

## SPECIES-SPECIFIC SPERM ADHESION IN SEA URCHINS

### A Quantitative Investigation of Bindin-mediated Egg Agglutination

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#### ABSTRACT

Bindin, a protein component of the acrosomal vesicle of sea urchin sperm, has been isolated from *Arbacia punctulata* and *Strongylocentrotus purpuratus*. Using this isolated bindin, we have devised a quantitative assay for bindin-mediated egg agglutination and compared the agglutination of bindin and eggs from *A. punctulata* and *S. purpuratus*. Bindin-mediated agglutination is species-specific in both species, although a measurable degree of heterotypic interaction is observed. Homotypic bindin-egg interactions differ significantly from heterotypic interactions both in the extent of agglutination and the size of the resulting aggregates. We also provide direct evidence that bindin particles agglutinate eggs by adhering to the surfaces of adjacent eggs. Although the *A. punctulata* bindin preparation displays the same functional properties and consists of one major polypeptide of the same apparent molecular weight as *S. purpuratus* bindin, its morphology is very different. Unlike the spherical aggregates observed with *S. purpuratus* bindin, *A. punctulata* bindin exists as lamellar vesicles and binds significant amounts of phospholipid and Triton X-100, suggesting that it may be tightly associated with the acrosomal membrane.

Having defined a number of the basic parameters of bindin-mediated agglutination, we examined the effect of a number of saccharides and glycopeptides on bindin-mediated egg agglutination. Carbohydrate-containing components derived from the egg cell surface by proteolysis were found to inhibit bindin-mediated egg agglutination at low concentrations, but this inhibition is not species-specific.

KEY WORDS specific adhesion · cell recognition · sea urchin fertilization · bindin

In a step prerequisite to fertilization, echinoid sperm undergo the acrosomal reaction, an exocytotic process that results in deposition of the contents of the acrosomal vesicle on the outer surface of the extended tip of the sperm (4, 5, 19). Recently, Vacquier and Moy (21) have isolated bindin, a major component of the acrosomal vesicle, and have shown that it exists as insoluble granules

in seawater. Further, it has been shown that these granules induce species-specific agglutination of eggs (9, 21). Trypsin treatment of eggs under conditions that cause a loss of their capability to be fertilized also results in the loss of their capability to be agglutinated by bindin. Basing their hypothesis on these observations, Vacquier and co-workers (10, 21, 22) proposed that bindin is the adhesive molecule that specifically binds sperm to eggs during fertilization.

For this study we have devised a quantitative

assay to examine the bindin-dependent agglutination of eggs. With this assay, we have examined the interaction of eggs and isolated bindin in *Arbacia punctulata* and *Strongylocentrotus purpuratus*, and have established the conditions for optimum egg agglutination. We have found that bindin is localized in the region of contact in agglutinated eggs and that bindin from both species preferentially agglutinates eggs of its species of origin, although measurable heterologous agglutination is also observed. We have observed that heterologous and homologous bindin-egg interactions differ in both the extent of agglutination and the size of the aggregate formed. Preliminary experiments suggest that quantitative measurement of bindin-mediated egg agglutination may be useful in elucidating egg surface components involved in sperm-egg adhesion.

## MATERIALS AND METHODS

*A. punctulata* was maintained at 18°C and *S. purpuratus* at 6°C in aquariums containing artificial seawater (Instant Ocean, Aquarium Systems, Eastlake, Ohio). Gametes were obtained from both species by injection of 0.5 M KCl or electric shock. Sperm were stored on ice until used. Bindin was isolated from sperm of both species by the Triton X-100 (Rohm and Haas Co., Philadelphia, Pa.) extraction method described by Vacquier and Moy (21). Isolated bindin was stored in calcium-free seawater (CFSW) at -70°C. Eggs from *S. purpuratus* were dejellied by titrating a 10% vol/vol suspension of eggs to pH 5 with 0.1 M HCl for 2 min and readjusting the pH to 7.9 with 1 M Tris-HCl. Because *A. punctulata* eggs often agglutinate following the acid dejelling procedure, they were mechanically dejellied as follows: a 1% vol/vol egg suspension was repeatedly passed through 90 µm Nitex (Tetko Inc., Elmsford, N. Y.) nylon mesh. Dejelling was monitored by light microscopy after addition of one drop of colloidal ink and judged complete when no more jelly coat was observed and >80% of the eggs were agglutinable by bindin.

### Quantitative Assay for Bindin-dependent Egg Agglutination

Egg agglutination was initiated by adding isolated bindin particles to a 200-µl suspension of 0.5% vol/vol dejellied eggs. The assay was performed in a polyethylene cap (11 × 11 mm, Wheaton R7475B, Wheaton Scientific Div., Wheaton Industries, Millville, N.J.). After addition of bindin, the suspension was rotated on a gyratory shaker (Arthur H. Thomas Co., Philadelphia, Pa.) at 90 rpm for 4 min. After rotation the eggs were gently suspended by the addition of 0.8 ml of seawater and transferred to a microscope slide. Single and agglutinated eggs and the number of eggs in an aggregate were scored using phase contrast microscopy at × 100. The background level of eggs that apparently agglutinated in the absence of bindin was generally <5%. Batches of eggs exhibiting a background level of agglutination of >10% were not used. The variation between duplicate assays was <± 10%.

### Labeling Bindin with Fluorescein Isothiocyanate (FITC)

Bindin (400 µg protein) was suspended by sonication in 400 µl CFSW. Then, 100 µl of 1 M NaHCO<sub>3</sub>, pH 9.1, followed by 20 µl of a 1-mg/ml solution of FITC in isopropanol, were added. The suspension was incubated 90 min at 0°C with frequent mixing. The reaction mixture was diluted to 40 ml with CFSW and centrifuged at 25,000 g for 20 min to pellet the bindin particles. The supernate was removed, and the pellet was washed twice by resuspension in 40 ml CFSW and centrifuged. After washing, the pellet was resuspended in 400 µl CFSW, and the bindin tested for agglutinating activity.

### Preparation of Egg Surface Components by Pronase Digestion

Acid dejellied eggs were suspended to 50% (vol/vol) in artificial seawater containing 50 mM Tris-HCl, pH 8, and 0.25 mg/ml pronase (Calbiochem-Behring Corp., American Hoechst Corp., San Diego, Calif.) that had been predigested for 15 min at 50°C. After 20 min incubation at 23°C, the eggs were pelleted by gentle hand centrifugation and the supernate further centrifuged at 25,000 g for 20 min. The supernate was removed and ethanol added to a final concentration of 5%. The mixture was incubated at 50°C for 24 h, after which time an additional 0.01 mg/ml pronase that had been predigested at 50°C for 15 min was added. The mixture was then incubated an additional 24 h. The digestion was stopped by boiling, and the digestion products were centrifuged at 25,000 g for 20 min. After lyophilization and resuspension in a minimum volume, the mixture was desalted by gel filtration on Biogel P-4 (Bio Rad Laboratories, Richmond, Calif.), and the carbohydrate-containing fractions pooled. No residual protease activity was detected by hydrolysis of *p*-tosyl-L-arginine methyl ester in the preparation.

### Other Procedures and Materials

Bindin samples for electron microscopy were fixed in 1% glutaraldehyde (vol/vol) in artificial seawater for 1 h at 20°C. The samples were postfixated in 1% OsO<sub>4</sub> (wt/vol) in artificial seawater for 1 h at 0°C, dehydrated with alcohol, and embedded in Epon (Shell Chemical Co., Houston, Tex.). Sections were cut with a diamond knife, stained with alcoholic uranyl acetate and lead citrate, and viewed with a Zeiss EM 10 or Siemens 1 electron microscope. SDS polyacrylamide gel electrophoresis was performed by the method of Laemmli (12). Protein was determined by the Folin technique (15). Carbohydrate was determined by the phenol-sulfuric acid method (7). Phospholipid phosphate was estimated after extraction of total lipids with chloroform:methanol (2:1) by the procedure of Bartlett (2).

Carbohydrates were purchased from Pfanzstiel Co (Waukegan, Ill.). The bovine thyroglobulin glycopeptides were a gift of Dr. James Elting of this laboratory.

## RESULTS

### Analysis of Bindin

Bindin was isolated from sperm of *A. punctulata* and *S. purpuratus* by the Triton X-100 extraction method described by Vacquier and Moy (21). SDS

polyacrylamide gel electrophoresis of bindin isolated from *S. purpuratus* (Fig. 1 A) revealed the presence of a single band of protein of an apparent molecular weight of 31,000, in agreement with the findings of Vacquier and Moy (21). Similar analysis of *A. punctulata* bindin showed that the major polypeptide component also has an apparent molecular weight of 31,000 (Fig. 1 B).

Electron microscopy of isolated *S. purpuratus* bindin preparations showed that the bindin particles exist in the form of spherical granules of varying size, ranging from 0.25 to  $>5 \mu\text{m}$  (Fig. 2 A). Remarkably, many of the spheres appear to be hollow. In contrast, electron micrographs of preparations fixed in the isolation medium containing Triton X-100 appear as more uniform granules of  $\sim 0.25\text{--}0.50 \mu\text{m}$  in diameter (*inset*). This suggests that after isolation in the absence of Triton X-100, bindin granules from *S. purpuratus* undergo extensive aggregation.

Electron micrographs of isolated bindin from *A. punctulata* revealed that the bindin is in the form of lamellar vesicles (Fig. 2 B) composed of varying numbers of lamellae. As many as seven lamellae can be resolved (*inset*). This lamellar morphology of *A. punctulata* bindin is retained in samples fixed in the Triton X-100-containing isolation medium, even after repeated Triton X-100 extractions. Determination of the amount of phospholipid extracted by chloroform:methanol (2:1) indicated that phospholipid accounts for 12% of the mass of *A. punctulata* bindin. 67% of the mass is protein as determined by the Folin reaction (15). This corresponds to an approximate ratio of eight phospholipid molecules to each bindin polypeptide. 14% of the mass of the *A. punctulata* bindin preparations was Triton X-100.

#### Quantitative Assay for Bindin-dependent Egg Agglutination

To quantitatively assess bindin-mediated agglutination of eggs, bindin particles were introduced into a suspension containing  $\sim 2,000$  dejellied eggs and gyrated at 90 rpm. During gyration the eggs collect at the center of the vial where they collide. As shown in Fig. 3 A and B, aggregation of eggs is dependent on the concentration of bindin and reaches a maximum at  $20 \mu\text{g}/\text{ml}$  ( $4 \mu\text{g}/\text{assay}$ ) for both *A. punctulata* and *S. purpuratus* bindin. The kinetics of bindin-mediated egg agglutination are shown in Fig. 4. Agglutination increases with time, reaching a maximum after 4 min of rotation. The

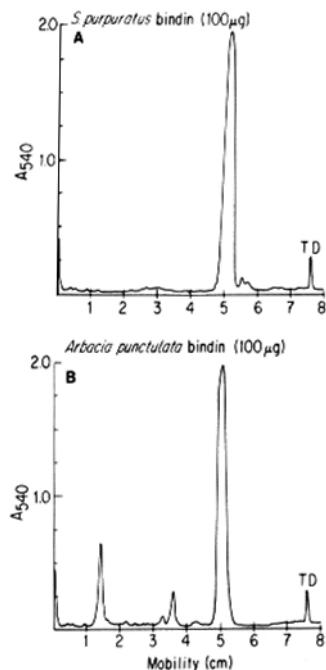
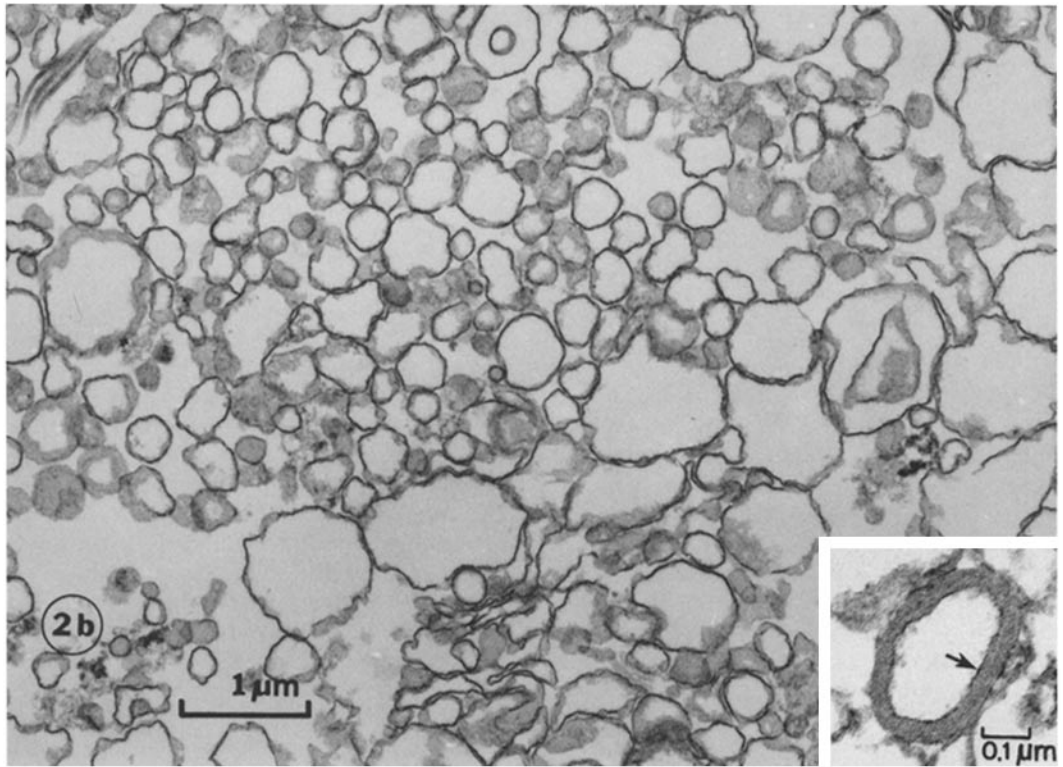
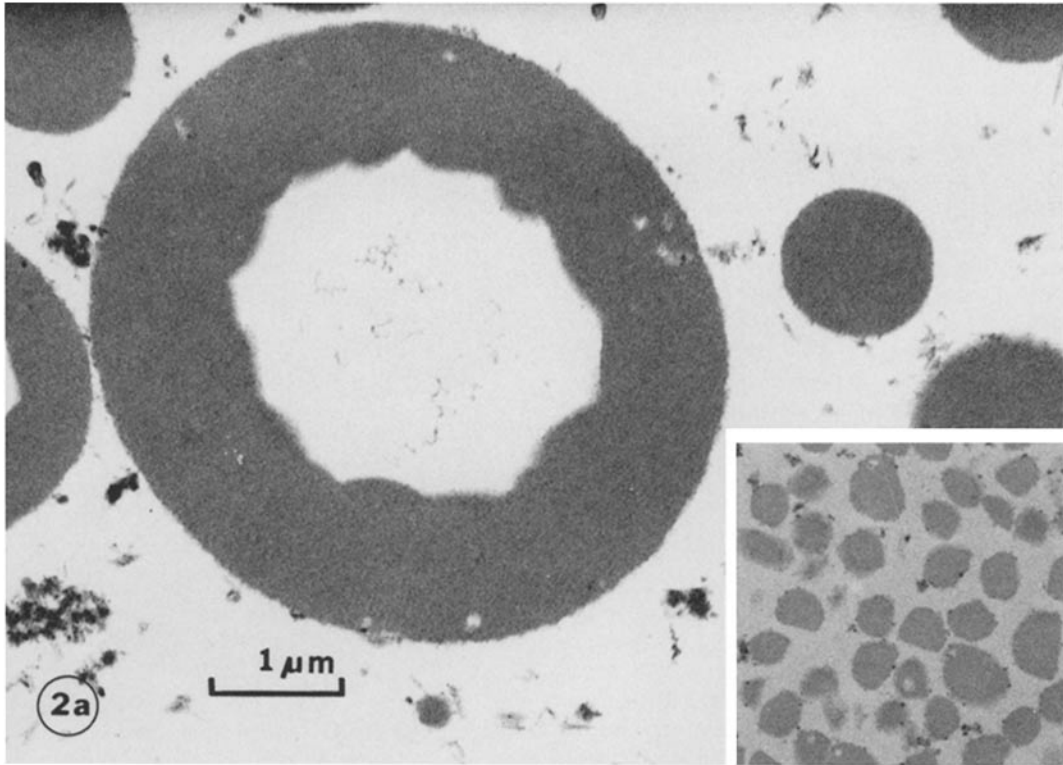


FIGURE 1 SDS polyacrylamide gel scans of bindin isolated from sperm. (A)  $100 \mu\text{g}$  of *S. purpuratus* bindin. (B)  $100 \mu\text{g}$  of *A. punctulata* bindin. The major component, which represents 83% of the Coomassie Blue stain, has the same relative mobility as *S. purpuratus* bindin. TD, tracking dye.

formation of aggregates by heterologous bindin (see below) has the same kinetics, displaying a maximum at 4 min (data not shown). The extent of agglutination does not depend on the speed of rotation within the range of 60–180 rpm.

Using this assay, we established that agglutination does not require exogenous divalent cations; eggs in  $\text{Ca}^{2+}$ - and  $\text{Mg}^{2+}$ -free seawater containing EDTA agglutinate to the same extent as control eggs in seawater (data not shown). The pH range for bindin-mediated egg agglutination is very broad, with agglutination occurring in the range of pH 4.5–9; the optimum is between pH 5.5 and 7 (Fig. 5).

To gain some insight into the physical basis of the bindin-mediated agglutination, fluorescein-labeled bindin was prepared by reaction with FITC. FITC-labeled bindin was found to have the same egg-agglutinating properties as unlabeled bindin (data not shown). Fluorescence microscopy of eggs agglutinated by fluorescein-labeled bindin revealed that bindin particles are invariably present



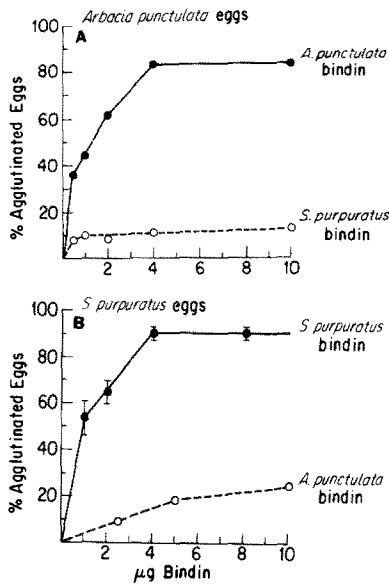


FIGURE 3 Egg agglutination by bindin from *A. punctulata* and *S. purpuratus*. (A) *A. punctulata* eggs are effectively agglutinated by *A. punctulata* bindin, but only limited agglutination is observed with bindin from *S. purpuratus*. (B) *S. purpuratus* eggs are effectively agglutinated by bindin of the same species. In contrast, *A. punctulata* bindin is less effective in agglutinating *S. purpuratus* eggs.

at the point of contact between eggs (Fig. 6). Bindin particles were observed in the region of contact between eggs in all 44 egg pairs examined. Some bindin particles were also present on areas of the egg surface not involved in contact. Fluorescent *S. purpuratus* bindin particles were also found at regions of contact between agglutinated *A. punctulata* eggs, suggesting that agglutination of heterologous eggs occurs by a mechanism similar to that of homologous eggs. Glutaraldehyde-fixed *S. purpuratus* eggs were agglutinated in an identical fashion, ruling out the possibility that agglutination is a dynamic response of the egg to bindin

FIGURE 2 Electron micrographs of isolated bindin. (a) Electron micrograph of *S. purpuratus* bindin after the final step of the isolation procedure.  $\times 18,000$ . Spherical granules are evident in the size range of from  $0.25 \mu\text{m}$  to  $>5 \mu\text{m}$ . Inset: electron micrograph of *S. purpuratus* bindin fixed in the isolation medium containing Triton X-100.  $\times 18,000$ . In this medium it is apparent that the bindin exists as a homogeneous population of granules of  $0.25 \mu\text{m}$  diameter. (b) Electron micrograph of isolated *A. punctulata* bindin.  $\times 18,000$ . Isolated *A. punctulata* bindin is in the form of large vesicles composed of varying numbers of lamellae. This vesicular morphology is seen even after repeated Triton X-100 extraction and after fixing in the Triton-containing isolation medium. Inset: as many as seven lamellae can be resolved (arrow).  $\times 60,000$ .

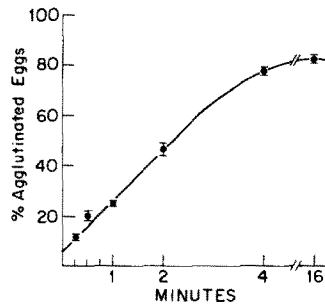


FIGURE 4 Kinetics of bindin-mediated egg agglutination. Agglutination increases with time and reaches a maximum after 4 min of rotation.

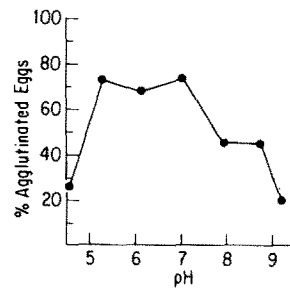


FIGURE 5 Dependence of bindin-mediated egg agglutination on pH. *S. purpuratus* eggs were suspended in artificial seawater containing 25 mM Tris-maleate at various pH values, and bindin was added to a concentration of  $12.5 \mu\text{g}/\text{ml}$ . Agglutination was scored after 4 min of rotation at 90 rpm.

stimulation (data not shown). These findings establish directly that the adhesion of bindin granules to components of the egg surface is responsible for the bindin-induced agglutination of eggs.

### Species-Specificity

Although, as shown in Fig. 3 A, bindin isolated from *A. punctulata* sperm effectively agglutinated eggs of this species, bindin from *S. purpuratus* was relatively ineffective in agglutinating *A. punctulata*

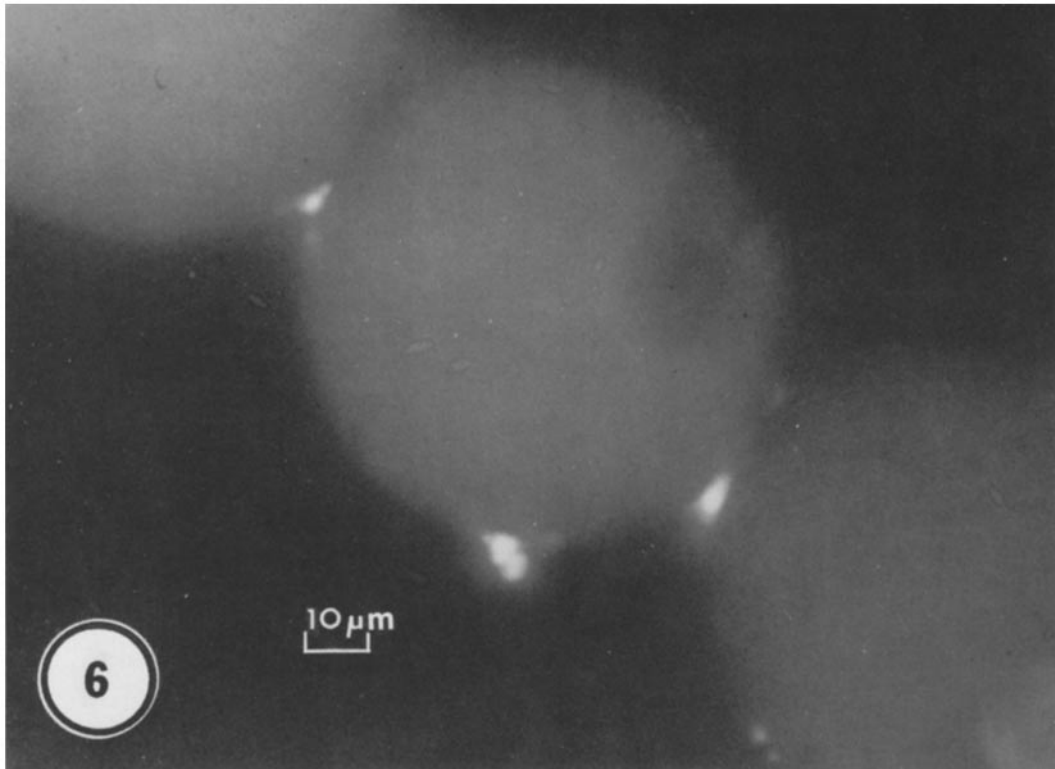


FIGURE 6 Fluorescence illumination light micrograph of *S. purpuratus* eggs agglutinated by fluorescein-labeled *S. purpuratus* bindin particles. Bindin particles are invariably present at points of contact between agglutinated eggs.

eggs. A maximum of 20% of the *A. punctulata* eggs could be agglutinated by *S. purpuratus* bindin; an increase in agglutination was not observed, even if the level of bindin was increased 10-fold over that shown in Fig. 3 A. Similar results were obtained in the opposite heterotypic cross (Fig. 3 B): *S. purpuratus* bindin effectively agglutinated *S. purpuratus* eggs, whereas *A. punctulata* bindin was relatively ineffective in promoting agglutination of *S. purpuratus* eggs.

Although a measurable degree of heterotypic bindin-induced agglutination was observed, the size of the aggregate formed with heterologous bindin was much smaller than that formed with homologous bindin. For example, *S. purpuratus* eggs formed large aggregates containing an average of seven or more eggs (Fig. 7), and aggregates containing >100 eggs were observed. In contrast, the aggregates formed with *S. purpuratus* eggs and *A. punctulata* bindin, or with *A. punctulata* eggs and *S. purpuratus* bindin (data not shown), consisted of very small aggregates containing only

pairs or trios of eggs (Fig. 7). This suggests that the forces involved in homologous interactions may be much greater than those involved in heterologous interactions.

Other evidence for the species-specificity of bindin-mediated egg agglutination comes from observations of the interaction of bindin with a mixed population of eggs. *A. punctulata* and *S. purpuratus* eggs are easily distinguished by their pigmentation. When limiting amounts of bindin from either species were added to an egg suspension containing equal numbers of eggs of both species, the majority of egg-egg associations were of the homologous type (Fig. 8 A and B). The next most frequent association was between the eggs of different species. Very few bindin-dependent associations of heterologous eggs were observed. Bindin preferentially associates with, and induces aggregation in, homotypic eggs.

#### *Inhibition of Agglutination*

Using the quantitative agglutination assay, we

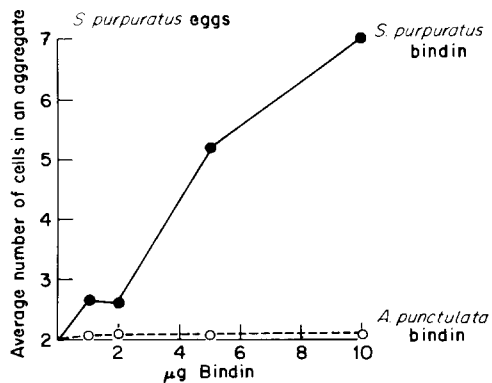


FIGURE 7 Average size of the aggregate formed by *S. purpuratus* eggs and bindin from *S. purpuratus* and *A. punctulata*. Eggs and bindin from *S. purpuratus* form large aggregates containing seven or more eggs. Aggregates formed of *S. purpuratus* eggs and *A. punctulata* bindin tend to consist of pairs or trios of eggs.

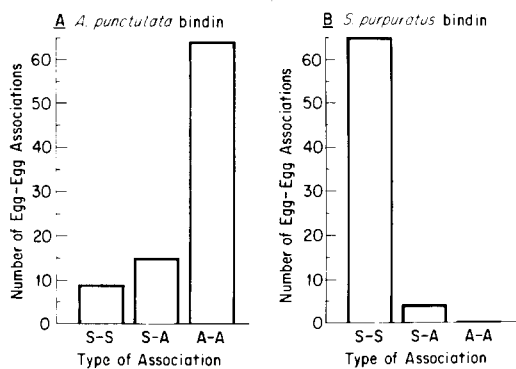


FIGURE 8 Distribution of the bindin-induced aggregates formed in a mixed population of eggs. A suspension containing equal numbers of eggs of *S. purpuratus* and *A. punctulata* was mixed with limiting amounts of bindin from one of the species. The resulting egg-egg associations are scored as homotypic with *S. purpuratus* (S-S) or *A. punctulata* (A-A), or as a heterotypic hybrid (S-A). (A) Distribution of egg associations formed with *A. punctulata* bindin. (B) Distribution of egg associations formed with *S. purpuratus* bindin.

have confirmed the observation that trypsin treatment of eggs renders them nonagglutinable by bindin (21). It is apparent that the egg surface components that interact with bindin are extremely sensitive to trypsin, because treatment of a 2% vol/vol suspension of dejellied eggs with 50 µg/ml trypsin at 4°C for 4 min reduced the level of agglutination to 10% of control values. In the hope of obtaining some information about the

nature of the surface component that interacts with bindin, we examined the possible inhibitory effect of a number of saccharides, glycopeptides, and components of the egg surface on bindin-mediated egg agglutination (Table I). As shown, the surface components released by Pronase digestion of intact eggs are effective inhibitors of bindin-mediated egg agglutination. However, this inhibition is not species-specific; preparations of egg surface material from both species inhibit agglutination in both species of eggs to the same extent and at similar concentrations (Table I). Other components of the egg cell surface such as the fucose sulfate polymer and the sialoprotein that make up the jelly coat (17) are inactive. Also inactive are monosaccharides and a complex type of glycopeptide from thyroglobulin. The only other compound tested that showed some inhibitory activity was a polymannose type of glycopeptide from thyroglobulin.

The inhibition of bindin-induced egg agglutination by the egg surface components does not appear to be the result of residual protease activity in these preparations because no protease activity was detected with synthetic substrates. Furthermore, pretreatment of the eggs with the surface

TABLE I  
Comparison of the Effect of Various Saccharides and Glycopeptides on Bindin-mediated Egg Agglutination

Source of egg surface glycopeptides	Assay species	Concentration required for >80% inhibition of agglutination (monosaccharide equivalents)
		mM
<i>S. purpuratus</i>	<i>S. purpuratus</i>	3.0
<i>A. punctulata</i>	<i>S. purpuratus</i>	1.0
<i>S. purpuratus</i>	<i>A. punctulata</i>	4.2
<i>A. punctulata</i>	<i>A. punctulata</i>	7.7

*S. purpuratus* fucose sulfate polymer and sialoprotein from egg jelly coat were inactive at 3.1 and 0.6 mM monosaccharide equivalents, respectively, concentrations well above that necessary to induce the acrosome reaction in sperm. Complex and polymannose type glycopeptide from bovine thyroglobulin were tested at 7.7 and 4.6 mM, respectively. Only the polymannose glycopeptide was inhibitory, but the inhibition never exceeded 20%. The monosaccharides tested included the α and β methyl glycosides of mannose, galactose, and glucose, as well as N-acetyl glucosamine, N-acetyl galactosamine, fucose, and N-acetyl neuraminic acid. None of these monosaccharides had any effect at 250 mM.

components for 1 h, followed by removal of the inhibitor by washing, had no effect on the subsequent agglutination of the eggs by bindin.

## DISCUSSION

Based on a number of lines of evidence, it has been suggested that the protein bindin, a component of the acrosomal vesicle, functions in sperm-egg adhesion (9, 10, 21, 22). Because the bindin-induced agglutination of eggs seems to be the result of interaction of bindin with the egg surface receptors that participate in sperm adhesion, we felt that this phenomenon might be a useful tool in the characterization of the receptor.

With this objective in mind, we first isolated bindin from the sperm of *S. purpuratus* and *A. punctulata* using the procedure previously reported for *S. purpuratus* bindin. Bindin had not been previously isolated from *A. punctulata* sperm, and therefore it was of interest to compare its composition and properties with those of bindin from *S. purpuratus*. Although isolated *A. punctulata* bindin was found to consist of one major polypeptide component of 31,000 mol wt that comigrated on SDS gels with *S. purpuratus* bindin, and although it displayed the same functional properties of egg agglutination (see below), its morphology is strikingly different from that of *S. purpuratus* bindin. Whereas, as has been previously reported (21), isolated *S. purpuratus* bindin in 1.67% Triton X-100 exists as discrete granules, *A. punctulata* bindin formed lamellar vesicles containing protein and phospholipid in a mass ratio of 6:1 after isolation in 1.67% Triton. This novel morphology of *A. punctulata* bindin persists when the bindin is fixed in a Triton X-100-containing isolation medium and after repeated extraction and removal of Triton X-100.

Partial amino acid sequencing of bindin from *S. purpuratus* and *S. franciscanus* has shown that the NH<sub>2</sub>-terminal region is rich in hydrophobic residues (22). Although no sequence information is available on *A. punctulata* bindin, it is perhaps even more hydrophobic than *S. purpuratus* bindin. In this connection, it should be noted that a number of lipid-free amphiphilic membrane proteins form micelles upon the removal of detergent (18). The lamellar morphology of *A. punctulata* bindin is perhaps a reflection of the amphiphilic structure of the polypeptide chain. Indeed, perhaps after the acrosomal reaction, bindin may become incorporated as an integral membrane protein into the membrane of the acrosomal process. It is possible

that the insertion of bindin into the acrosomal process membrane provides the extra surface area needed to cover the extending acrosomal filament.

As noted in Results, we have found that in the absence of Triton X-100, isolated bindin granules from *S. purpuratus* extensively coaggregate to form large particles >5 μm in diameter. This finding may provide a molecular explanation for the observation of Collins (3). He observed that *S. purpuratus* sperm form rosettes after induction of the acrosome reaction; this process appeared to be mediated by a "sticky" substance that coats the acrosome filament. The formation of rosettes is perhaps the result of the coaggregation of bindin on adjacent sperm.

With bindin from two species available, we developed a quantitative, microscopic assay for bindin-induced agglutination of eggs. We have extended earlier qualitative observations showing that bindin-mediated egg agglutination in *S. purpuratus* and *S. franciscanus* is species-specific (9). Using a different combination of species, we have demonstrated that bindin-mediated agglutination with *A. punctulata* and *S. purpuratus* shows marked, but not absolute, species-specificity. It is not clear whether the differences in observed specificity between this study and the earlier report (9) are the result of differences in the two methods of assessing egg agglutination or of differences in the actual extent of cross reaction between bindin and eggs. We have established that the size of the aggregate formed by heterologous bindin is much smaller than that of aggregates formed by homologous bindin. It seems possible that this cross-reaction could be overlooked in a qualitative assay based on macroscopic observations.

The difference in aggregate size is consistent with the possibility that the adhesive strength of homologous bindin-egg interactions is much greater than the heterologous interactions. Also consistent with this idea are our observations of bindin interactions in a mixed population of eggs. In a mixture of the two species of eggs, the aggregates formed with bindin of one species occurring most frequently are those composed solely of eggs homotypic to the bindin. Finally, in the context of bindin-induced adhesion, it should be noted that the finding that fluorescein-labeled bindin is invariably found localized at the junction between aggregated cells provides the first direct evidence that bindin indeed is an "adhesive" molecule.

We have found that bindin-mediated egg agglutination occurs in the absence of divalent cations



and at pH 7, and that this process is not inhibited by concanavalin A. However, all of these conditions and treatments are known to inhibit sperm binding or fertilization (1, 11, 13, 14, 16, 23, 24). Our interpretation of this apparent contradiction is that these conditions and treatments may inhibit the occurrence of the acrosome reaction that is a prerequisite for sperm binding but do not affect the interaction of bindin and the egg surface per se. It is known that  $\text{Ca}^{2+}$  (5) and alkaline pH (6) are required for the acrosome reaction, and that sperm can fertilize eggs in CFSW if they are treated with egg jelly to preinduce the acrosome reaction (20).

We have confirmed the observations of Vacquier and Moy (21) that trypsin treatment of the egg abolishes bindin agglutination ability, and that egg surface glycopeptides inhibit bindin-mediated agglutination. Recently, it has been reported that bindin agglutinates trypsin-treated, formaldehyde-fixed red blood cells, and that preliminary data suggest that bindin is specific for terminal galactose residues (22). These observations and other data (10, 21) have been interpreted to suggest that bindin-egg interaction is another example of lectin-glycoprotein "receptor" interaction. We have also observed inhibition of bindin-mediated agglutination by cell surface glycopeptides and have shown that this inhibition is not species-specific. This could mean that these glycopeptides are not derived from the egg cell surface molecules that are involved in the binding of bindin. Alternatively, the glycopeptides may have lost specificity upon cleavage from the intact receptor. If the lectin-glycoprotein hypothesis is true, the specificity may be a result of the three-dimensional orientation of accessibility of oligosaccharides on the cell surface (8).

Glabe and Vacquier (10) reported a  $^{125}\text{I}$ -labeled egg surface component that bound to bindin particles, and they presented evidence that this binding was species-specific. In this report, the authors pointed out that it was not possible to distinguish whether the observed specificity was a result of differences in concentration of "receptor" in the crude preparations or of actual species-specific interaction. The present report suffers from the same ambiguity. Perhaps species-specific differences in the affinity of the glycopeptides for bindin exist, but we are unable to detect these differences in affinity, possibly because the unfractionated preparations may differ in the concentration of active glycopeptide. We hope further studies using

the quantitative assay described in this paper for studying the effect of purified egg surface components on bindin-dependent agglutination will provide additional insight into the molecular basis of adhesion and species-specificity in fertilization.

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## REFERENCES

- AKETA, K. 1975. Physiological studies on the sperm surface component responsible for sperm-egg bonding in sea urchin fertilization. *Exp. Cell Res.* **80**:439-441.
- BARTLETT, G. R. 1959. Phosphorus assay in column chromatography. *J. Biol. Chem.* **234**:466-468.
- COLLINS, F. 1976. A reevaluation of the fertilizin hypothesis of sperm agglutination and the description of a novel form of sperm adhesion. *Dev. Biol.* **49**:381-394.
- DAN, J. C. 1954. Studies on the acrosome. II. Acrosome reaction in starfish spermatozoa. *Biol. Bull. (Woods Hole)*. **107**:203-209.
- DAN, J. C. 1954. Studies on the acrosome. III. Effect of calcium deficiency. *Biol. Bull. (Woods Hole)*. **107**:209-215.
- DECKER, G. L., D. B. JOSEPH, and W. J. LENNARZ. 1976. A study of factors involved in the induction of the acrosomal reaction in sperm of the sea urchin *Arbacia punctulata*. *Dev. Biol.* **53**:115-125.
- DUBOIS, M., K. A. GILLES, J. K. HAMILTON, P. A. REBERS, and F. SMITH. 1956. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* **28**:350-356.
- GLABE, C. 1979. A supramolecular theory for specificity in intracellular adhesion. *J. Theor. Biol.* **78**:417-423.
- GLABE, C. G., and V. D. VACQUIER. 1977. Species specific agglutination of eggs by bindin isolated from sea urchin sperm. *Nature (Lond.)*. **267**:836-837.
- GLABE, C. G., and V. D. VACQUIER. 1978. Egg surface glycoprotein receptor for sea urchin sperm bindin. *Proc. Natl. Acad. Sci. U. S. A.* **75**:881-885.
- HOWE, C. W. S., and C. B. METZ. 1972. Multivalent and univalent concanavalin A as a probe for studying sperm-egg interactions. *Biol. Bull. (Woods Hole)*. **143**:465.
- LAEMMLI, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (Lond.)*. **227**:680-685.
- LALLIER, R. 1972. Effect of concanavalin A on the development of the sea urchin egg. *Exp. Cell Res.* **72**:157-163.
- LOEB, J. 1915. On the nature of the conditions which determine or prevent the entrance of the spermatozoa into the egg. *Am. Nat.* **49**:257-269.
- LOWRY, O. H., N. J. ROSEBROUGH, A. L. FARR, and R. J. RANDALL. 1951. Protein measurements with the Folin phenol reagent. *J. Biol. Chem.* **193**:265-275.
- SCHMELL, E., B. J. EARLES, C. BREAUX, and W. J. LENNARZ. 1977. Identification of a sperm receptor on the surface of the eggs of the sea urchin *Arbacia punctulata*. *J. Cell Biol.* **72**:35-46.
- SEGALL, G., and W. J. LENNARZ. 1979. Chemical characterization of the component of the jelly coat from sea urchin eggs responsible for induction of the acrosome reaction. *Dev. Biol.* **71**:33-48.
- SIMONS, K., A. HELENIUS, K. LEONARD, M. SARVAS, and M. J. BETHING.

1978. Formation of protein micelles from amphiphilic membrane proteins. *Proc. Natl. Acad. Sci. U. S. A.* **75**:5305-5310.
19. SUMMERS, R. G., and B. L. HYLANDER. 1975. Species specificity of the acrosome reaction and primary gamete binding in echinoids. *Exp. Cell Res.* **96**:63-68.
  20. TAKAHASHI, Y., and M. SUGIYAMA. 1975. Relationship between the acrosome reaction and fertilization in the sea urchin. I. Fertilization in Ca-free sea water with egg-water-treated spermatozoa. *Dev. Growth Differ.* **15**:261-267.
  21. VACQUER, V. D., and G. MOY. 1977. Isolation of bindin: the protein responsible for adhesion of sperm to sea urchin eggs. *Proc. Natl. Acad. Sci. U. S. A.* **74**:2456-2460.
  22. VACQUER, V. D., and G. MOY. 1978. Macromolecules mediating sperm-egg recognition and adhesion during sea urchin fertilization. In ICN-UCLA Symposia on Molecular and Cellular Biology. E. R. Dirksen, D. Prescott, and C. F. Fox, editors. Academic Press Inc., New York. **12**: 379-389.
  23. VACQUER, V. D. and J. E. PAYNE. 1973. Methods for quantitating sea urchin sperm-egg binding. *Exp. Cell Res.* **82**:227-235.
  24. VERON, M., and B. M. SHAPIRO. 1977. Binding of concanavalin A to the surface of sea urchin eggs and its alteration upon fertilization. *J. Biol. Chem.* **252**:1286-1292.