CHARACTERIZATION AND COMPARISON OF "BINDIN" ISOLATED FROM SPERM OF TWO SPECIES OF SEA URCHINS

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SUMMARY. The insoluble acrosome granule content of sea urchin sperm consists of a single 30,500 dalton protein named bindin. Bindin mediates species-specific recognition and adhesion of sperm to the egg surface. Bindin from Strongylocentrotus purpuratus (Sp) and Strongylocentrotus franciscanus (Sf) have tyrosine as their single N-terminal amino acid. The pI of Sp bindin is 6.62 and of Sf 6.59. Amino acid analysis reveals almost identical composition between the two species for 16 amino acids. Only two (or three) amino acids, Pro and Asx, show large species differences. Tryptic peptide maps of the two species of bindin show very similar patterns with 24 spots of identical correspondence.

INTRODUCTION. The acrosome granule of sea urchin sperm contains an insoluble protein which appears to mediate species-specific recognition and adhesion of sperm to the egg surface[1,2]. The protein is isolated by exposure of sperm to 2.5% Triton-X-100, which detaches the acrosome granule from the sperm, followed by sieving in a glass fiber column which allows passage of the freed granules while trapping whole sperm, sperm heads and broken flagella[1]. The acrosome granules are collected by centrifugation and the Triton removed by several washings of the pellet. Electron microscopy of the pellet shows it to consist solely of acrosome granules which have lost their limiting biomembranes during Triton extraction[1]. Electrophoresis of the pelleted granules dissolved in either sodium dodecyl sulfate (SDS) or acid-urea on polyacrylamide gels yields a single Coomassie Blue staining band indicating that the isolated granules are composed of only one polypeptide chain[1].

Evidence that this protein is involved in binding sperm to the egg surface comes from the following observations. When the pelleted granules are suspended in sea water and mixed with unfertilized eggs, the eggs

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agglutinate[1]. This agglutination is species-specific at high concentrations of the protein, showing that at the molecular level the recognition event between the protein and the egg surface must be quite different in these two closely related species[2]. The egg-agglutinating property of the protein is lost if it is mixed with protease-generated glycopeptides from egg surfaces. Eggs whose surfaces have been treated with proteases or $NaIO_4$ are not agglutinated by the protein[1]. These data suggest that the protein may recognize specific carbohydrate portions of egg surface glycoproteins; sperm-egg adhesion may thus be another example of intercellular adhesion based on a protein-carbohydrate interaction. Indeed, we have recently identified a high molecular weight egg surface glycoprotein which has species-specific affinity for the acrosome granule protein[3]. Lastly, antibody to the protein, conjugated to horseradish peroxidase, localizes the protein on the surfaces of the sperm acrosomal process and the egg microvillus to which the sperm is bound[4]. Because of its suspected function in sperm-egg binding, we have referred to this protein as "bindin" and have proposed that "sperm bindins" may be a new class of proteins which bind sperm to eggs in many diverse animal groups[1].

To our knowledge, sea urchin sperm bindin is the first protein to be isolated, in essentially pure form and mg quantities, that mediates a specific intercellular recognition and adhesion of a metazoan. Therefore, as the first step to understanding the molecular mechanism of sperm-egg adhesion during fertilization, it seemed important to characterize the bindins from the two sea urchin species which have been shown to act as species-specific agglutinins of eggs[2].

METHODS. Bindin was isolated from sperm of Strongylocentrotus purpuratus (Sp) and Strongylocentrotus franciscanus (Sf) by the published method[1] and stored at -20°C. Apparent molecular weights were determined by dissolving the protein in 2% SDS-2% mercaptoethanol and electrophoresing it in 12.5% polyacrylamide slab gels containing 0.1% SDS[5]. Protein standards used were: bovine serum albumin, 66,000; hexokinase, 55,000; ovalbumin, 43,000; lactic dehydrogenase, 35,000; trypsinogen, 24,000 and hemoglobin, 17,000. Coomassie Blue-stained tube gels were scanned with a Gilford Linear

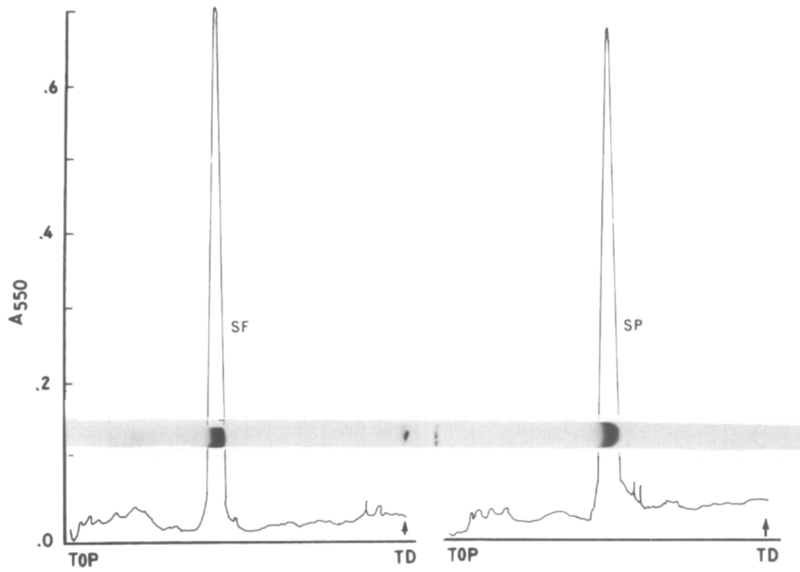

Figure 1. 12.5% polyacrylamide -0.1% SDS gels containing 50 µg acrosome granule pellet. The single Coomassie Blue band and the scans of the stained gels at 550 nm show the isolated acrosome granules to be a single protein. TD=tracking dye.

Transport. All reagents were from Sigma. Isoelectric focusing was performed in gel tubes 0.5 X 10 cm[6]. The gels contained 6M ultra-pure urea, 2% ampholines pH 3.5-10 (LKB) and 5% mercaptoethanol. After 8 hr at 0.1 watt / gel, one gel was stained with Coomassie Brilliant Blue and an identical gel sliced into 1 mm sections and each section eluted 1 hr in 10mM KCl (pH7) followed by reading the pH of the KCl with a combination electrode. For N-terminal analysis bindin was dansylated and the dansylated amino acids chromatographed on polyamide thin layer plates[7]. For amino acid analysis 200µg salt free lyophilized bindin was suspended in 1 ml 6N HCl, 0.05% mercaptoethanol and 10 mg phenol, the vial bubbled 15 min with N_2, sealed and then heated 24 hr at 110°C[8]. Amino acids were determined with a Durrum D-500 AA analyzer. Cysteine[9] and tryptophan [10] were determined by colorimetric procedures. Trypsinization of 10mg bindin in 1 ml 50mM NH_4HCO_3 (pH8) was done by addition of 0.1 mg TPCK-trypsin (Worthington) at 37°C for 18 hr. The tryptic peptides were lyophilized, resuspended in H_2O and relyophilized 3 times to volatilize the buffer. Two dimensional tryptic peptide maps[11] were made using water: pyridine: acetic acid (380:15:5) as the electrophoresis buffer. Approximately 60 µg bindin peptides were spotted on 10 X 10 cm Polygram Cel 300 cellulose thin layer plates (Brinkmann), the plates sprayed with electrophoresis buffer, placed in a Desaga/Brinkmann 171D double chamber thin layer electrophoresis apparatus precooled to 4°C and 500 volts was applied for 30 min. The plates were then dried and chromatographed in the second dimension in butanol: water: acetic acid: pyridine (4:1:6:4). Peptide spots were visualized by U.V. light after washing the plates with acetone followed by 30 min in acetone containing 3 mg/ml fluorescamine (Roche Diagnostics).

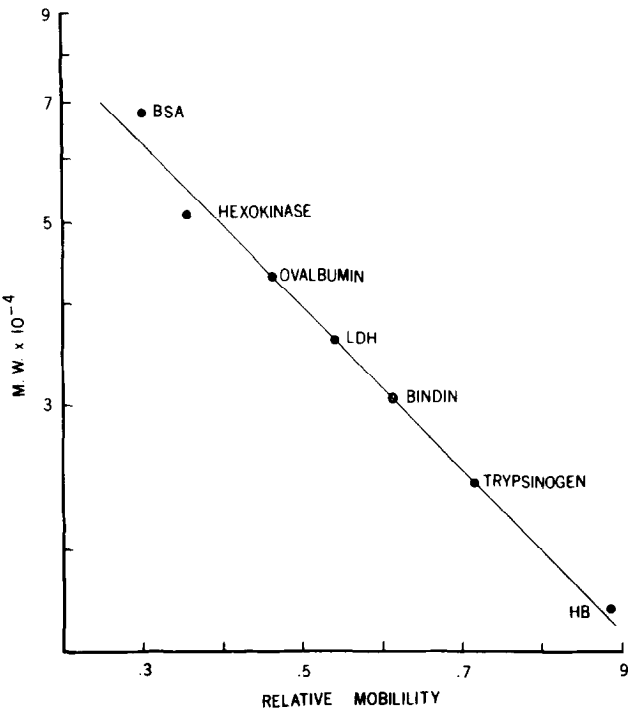

Figure 2. Determination of apparent molecular weight of bindins on 12.5% polyacrylamide -0.1% SDS gels. Both sea urchin bindins run at 30,500 daltons and are indistinguishable when mixed together and run at low protein concentrations.

RESULTS AND DISCUSSION. By dry weight bindin is 105% protein as measured[12] against a standard of bovine serum albumin[1]. SDS-polyacrylamide gels and gel scans of the bindin preparations used for analysis appear in Figure 1. Bindin from both species migrates as a single sharp band (50 µg protein/gel) which appears to be essentially pure by this criterion. An additional criterion for purity is the finding that tyrosine is the only N-terminal[7] amino acid present in both bindins. The apparent molecular weight of both bindins is 30,500 daltons (Figure 2); both run as a single band when mixed together and electrophoresed at low concentrations. The isoelectric point of Sp bindin averages 6.62 (9 runs ranging from 6.28 to 6.89) and for Sf 6.59 (10 runs ranging from 6.28 to 6.89).

Table 1.

Amino Acid Composition of Bindin

	S. purpuratus		S. franciscanus	
non-polar				
Ala	10.2	(29)	11.1	(32)
Val	4.5	(13)	4.6	(13)
Leu	5.2	(15)	5.4	(15)
Ile	2.6	(7)	3.1	(9)
* Pro	9.2	(26)	5.0	(14)
Met	4.5	(13)	3.3	(9)
Phe	1.3	(4)	1.3	(4)
Try	2.1	(6)	2.1	(6)
polar				
Gly	15.6	(44)	16.4	(47)
Ser	5.3	(15)	5.3	(15)
Thr	3.2	(9)	2.6	(7)
Cys	1.8	(5)	1.1	(3)
Tyr	2.3	(7)	2.7	(8)
* Asx	6.9	(20)	10.6	(30)
Glx	12.0	(34)	12.2	(35)
positive				
Lys	5.3	(15)	4.9	(14)
Arg	6.0	(17)	6.6	(19)
His	2.0	(6)	1.7	(5)

Amino acid composition of the two bindins, the average of three determinations per species. Number on left = % composition; number on right in parentheses = number of residues per molecule (calculated on the basis 285 residues per molecule and an average mol.wt. per residue as 107 daltons).

From the averages of three amino acid analyses for each species (Table 1) we calculate the average molecular weight per amino acid to be 107 daltons, with 285 residues per molecule. The two bindins are remarkably similar for 16 amino acids, the average difference in residues per molecule between the two being 1.3. However, two amino acids, Pro and Asx (asterisks) are strikingly different. Sp has 26 and Sf 14 Pro per molecule. Sp has 20 and Sf 30 Asx per molecule. It will be interesting to see if bindins from

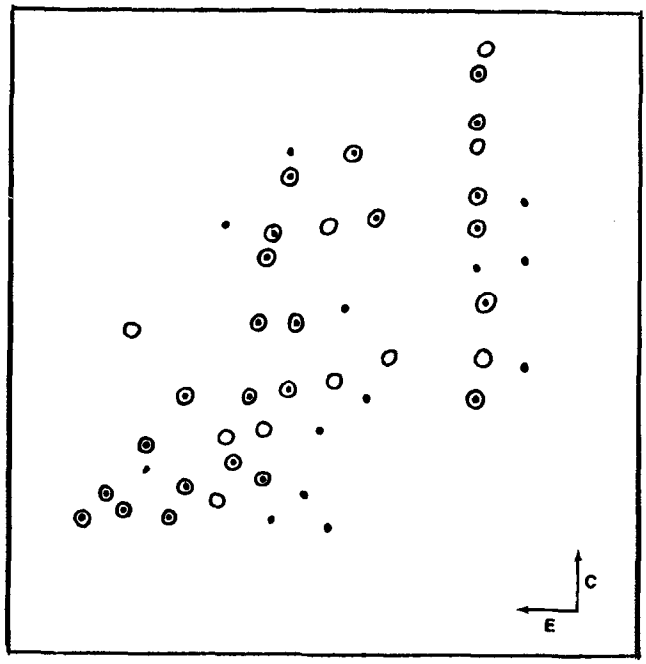

Figure 3. Two-dimensional tryptic peptide maps of the two bindins visualized by fluorescamine. S. purpuratus, ● ; S. franciscanus, ○. The size of the spots was the same for both species and is shown by the open circles for Sf.

other sea urchin species show major differences in only these two amino acids. The native structure of bindin, which confers the species specificity of cell surface recognition, might be governed by the number and placement of these two amino acids along the polypeptide chain. For percentages nonpolar amino acids Sp has 40.6 and Sf 35.9%; for polar amino acids Sp has 47.1 and Sf 50.9%; for positively charged amino acids Sp has 13.3 and Sf 13.2%.

Two dimension tryptic peptide maps[11] of the two bindins (Figure 3) resolve 37 spots for Sp and 34 for Sf. The general patterns of the two maps are similiar with 24 spots of identical correspondence. Thirteen spots are unique to Sp and 10 to Sf. Visually, the most prominent differences are the 3 Sp spots on the far right and the 1 Sf spot on the far left of the chromatogram.

In the future we hope to study the native structure of bindins in an attempt to discover the basis for their species-specific interaction with the egg cell surface. One difficulty in this project is the relative insolubility of bindin. SDS, 6-8 M urea and 6 M urea-10% acetic acid are the only ways we have found to obtain complete solubilization. However, bindin is sparingly soluble in pure water titrated to pH 9.0 with NH_4OH[13]. Disulfide cleaving reagents and chelators have no effect on solubility.

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