SUGAR SELECTIVITY AND OPSONIZATION POTENTIAL OF A Ca^{2+}-INDEPENDENT SERUM LECTIN ISOLATED FROM THE SPINY DOGFISH (SQUALUS ACANTHIAS)

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Soluble mannose-binding proteins (MBP) have been isolated from human serum (Taylor and Summerfield, Biochim. Biophys. Acta 915: 60-67, 1987) and are believed to act as primitive immune molecules. MBP serve as opsonins and initiate the complement cascade (Super, et al., Clin. Exp. Immun. 79: 144-150, 1990). Low levels of MBP are found in 5-7 percent of the human population and have been clinically correlated with chronic diarrhea, ear infections, failure to thrive, and severe recurrent infections in young children (Summerfield, Biochem. Soc. Trans. 21: 473-477, 1993). In humans, MBP seem to be accessory molecules, most important at times when antibody levels are low.

Since the immune system of the shark is less well-developed than that of mammals, mannose-binding proteins might be more important in the defense mechanisms of the shark. We have previously detected a mannose-binding lectin (MBL) in the serum of the spiny dogfish shark (Newton and Brown, Bull. Mount Desert Island Biol. Lab. 32: 115-117, 1993), and have compared its structure to that of MBP found in mammals (Newton et al., Bull. Mount Desert Island Biol. Lab. 33: 12-14, 1994). In the present study, our objectives were to examine the sugar selectivity of MBL and to evaluate its opsonization potential.

Dogfish were caught by gill nets from Frenchman Bay, ME, and held in floating live cars for several days prior to use. Serum was obtained from blood collected from the caudal artery. Lectins binding mannose were isolated by affinity chromatography on a mannose-Sepharose 6B column. Ca^{2+} dependence of binding was determined by comparing the amount of lectin binding to the affinity column in the presence of 15 mM Ca^{+2} with that bound in the presence of 2 mM EGTA. Sugar selectivity was investigated using inhibition binding assays in which purified MBL was incubated with mannose-Sepharose resin in the presence of various concentrations of free monosaccharides; the amount of MBL bound to the resin as a percentage of the amount of MBL bound to the resin in the absence of added free sugar served as an index of competition. For opsonization studies, adherent cells were harvested from the peritoneal cavities of several sharks and plated out in Falcon 3.3 cm petri dishes. The cells were maintained at 15° C. in shark Ringer's solution. Yeast cells (10^8 cells/dish) were overlaid on the adherent cell monolayer and allowed to incubate for 2 hr. Following the incubation period, monolayers were washed free of excess yeast cells and examined by inverted phase contrast microscopy to determine the percentage of effective phagocytic cells, based on ingestion of yeast.

Purified MBL was able to bind equally well to mannose-Sepharose resin in the presence of either 15 mM Ca^{+2} or 2 mM EGTA and so the binding appeared to be Ca^{+2}-independent. Inhibition assays (Fig. 1) showed that galactose and D-fucose were ineffective inhibitors, having essentially no effect on the binding of MBL to the affinity resin. N-acetylglucosamine and glucose were moderately effective as inhibitors, reducing the binding by about 50%, and mannose and L-fucose were strong inhibitors, reducing the binding to less than 20%. The relative affinities of

the three sugars showing the greatest effect on MBL are apparent in Fig. 2, with mannose having an affinity intermediate between that of L-fucose and that of glucose.

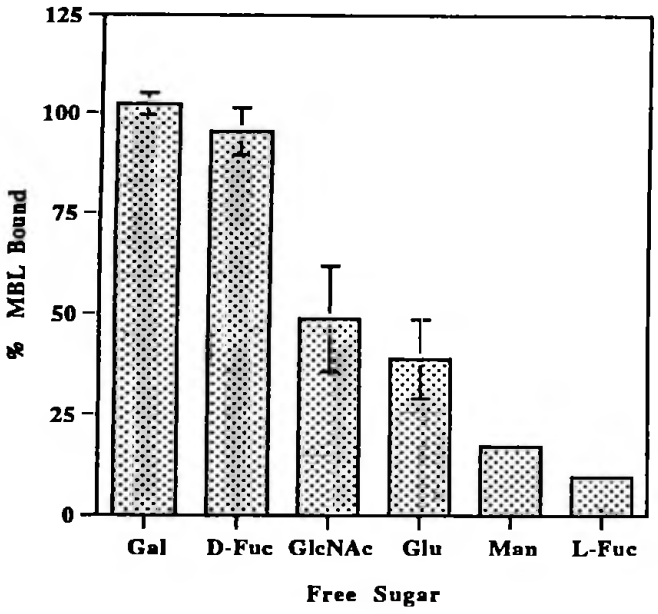

Fig. 1. % of MBL bound to mannose-Sepharose beads in the presence of 0.1 M free sugars. Mean values from three experiments +/- Std. Dev.

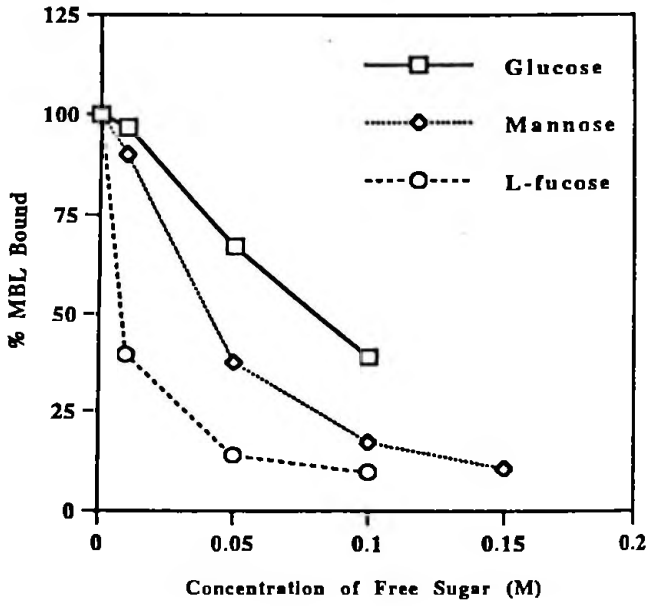

Fig. 2. % MBL bound to mannose-Sepharose beads as a function of free sugar concentration.

Results of the opsonization study are shown in Table I. Adherent cells scored as positive appeared swollen with numerous internalized yeast particles, but we did not attempt to rigorously distinguish between attached and ingested organisms. Maximal opsonization was given by pretreatment of yeast with shark serum. Little phagocytosis was observed with yeast preincubated in shark Ringer's solution, probably due to lack of complement proteins. The small increase in phagocytosis seen with the addition of MBL to shark Ringer's solution may indicate that MBL has a minor role as an opsonin in the absence of complement, though further studies are required to ascertain its significance. The depleted serum showed a decline in opsonization potential that was not restored by the addition of MBL, indicating that some other serum component required for phagocytosis was also removed or inactivated by this treatment. Anders et al. (J. Gen. Virol. 75: 615-622, 1994) have indicated that complement proteins are decreased by about 50% by incubation with mannose-Sepharose.

Yeast Pretreatment	% Adherent Cells Showing Phagocytosis
Shark Serum	83.5
Shark Ringer's Solution	2.8
Ringer's Solution + MBL	7.5
Depleted Serum	69.6
Depleted Serum + MBL	61.8

Table I. % Adherent Cells Ingesting Pretreated Yeast
Yeast were pretreated for 30 min before overlaying on adherent cells. Serum depleted of MBL was prepared by incubating serum with mannose-Sepharose beads for 12 hr at 4^{0} C, followed by centrifugation to remove the depleted serum from the resin. MBL was added in some cases to give a final concentration of 1 mg/ml.

In comparing our sugar selectivity data to those obtained in mammalian systems, we find an overall similarity between shark MBL and MBP-1 found in human serum (Taylor and Summerfield, op. cit., 1987). Both lectins bind strongly to L-fucose and mannose, moderately to glucose, and weakly or not at all to galactose. Unlike MBL, the human MBP-1 also binds strongly to N-acetylglucosamine. Our data indicate that the configurations of the hydroxyl groups at both the C2 and C4 positions are important for binding to MBL. Maximal binding occurs with a C2 axial hydroxyl group and a C4 equitorial hydroxyl group, and little or no binding occurs when both hydroxyl groups have the opposite orientations; moderate binding occurs when both the C2 and C4 hydroxyl groups are equitorial. The analysis of a co-crystal of rat serum MBP and an oligomannose oligosaccharide confirmed the importance of equitorial C3 and C4 hydroxyl groups and the axial C2 hydroxyl group for optimal binding of MBP in mammals (Weis, et al., Nature 360: 127-134, 1992). Weis noted that L-fucose has an axial C4 hydroxyl group in the same position as the C2 hydroxyl group in mannose when the ring structures are superimposed in equivalent chair conformations. A recent NMR analysis of mannose binding by MBP following site-directed mutagenesis (Iobst, et al., J. Biol. Chem. 269: 15505-15511, 1994) emphasized the importance of the equitorial C4 hydroxyl group and attributed lesser importance to the axial C2 hydroxyl group. The data also suggested an alternative fucose orientation.

Our opsonization data do not support the hypothesis that MBL has a major role as an opsonin for yeast uptake by adherent cells, as is known to occur with the MBP of human serum (Super, et al., op. cit., 1990). However neither the kinetics of uptake nor the degree of cell activation were examined in this study. In future work we will employ a specific antibody to MBL in the depletion step to avoid the general inactivation that seemed to result from our use of the mannose-Sepharose beads. The possible presence of membrane-bound mannose receptors on peritoneal phagocytes could facilitate uptake of yeast through recognition of mannan (or mannan with complement) in the absence of MBL. If such a receptor is present, soluble MBL would have little, if any, effect on yeast uptake. In future studies we will use circulating neutrophils, as they have been shown not to contain mannose receptors in humans (Shepherd, et al., J. Reticuloendothel. Soc. 32: 423-431, 1982).

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