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# Carbohydrate Specificity of Sea Urchin Sperm Bindin: a Cell Surface Lectin Mediating Sperm-Egg Adhesion

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**ABSTRACT** We have examined the carbohydrate specificity of bindin, a sperm protein responsible for the adhesion of sea urchin sperm to eggs, by investigating the interaction of a number of polysaccharides and glycoconjugates with isolated bindin. Several of these polysaccharides inhibit the agglutination of eggs by bindin particles. An egg surface polysaccharide was found to be the most potent inhibitor of bindin-mediated egg agglutination. Fucoidin, a sulfated fucose heteropolysaccharide, was the next most potent inhibitor, followed by the egg jelly fucan, a sulfated fucose homopolysaccharide, and xylan, a  $\beta(1\rightarrow4)$  linked xylose polysaccharide. A wide variety of other polysaccharides and glycoconjugates were found to have no effect on egg agglutination. We also report that isolated bindin has a soluble lectinlike activity which is assayed by agglutination of erythrocytes. The bindin lectin activity is inhibited by the same polysaccharides that inhibit egg agglutination by particulate bindin. This suggests that the egg adhesion activity of bindin is directly related to its lectin activity. We have established that fucoidin binds specifically to bindin particles with a high apparent affinity ( $K_d = 5.5 \times 10^{-8}$  M). The other polysaccharides that inhibit egg agglutination also inhibit the binding of  $^{125}\text{I}$ -fucoidin to bindin particles, suggesting that they compete for the same site on bindin. The observation that polysaccharides of different composition and linkage type interact with bindin suggests that the critical structural features required for binding may reside at a higher level of organization. Together, these findings strengthen the hypothesis that sperm-egg adhesion in sea urchins is mediated by a lectin-polysaccharide type of interaction.

A growing body of evidence indicates that carbohydrates of the cell surface serve as specific recognition determinants in intercellular adhesion phenomena (5, 13). Sea urchin fertilization represents an example of specific cell recognition and adhesion, the outcome of which is the fusion of the gametes and subsequent activation of the zygote. Sperm adhesion to the egg vitelline layer is mediated by the protein bindin, the major component of the sperm acrosome granule, which is exposed by exocytosis during the acrosome reaction (29, 32, 33). Two lines of evidence support the hypothesis that bindin mediates sperm-egg adhesion. Immunocytochemistry with anti-bindin localizes bindin to the site of sperm-egg adhesion (19), and insoluble bindin particles agglutinate sea urchin eggs species specifically (6, 8). This specificity of egg agglutination by bindin corresponds to the specificity observed for sperm adhesion. The available evidence suggests that egg surface glycoconjugates serve as receptors for bindin (7-9, 32). The egg

surface glycoconjugate implicated in the adhesion of sperm to eggs is a high molecular weight polysaccharide containing fucose, xylose, galactose, and glucose (9).

In a preliminary communication, Vacquier and Moy (34) reported that bindin agglutinates erythrocytes and that the hemagglutination activity is inhibited by several simple sugars. We have not been able to consistently reproduce this observation with particulate bindin; however, we have found that bindin suspensions contain a soluble hemagglutination activity that is reproducibly inhibited by simple sugars. Thus bindin satisfies the classical definition of a lectin; it agglutinates erythrocytes and this agglutination is inhibited by specific sugars (12). We have further defined the carbohydrate-binding specificity of bindin in this report by using a series of polysaccharides and neoglycoproteins. The results of these investigations demonstrate that the egg agglutinating activity of bindin is directly related to its lectin activity.

## MATERIALS AND METHODS

Gametes of *Strongylocentrotus purpuratus* were obtained by injection of 0.5M KCl. Bindin was isolated as described (33) with the following modifications: the isolation medium contained 0.3 M sucrose and, after the first high-speed centrifugation, the bindin pellet was resuspended in 2 M NaCl, 50 mM NaPO<sub>4</sub>, pH 6 containing 0.1% Triton X-100. After extraction for 30 min, the bindin particles were pelleted and washed twice with 0.5 M NaCl, 50 mM Tris pH 7.4. Bindin prepared in this manner is >95% pure as judged by densitometry of Coomassie Blue-stained SDS polyacrylamide gels (8, 33). Silver-stained gels reveal several residual contaminating proteins, with the major contaminant of a molecular weight corresponding to that of tubulin. Isolated bindin was stored at -20°C. Eggs were dejelled by titrating a 10% suspension to pH 5.0 with 0.1 M HCl for 2 min and readjusting the pH to 7.9 with 1 M Tris HCl pH 8.0 and washed three times with 100 vol of sea water. Fucoidin was purchased from ICN-K&K chemicals (Plainview, NY). Other chemicals were obtained from Sigma Chemical Co. (St. Louis, MO). Egg jelly sulfated fucose polysaccharide was isolated as described by SeGall and Lennarz (26). Guaran was a generous gift of Dr. I. J. Goldstein (University of Michigan). The mnn<sub>2</sub> yeast mannan was a gift of Dr. C. Ballou (University of California, Berkeley). Neoglycoproteins were prepared by reacting aminophenylglycoside isothiocyanate derivatives with bovine serum albumin (BSA) as previously described (15). Substitution ratios of the neoglycoproteins were in the range of 15–20 moles of saccharide per mole of BSA. Fucoidin was desulfated by solvolysis as described (31).

The agglutination of eggs by bindin was quantified as previously described (8). Bindin was routinely sonicated to disperse frozen aliquots. Soluble bindin hemagglutinin was prepared from a 1 mg/ml suspension of bindin particles in 0.5M NaCl, 50 mM Tris HCl, pH 7.4, by centrifuging at 100,000 g for 1 h at 4°C. The resulting supernatant, which contained 25–50% of the total hemagglutination activity and 5% of the protein, gave a hemagglutination titer of 128 at 50 µg/ml protein. Glutaraldehyde-fixed, trypsin-treated rabbit erythrocytes (GTRs) were prepared as previously described (10). Hemagglutination assays were carried out in microtiter V-shaped multitest plates (Dynatech Laboratories): One drop (25 µl) of serially diluted soluble bindin was added to a well containing 1 drop of BSA-saline (1 mg/ml BSA in 0.15 M NaCl) with or without inhibitors. 1 drop of GTRs (2.5% vol/vol in CMF) is added, the dish is shaken, the erythrocytes are allowed to settle for 45 min, and the endpoint (highest dilution of extract still producing hemagglutination) is recorded.

Fluorescent fucoidin was prepared as will be described in detail (Glabe et al. Manuscript in preparation). Briefly, 20 mg of fucoidin in 2 ml of water was activated with 20 mg of CNBr by titrating to pH 11 for 8 min. The solution was desalted by gel filtration on a 1 × 40 cm column of Sephadex G-50 (Pharmacia Fine Chemicals, Piscataway, NJ) in 0.2 M Na Borate pH 8.0. The fucoidin-containing fractions were immediately pooled and reacted with 2 mg of fluorescein amine for 24 h, after which the derivatized fucoidin was separated from the unincorporated fluorescein by gel filtration on Sephadex G-50 in Ca<sup>2+</sup>, Mg<sup>2+</sup>-free Dulbecco' phosphate-buffering saline (CMF). The fluorescein-derivatized fucoidin (fl-fucoidin) contained 1 mole of fluorescein per 1,000 moles of fucose residues and retained full potency in inhibiting hemagglutination as compared to the underivatized fucoidin. Fl-fucoidin was iodinated by reacting 150 µg of fl-fucoidin and 0.2 mCi of <sup>125</sup>I (New England Nuclear, Boston, MA) in 0.2 ml of Na borate buffer pH 8.0 with a 4-µg film of Iodogen (Pierce Chemical Co., Rockford, IL) as previously described (4). <sup>125</sup>I-fl-fucoidin was purified by gel filtration on Sephadex G-50, and a specific activity of 2 × 10<sup>5</sup> CPM per µg fucoidin was obtained. Binding of <sup>125</sup>I-fl-fucoidin to bindin was performed by the filtration assay previously described (7). The binding of <sup>125</sup>I-fl-fucoidin was linearly dependent on bindin concentration and, with bindin in excess, 50% of the total CPM bound to bindin.

Carbohydrates were determined by the phenol-sulfuric acid assay using L-fucose as a standard (3). Protein was quantified by absorption at 280 nm with BSA as standard.

## RESULTS

### *Inhibition of Bindin-mediated Egg Agglutination*

On the basis of the previous characterization of the egg surface glycoconjugate implicated in sperm adhesion (9), we have examined the interaction between polysaccharides of similar composition and defined structure and bindin particles. As indicated in Table I, a number of polysaccharides are potent inhibitors of the agglutination of eggs by bindin. The egg surface glycoconjugate, released by Pronase digestion, is the best inhibitor of bindin-mediated egg agglutination, followed by fucoidin (a sulfated heteropolysaccharide consisting pre-

dominantly of L-fucose; 16, 17, 21) and xylan (a β(1 → 4) linked xylose polysaccharide; 30). The inhibition of egg agglutination by these polysaccharides is not due to an effect on the egg surface, since pretreatment of the eggs with the polysaccharides followed by washing has no effect on the ability of the eggs to be subsequently agglutinated by bindin.

Sulfation appears to be required for the inhibitory activity of fucoidin, since desulfated fucoidin, containing <10% of the esterified sulfate of fucoidin, is inactive as an inhibitor of bindin-mediated egg agglutination. However, the inhibition by fucoidin cannot be ascribed solely to an ionic interaction between the charged sulfate groups and bindin, since other sulfated polysaccharides such as heparin and chondroitin sulfate have no effect on bindin-mediated egg agglutination. The egg jelly fucose polysaccharide, a sulfated fucose homopolymer (26, 27), also inhibits egg agglutination but at concentrations slightly higher than fucoidin. A wide variety of polysaccharides listed in Table I, as well as simple saccharides, have no effect on egg agglutination by bindin, confirming previous observations (8).

### *Inhibition of the Hemagglutination Activity of Bindin*

We found that bindin has a soluble (100,000 g, 1 h) lectinlike activity which is assayed by agglutination of erythrocytes. The soluble hemagglutination activity represents a substantial proportion (25–50%) of the total activity of a bindin suspension after sonication. After resuspending the original pelleted bindin particles in fresh media, followed by centrifugation at 100,000 g for 1 h, an additional quantity of hemagglutination activity is recovered in the supernatant (data not shown). This suggests the possibility that a soluble form of bindin may exist in equilibrium with particulate bindin. The existence of this soluble form of bindin can also explain the observation that bindin diffuses in agarose gels and forms a precipitin line with antibindin antibody (19).

As shown in Table II, the same polysaccharides that inhibit bindin-mediated egg agglutination also inhibit the hemagglutination activity of bindin. The most potent inhibitor of hemagglutination is the egg surface glycoconjugate, which is also the best inhibitor of bindin-mediated egg agglutination. Fucoidin, the egg jelly polysaccharide, and xylan are also excellent inhibitors of bindin hemagglutination as they are of bindin-mediated egg agglutination. The most effective of the synthetic neoglycoproteins (Materials and Methods) is BSA substituted with L-fucose, which is fivefold more active than neoglycoproteins containing α D-galactose or lactose; mannose and β D-

TABLE I  
*Inhibition of Bindin-mediated Egg Agglutination by Polysaccharides*

Polysaccharide	Concentration required for 50% inhibition*
Egg surface glycoconjugate	11
Fucoidin	15
Egg jelly fucose polysaccharide	45
Xylan	150
Dextran sulfate	630

The following polysaccharides and glycoconjugates were inactive at 15 mM monosaccharide eq.: Heparin, Chondroitin sulfate, Hyaluronic acid, yeast Mannan, Dextran, Guaran, and desulfated Fucoidin.

\* µM monosaccharide equivalent.

TABLE II  
Inhibition of Bindin Hemagglutination

	Concentration required for 50% inhibition*
Polysaccharide	
Egg surface polysaccharide	0.9
Fucoidin	1.0
Egg jelly fucose polysaccharide	2.4
Xylan	4.2
Dextran sulfate	20.0
Yeast mannan mnn2	110.0
Neoglycoprotein	
Fucose-BSA	58
galactose-BSA	290
Lactose-BSA	290
Saccharides	
Lactose	33,000
L-fucose	67,000
D-galactose	67,000

The following compounds did not inhibit hemagglutination: Heparin, Chondroitin sulfate, Dextran (20mM monosaccharide eq.);  $\alpha$  mannose-BSA,  $\beta$ -galactose-BSA (290  $\mu$ M monosaccharide eq.); glucose, D-fucose, N-Acetyl glucosamine, mannose, N-acetyl neuraminic acid (67 mM).

\*  $\mu$ M saccharide equivalent.

galactose-BSA are inactive. Although the particulate and soluble bindin hemagglutination activities are equally sensitive to fucoidin inhibition (data not shown), only the soluble activity is inhibited by high concentrations of simple sugars (Table II). We find that lactose is the most potent monosaccharide hemagglutination inhibitor, followed by fucose and galactose. On a sugar concentration basis, the neoglycoproteins are 250 to 500-fold more effective than the corresponding free saccharides. The aminophenyl sugars used in the synthesis of the neoglycoproteins were found to have a hemagglutination inhibitory potency similar to that of the free sugars (data not shown). The rank order of effectiveness of the inhibitory polysaccharides is the same in the hemagglutination and egg agglutination assays.

The initial hemagglutination observations were made with particulate bindin samples contaminated with sperm histone H-1. Since we discovered that purified histone H-1 (22) is a potent agglutinin of GTRs, we have investigated the possibility that the hemagglutination activity of bindin is due to this contamination. We have compared the polypeptide composition of equal amounts of hemagglutination activity from histone and soluble bindin (Fig. 1). No H-1 histone is detected in the soluble bindin; therefore the histone is not soluble under these conditions of low ionic strength extraction (Materials and Methods). The major polypeptide in the soluble bindin has the same molecular weight (30,500) as the particulate bindin polypeptide. Although the polysaccharide specificities of bindin and histone are similar, the histone hemagglutination activity is preferentially inhibited by DNA (manuscript in preparation). In addition, we found that H-1 histone is quantitatively extracted from particulate bindin by 2 M NaCl. To demonstrate that the activities ascribed to bindin are not due to contaminating histone, we have determined that the histone-free bindin prepared by this procedure has the same egg agglutination activity and polysaccharide specificity observed for preparations from histone-contaminated bindin. In addition, soluble bindin prepared from histone-free bindin retains identical hemagglutination properties.

### Interaction of Fucoidin with Bindin Particles

We have studied the interaction of fucoidin with bindin particles by the use of fluorescein-derivatized fucoidin (fl-fucoidin) and  $^{125}$ I-labeled fl-fucoidin. As shown in Fig. 2, fluorescent fucoidin binds specifically to the surface of bindin particles. The binding of fluorescent fucoidin is inhibited by a 1,000-fold excess of unlabeled fucoidin but not by a 1,000-fold excess of heparin or chondroitin sulfate. In addition, a fluorescein derivative of chondroitin sulfate does not bind to bindin particles, further indicating the specificity of this interaction. These findings also demonstrate the potential usefulness of fluorescent polysaccharides for the localization of cell surface lectins.

The binding of  $^{125}$ I-fl-fucoidin to bindin approaches saturation; 1  $\mu$ g of bindin binds a maximum of 45 ng of fucoidin (Fig. 3). The association kinetics of fucoidin are quite rapid, with a half-time of  $\sim$ 15 s. The dissociation kinetics of bound fucoidin are biphasic in the absence of excess unlabeled fucoidin: one-third of the fucoidin dissociates with a half-time of 4 min, while the remaining two-thirds dissociates with a half-time of 2d (data not shown). Scatchard plot analysis of the binding data yields a slightly curved function (Fig. 3). This suggests the possible existence of binding sites with different affinities for fucoidin.

This interpretation is also consistent with the biphasic dissociation kinetics previously mentioned. Possible explanations

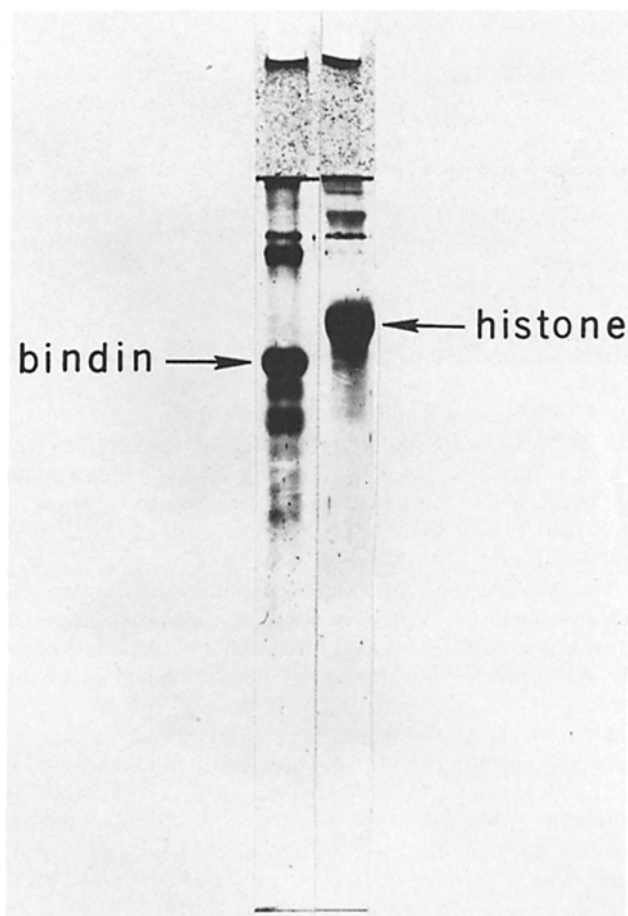


FIGURE 1 SDS polyacrylamide gel of bindin and H-1 histone hemagglutinins. 640 U of hemagglutination activity was loaded on each lane. The 15% gel was silver stained to maximize detection of trace contamination (18). No H-1 histone is detected in the bindin sample.

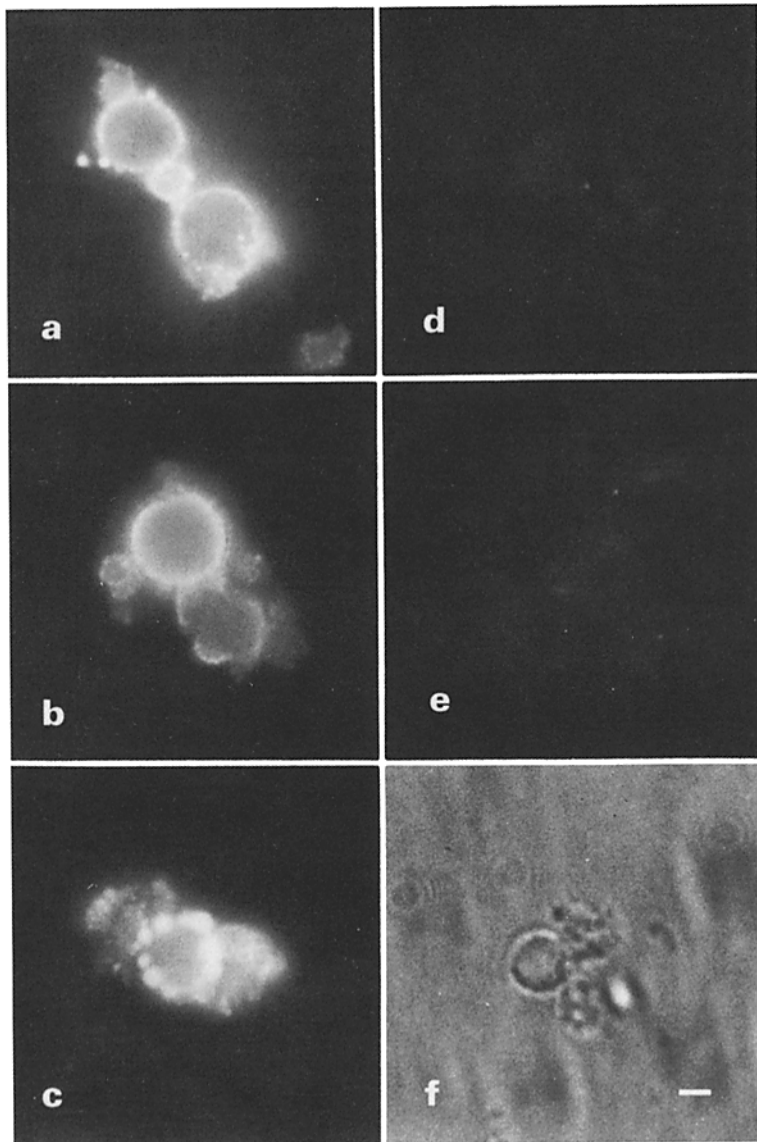


FIGURE 2 Binding of fluorescein-derivatized fucoidin (fl-fucoidin) to bindin particles. (a) 1  $\mu$ g of fl-fucoidin was mixed with 50  $\mu$ g of bindin particles in a total volume of 160  $\mu$ l of CMF, incubated for 5 min, and viewed under fluorescence illumination. (b) 1  $\mu$ g of fl-fucoidin was mixed with 1 mg of chondroitin sulfate, after which 50  $\mu$ g of bindin particles were added. After 5-min incubation, the sample was viewed under fluorescence illumination. (c) Same as above but in the presence of 1 mg of heparin. (d) Same as above but in the presence of 1 mg of unlabeled fucoidin. (e) 1  $\mu$ g of fluorescein-derivatized chondroitin sulfate mixed with 50  $\mu$ g of bindin particles and viewed under fluorescence illumination. (f) Bright-field light micrograph of bindin particles. Bar, 1  $\mu$ m.

for the existence of binding sites of different apparent affinity will be considered in the discussion. The apparent affinity of fucoidin binding is  $5.5 \times 10^{-8}$  M (Fig. 3). This dissociation constant is close to those reported for the interaction between other cell surface carbohydrate binding proteins and their specific ligands (14, 24, 28).

The specificity of  $^{125}$ I-fl-fucoidin binding to bindin particles was investigated further by testing the same polysaccharides examined previously as competitive inhibitors of fucoidin binding. As shown in Fig. 4, unlabeled fucoidin competes for the binding of  $^{125}$ I-fucoidin to bindin. Xylan and egg jelly fucose polysaccharide also compete effectively for the binding of fucoidin, suggesting that these substances all inhibit egg agglutination and hemagglutination by competing for the same sites on bindin. In contrast, chondroitin sulfate and yeast mannan compete very poorly for fucoidin binding, and none of the monosaccharides have any effect on fucoidin binding at 0.2 M (data not shown). The  $K_i$  obtained for fucoidin is  $2.5 \times 10^{-8}$  M, which compares well with the  $K_d$  of  $5.5 \times 10^{-8}$  M. Thus, the specificity of the interaction between fucoidin and bindin is similar to the specificity of bindin-mediated egg agglutination and the hemagglutination activity of bindin.

## DISCUSSION

Several lines of evidence suggest that bindin is a sperm surface lectin that mediates the adhesion of sea urchin sperm to eggs by binding to carbohydrate-containing components of the egg surface (7-9, 32-34). Here we report that fucoidin and xylan are potent inhibitors of the bindin-mediated agglutination of eggs. This is the first report of compounds of defined structure that inhibit the interaction between bindin and the egg surface. We have established that fucoidin inhibits egg agglutination through a direct interaction with the bindin particles. The interaction between fucoidin and bindin is specific as shown both qualitatively (Fig. 2), by the binding of fl-fucoidin and quantitatively, as measured by the binding of  $^{125}$ I-labeled fucoidin (Fig. 4). This interaction is of moderately high affinity and is inhibited selectively by the same polysaccharides that inhibit the agglutination of eggs by bindin.

In this report, we relate the hemagglutination activity of bindin to its egg agglutination activity by showing that the same polysaccharides that inhibit the agglutination of eggs are also potent inhibitors of hemagglutination. The hemagglutination activity of bindin is inhibited 50% by a concentration of polysaccharide that is nearly 100-fold less than that which is required to inhibit egg agglutination by 50%. This indicates

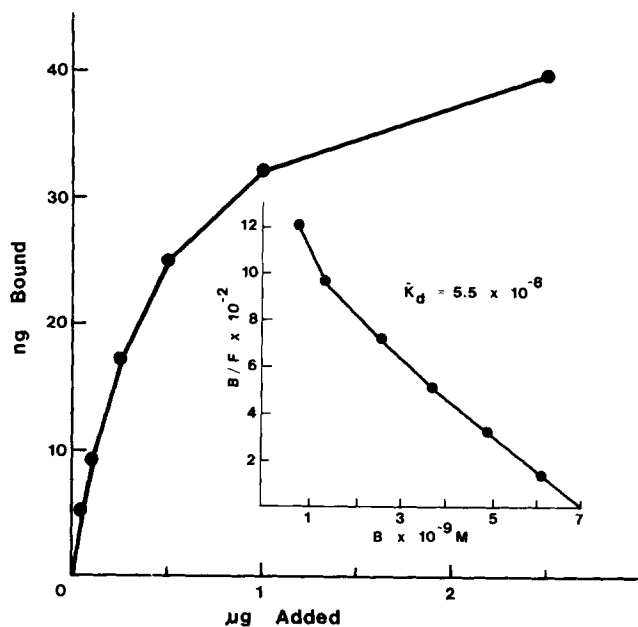


FIGURE 3 Binding of  $^{125}\text{I}$ -fucoidin to bindin particles. 1  $\mu\text{g}$  of bindin particles was mixed with various amounts of  $^{125}\text{I}$ -fucoidin in a total volume of 0.2 ml of CMF. After 5-min incubation at  $0^\circ\text{C}$ , the sample was filtered on a Whatman GF/c glass fiber filter, washed twice with 3 ml of CMF,  $0^\circ\text{C}$ , and the amount of bound  $^{125}\text{I}$ -fucoidin was determined. Inset: Scatchard plot of the data obtained from the binding assay.

that the hemagglutination assay is much more sensitive than the egg agglutination assay. In this regard, several polysaccharides, monosaccharides, and neoglycoproteins that inhibit hemagglutination do not inhibit egg agglutination even at high concentrations. A possible explanation for this finding is that bindin may possess a much higher affinity for the egg surface glycoconjugate than the erythrocyte receptor, since the interacting components of the erythrocyte may be qualitatively different than those on the egg surface. Thus, for simple saccharides and neoglycoproteins which may interact only weakly with bindin, extremely high concentrations would be required to inhibit the high affinity interaction between bindin and the egg surface. It is interesting to note that the neoglycoproteins are 250 to 500-fold more potent than the corresponding free saccharides or aminophenyl derivatives as hemagglutination inhibitors. This suggests that multivalency of these neoglycoproteins and perhaps the other inhibitors may dramatically augment their interaction with bindin, although there may be steric contributions of the polypeptide.

The curvilinear Scatchard plot may indicate the existence of discrete binding sites of different affinity for fucoidin on the binding particles. Alternatively, the multivalency of fucoidin can also explain the apparent existence of different affinity sites. The polysaccharides that bind first may interact at multiple sites and thus limit the availability of sites for molecules that bind subsequently. Therefore, the different affinities would be a reflection of the different number of interaction that each polysaccharide makes with a bindin particle.

The egg jelly fucan, fucoidin, and xylan have different molecular weights ( $10^6$ ,  $10^5$ , and  $10^4$ , respectively). If these polysaccharides make different numbers of interactions with bindin particles due to their different sizes, then the apparent  $K_d$ 's may not provide a fair comparison of the relative affinities of the active site of bindin for the recognition determinants of

the polysaccharides. These problems and others concerning the specificity of the active site of bindin are presently the subject of more detailed investigations.

Previous investigations have implicated a high molecular weight egg surface glycoconjugate in sperm-egg adhesion (9). This glycoconjugate was found to be composed primarily of fucose (32%), xylose, galactose, and glucose. Fucoidin is composed primarily of fucose but in addition contains some xylose, glucose, galactose, and glucuronic acid (16). Fucoidin is believed to contain an  $\alpha(1 \rightarrow 2)$  linked fucose backbone which is sulfated at C-4 with a small degree of branching at C-3. In addition, xylose and glucuronic acid are believed to be terminal (17). The fact that fucoidin and the egg surface glycoconjugate are the most potent inhibitors of agglutination and have similar compositions suggests the possibility of structural similarities. The egg jelly fucan, which is the next best inhibitor, is a sulfated fucose homopolysaccharide (26, 27). Xylan was also found to be a potent inhibitor of egg agglutination. Xylan is a  $\beta(1 \rightarrow 4)$  linked xylose polysaccharide containing 4-O methylglucuronic acid residues as branches every ten xylose residues (20, 30). Dextran sulfate (*Leuconostoc mesenteroides*), which also inhibits egg agglutination, is composed of a linear  $\alpha(1 \rightarrow 6)$  linked glucose polymer containing a small number of (1  $\rightarrow$  3) glucose branches. Although no simple conclusion can be made about the carbohydrate specificity of bindin on the basis of sugar composition, the three most potent polysaccharides are fucose-rich. The other inhibitory polysaccharides, xylan and dextran sulfate, are structurally related to the fucans by the fact that they are negatively charged, and the backbone saccharides lack a free hydroxyl group at C-6 and have the same configuration about C-4. Possibly the higher order, three-dimensional structure of the polysaccharide is critical in determining the specificity of binding, and the structural requirements can be satisfied by polysaccharides of different sugar composition.

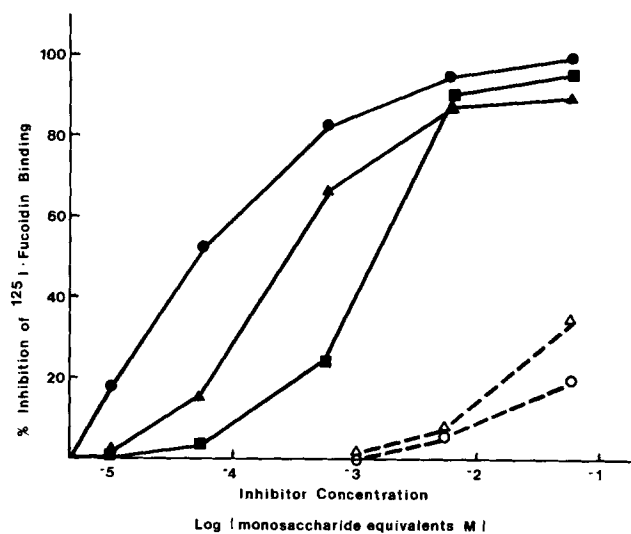


FIGURE 4 Competition for the binding of  $^{125}\text{I}$ -fl-fucoidin to bindin particles by polysaccharides. 500 ng of  $^{125}\text{I}$ -fl-fucoidin was mixed with various amounts of unlabeled polysaccharides in a total volume of 0.1 ml. 1 mg of bindin particles was added, and the mixture was incubated for 5 min at  $0^\circ\text{C}$ . After the incubation period, the mixture was filtered, the filters were washed, and the amount of bound  $^{125}\text{I}$ -fucoidin was determined. The percent of inhibition is expressed relative to the amount of  $^{125}\text{I}$ -fl-fucoidin bound in the absence of any unlabeled polysaccharide: Fucoidin (●), egg jelly fucan (▲), xylan (■), Mannan MNN<sub>2</sub> (Δ), and chondroitin sulfate (○).

The fact that the egg jelly fucan interacts with bindin raises an apparent paradox. The egg jelly fucan is known to induce the acrosome reaction in sperm (26). If the fertilizing sperm undergo the acrosome reaction in the jelly coat, why doesn't the egg jelly fucan inhibit the binding of sperm to the egg surface? One possible explanation is that the successful sperm undergo the acrosome reaction at the egg surface, as has been suggested by Aketa and coworkers (1). In the native state the egg jelly fucan is in a gel form, presumably due to its interaction with the other major component of jelly, the sialoglycoprotein (26). Another possibility is that in this gel state the fucan is unable to interact with bindin. We have also investigated the possibility that fucoidin might be able to inhibit fertilization under conditions where the extent of fertilization is linearly dependent on the sperm concentrations. We found no effect of fucoidin on fertilization at concentrations of 1 mg/ml. This failure to inhibit fertilization can also be explained by the fact that the bindin is exposed only after the acrosome reaction is induced at the surface of these dejellied eggs. Under these conditions, the concentrations of fucoidin and the time required for it to compete for bindin with the egg surface are presumably insufficient to inhibit fertilization.

It is interesting to note that a fucoidinlike glycoconjugate may serve as an egg surface receptor for a sperm carbohydrate-binding protein in gametes of *Fucus*, the genus of algae from which fucoidin is obtained (2). Fucose residues are implicated in the interaction of sperm and eggs from the tunicate *Ciona* (25). We have recently observed that fucoidin specifically interacts with a cell surface lectin in mouse teratocarcinoma stem cells and inhibits intercellular adhesion (11). Thus fucoidinlike polysaccharides may participate in other types of intercellular adhesion.

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*Note Added in Proof:* Runnström and Immers have previously reported that fucoidin has no fertilization inhibitor activity in sea urchins. 1958. *Exp. Cell Res.* 10:354-363.

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