.

$^d$ Expressed as per cent of Con A bound per fertilized egg.

in the membrane, with exposure of previously masked binding sites. If this is so, the receptors are exposed only slowly, for the increase in binding is a gradual one.

We have demonstrated two classes of Con A binding sites on the egg surface, distinct with respect to their affinity for the lectin, one with an affinity around 6 times higher than the other. Both values are in the range of the affinity usually

DISCUSSION

Localization of Con A Binding Sites—We have shown that Con A binds specifically to sea urchin eggs and that this binding is modified at fertilization. Binding is initially rapid, followed by a slow increase that does not seem to be due to endocytosis of Con A, because it is not sensitive to inhibition by sodium azide, nor is it affected by alterations in temperature from 0-10°. The Con A ligand, α-methyl mannoside, can displace all of the bound lectin. Thus the slow increase in Con A binding may correspond to rearrangements of receptor sites
found for the specific binding of Con A to cell surfaces (19–23). Although useful in the description of the data, the classification of Con A receptor sites into two classes is likely to be a gross oversimplification. Each class is probably heterogeneous and composed of distinct glycoproteins, each having a unique affinity for Con A, depending on its tertiary structure and membrane localization. Nevertheless, the ability to distinguish between these two general classes of receptor sites has been useful for studying their localization in the different layers of the complex egg surface.

Although the jelly coat contains a large amount of glycoprotein (37), it does not appear to contain Con A receptor sites. Indeed, the number of sites present on jelly-free eggs is about the same as that on native eggs. Experiments with fluorescein-labeled Con A showed that no binding occurs to the jelly, for all the fluorescence was found at the egg surface (38). In contrast with the jelly coat, a large number of Con A receptor sites is found in the vitelline layer. This is shown by the fact that partial removal or alteration (or both) of this layer with dithiothreitol reduces the affinity and the number of high affinity sites. Indeed, some dithiothreitol-treated batches of eggs did not have any high affinity binding sites, whereas the low affinity sites were not affected. The proposed localization of high affinity sites in the vitelline layer is in agreement with the finding that the only sites detected on fertilized eggs are of the high affinity type. Since the fertilization membrane is derived from the vitelline layer, it seems reasonable to propose that the Con A receptor sites titrated on the elevated fertilization membrane are of vitelline origin. The absence of any binding to the low affinity sites of fertilized eggs is probably caused by the inhibition of access of the lectin to the plasma membrane after the fertilization membrane hardens, rather than to a loss of low affinity sites after fertilization. The number of low affinity sites actually doubles at fertilization, as estimated with dithiothreitol-treated eggs (Fig. 5), where a fertilization membrane does not form. Thus, it appears that the hardened fertilization membrane prevents the diffusion of Con A into the perivitelline space, thus preventing the interaction of Con A with the receptor sites present on the plasma membrane. Indeed, we have indications that agents that inhibit the hardening process allow access of Con A to plasma membrane binding sites (38).

**Exocytosis and Increase in Low Affinity Con A Binding Sites** — The plasma membrane surface area of the unfertilized egg is around $6 \times 10^6 \mu m^2$, assuming a diameter of 80 μm for the egg, and taking into account the presence of about 2 $\times 10^5$ microvilli/egg (4). The density of low affinity sites is thus calculated to be about 7500 sites/μm$^2$, a rather low number compared with other systems. BHK cells have around 130,000 sites/μm$^2$ (18); Dictyostelium, 80,000 sites/μm$^2$ (19); and fat cells from rat between 10$^4$ and 10$^5$ sites/μm$^2$, depending on the diet of the animal (39).

To compare Con A binding to the plasma membrane of fertilized and unfertilized eggs it was necessary to prevent elevation of the fertilization membrane. When this was done by dithiothreitol treatment of unfertilized eggs, it was shown that fertilization results in a doubling of the number of Con A receptors present in the plasma membrane. The increase in the number of Con A binding sites paralleled the kinetics of the cortical reaction (Fig. 6) and is not observed with ammonia activation, which does not trigger the cortical reaction but activates protein synthesis (14). Therefore, the new sites do not appear to arise from activation of the egg per se, but rather from a cortical reaction-linked process. These sites may arise from the new membrane surface that results from fusion of the cortical vesicle membrane with the plasma membrane, leading to a doubling of the surface area (4). The increase in Con A sites is not caused by hyaline material released from the cortical vesicles, since the doubling occurs in Ca$^{2+}$-free sea water containing EGTA, where the hyaline layer is dispersed. We conclude that the internal side of the membrane of the cortical vesicles contains Con A receptor sites of the low affinity type that enter the plasma membrane with the insertion of cortical vesicle membrane. Thus, the cortical vesicle membrane appears to contain Con A binding glycoproteins. An alternative explanation for the increase in Con A binding sites at fertilization is that generalized membrane rearrangement occurs, exposing previously cryptic sites. This is intuitively less attractive as an hypothesis, and does not account quantitatively for the increase as well as does the hypothesis of membrane insertion.

**Release of Con A Binding Sites at Fertilization** — We have also studied the effect of egg activation on Con A that had been bound prior to fertilization. A small amount of Con A is released spontaneously, even in the absence of any activating agent. This release is not surprising, since the binding of Con A to its receptor is not covalent, and probably corresponds to dissociation of $^{125}$I-Con A from a fraction of its binding sites. In contrast, the release of radioactivity specifically induced by fertilization or by the divalent ionophore A23187, is likely to reflect an actual cleavage of receptor sites from the surface. This phenomenon was best studied with ionophore activation, since this reagent triggers the cortical reaction, even in the presence of saturating concentrations of Con A (Fig. 7). Release occurs concomitantly with the cortical reaction; solubilization of radioactivity is probably due to the cleavage of surface Con A binding glycoprotein by the cortical proteases in a limited proteolysis of egg surface components (10). Our results show that Con A binding sites on surface glycoproteins are also removed at fertilization. Whether or not this release plays a role in the activation of development is not clear. It has been reported that the metabolic activation of sea urchin eggs observed upon treatment with ammonia is related to the release of a surface glycoprotein (30). This glycoprotein does not appear to be a Con A binding component, since ammonia treatment did not induce any release of $^{125}$I-Con A-linked radioactivity.

The release of Con A binding sites continues for only 20 min, which may indicate that by this time, all the surface glycoproteins susceptible to the limited proteolysis have been cleaved. This time course agrees with the kinetics of cleavage of total $^{125}$I-labeled surface proteins upon fertilization (10).

**Vitelline Layer Binding Sites and Inhibition of Fertilization** — Incubation of the eggs with more than 0.1 mg/ml of Con A inhibits fertilization of Strongylocentrotus purpuratus, as reported earlier for Paracentrotus lividus (25). Therefore, the study of the release of Con A binding sites upon fertilization was possible only with very low Con A binding; nonetheless, the results obtained were qualitatively analogous to those with ionophore activation. The inhibition of fertilization upon saturation of Con A receptor sites of the cell surface is not a general feature of sea urchin eggs: indeed, no such phenomenon has been observed in the species Asthenocidaris and Hemicentrotus (26). As suggested by Aketa (26), this specificity of Con A interaction with given species may relate to the species specificity of fertilization. In the case of S. purpuratus, inhibition of fertilization occurs when about half of the high affinity (vitelline layer) binding sites are bound by Con A, at a concen-
tration substantially lower than that needed to bind to the low affinity sites. The data suggest that the interaction of sperm with vitelline layer binding components may be an important step in the cortical binding reactions of fertilization, and one that occurs prior to and independently of plasma membrane binding.

Acknowledgments – We are grateful for useful discussions with E. Davie, E. M. Eddy, and C. Foerder.

REFERENCES
Binding of concanavalin A to the surface of sea urchin eggs and its alteration upon fertilization.

M Veron and B M Shapiro


Access the most updated version of this article at http://www.jbc.org/content/252/4/1286

Alerts:

• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/252/4/1286.full.html#ref-list-1