CHARACTERIZATION OF A MANNOSE-BINDING LECTIN IN THE SERUM OF 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 bind to mannosylated glycoproteins that are prevalent on the surfaces of certain bacteria, fungi, and viruses. When MBP bind to an invading organism, they facilitate attack by the phagocytes just as antibodies do. This opsonization may be done directly by the MBP (Kuhlman, et al., J. Exp. Med. 169: 1733-1745, 1989), or by activating the complement cascade system either by the classical (Super, et al., Clin. Exp. Immun. 79: 144-150, 1990) or alternative (Schweinie, et al., J. Clin. Invest. 84: 1821-1829, 1989) pathway. MBP have an overall structural organization similar to C1q (Drickamer, et al., J. Biol. Chem. 261: 6878-6887, 1986) and so the binding of MBP to a mannosylated glycoprotein may initiate effector functions analogous to those initiated by the more specific interaction of C1q with an antibody-antigen complex. The receptor for C1q on macrophages has recently been shown to bind to MBP as well (Malhotra et al., J. Exp. Med. 172: 955-959, 1990) and this interaction may initiate phagocytosis. MBP also attach to Influenza A viruses (Hartshorn, et al., J. Clin. Invest. 91: 1414-1420, 1993) and to HIV (Ezekowitz, et al., J. Exp. Med. 169: 185-196, 1989) through the coat glycoproteins, thereby inhibiting the ability of these viruses to infect host cells.

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 (Super, Lancet ii: 1236-1239, 1989). The presence of the MBP seems to be particularly important between the ages of 6 and 24 months, a time when the maternal supply of antibodies have disappeared and the immune system of the infant is beginning to produce its own antibody repertoire. In humans, therefore, 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). In the present study, our objective was to compare the structure and properties of the MBL found in shark serum with the MBP found in mammals.

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. Estimates of subunit molecular weight were determined by SDS-PAGE (Laemmli, Nature 227: 680-685, 1970) on 10% gels under reduced (with 2-mercaptoethanol) or nonreduced conditions, while native molecular weight estimates were obtained by gel filtration chromatography on a Sepharose 6B column. The degree of glycosylation was determined by PAS staining of gels (Segrest and Jackson, Meth. Enzym. 28: 54-63, 1972). The Ca^{2+} dependence of binding was determined by elution of protein from the affinity column with 10 mM EDTA.

We found the concentration of MBL in shark serum to be about 12 mg/100 ml serum, making up about 0.4% of the total protein in the serum. The subunit molecular weight is 124 kD under both reduced (Fig. 1) and nonreduced conditions (data not shown) as determined by SDS-PAGE, and the native molecular weight is estimated as 525 kD (Fig. 2). Shark serum is known to contain 50-60% globulin in the form of IgM (Marchalonis et al., Dev. Comp. Immunol.17: 41-53, 1993) and it has been reported that IgM can bind to mannose-Sepharose resins in a Ca^{+2}-independent manner (Summerfield and Taylor, Biochim. Biophys. Acta 883: 197-206, 1986). It is therefore of interest to note that little protein in the total serum is found in the 124 kD region. The heaviest bands in the serum are at approximately 43 kD and 63 kD, with considerable staining throughout the 59-84 kD range.

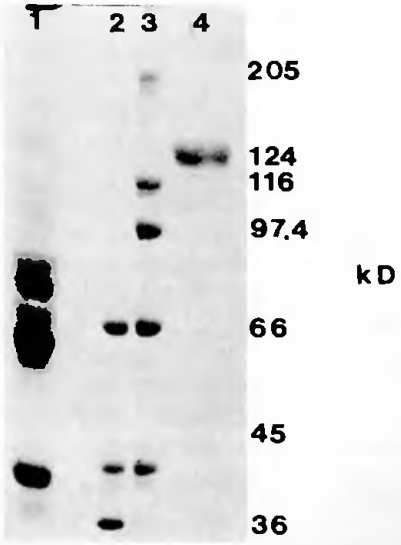

Figure 1

SDS-PAGE of isolated protein. Lane 1, Shark Serum. Lane 2, Low Molecular Weight Marker. Lane 3, High Molecular Weight Marker. Lane 4, MBL.

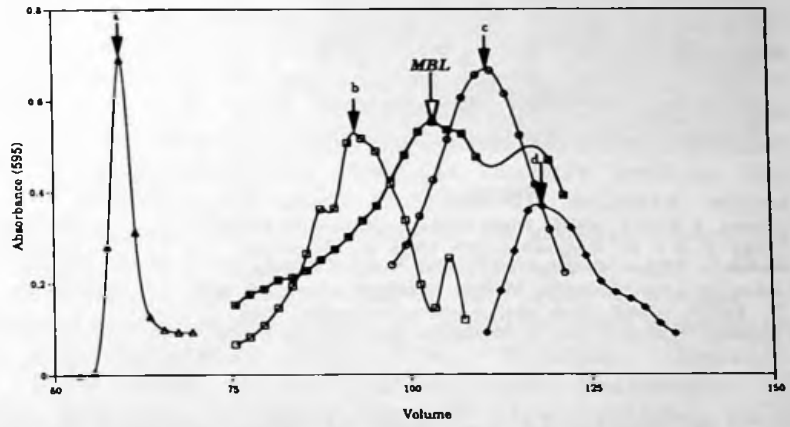

Figure 2

Native Molecular Weight Standards and Mannose-Binding Lectin (MBL). a) Blue Dextran (2,000 kD), b) Thyroglobulin (669 kD), c) Apoferritin (443 kD), d) Alcohol Dehydrogenase (150 kD), and MBL (525 kD).

To evaluate the specificity of the interaction of the MBP with the mannose-Sepharose beads, we performed identical isolations using either mannose-Sepharose beads or Sepharose beads alone, and eluted each column with either 0.2 M mannose or 10 mM EDTA. The results (Fig. 3) indicated that the 124 kD protein specifically recognized and bound to mannose, but could not be eluted from the column with EDTA, indicating that the MBL binding to the column was not calcium-dependent. A 45 kD band which we had seen earlier in some of our isolations as a contaminant, bound to Sepharose beads alone and was eluted by EDTA but not by free mannose.

PAS staining of gels (Fig. 4) showed that the shark MBL was lightly glycosylated as compared to the two standards we used, fetuin (21.4 mg carbohydrate/100 mg protein) and ovalbumin (3.4 mg/100 mg protein). From the intensity of the stain, we estimated that the MBL contained less than half the carbohydrate found in ovalbumin. The 45 kD band that bound to Sepharose alone was highly glycosylated, similar to the fetuin standard.

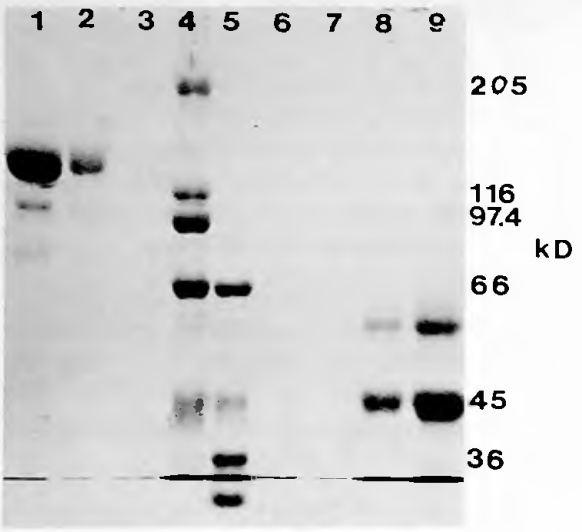

Figure 3

Lanes 1 and 2, MBL from mannose-Sepharose beads. Lane 3, 0.2 M mannose eluate from the Sepharose column. Lane 4, High Molecular Weight Standard. Lane 5, Low Molecular Weight Standard. Lanes 6 and 7, EDTA eluate from the mannose-Sepharose bead column. Lanes 8 and 9, Sepharose bead EDTA eluate.

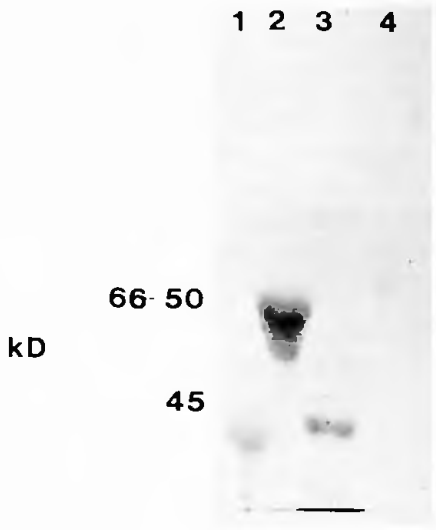

Figure 4

PAS-stain of glycoprotein standards and MBL. Lane 1 Ovalbumin(45 kD). Lane 2 Fetuin (50-66 kD). Lane 3 Sepharose bead EDTA eluate. Lane 4 MBL. Each lane contained 100 ug protein.

In comparing our data to those obtained in mammalian systems for MBP, we have observed several differences. We have consistently obtained over 10 mg of protein for 100 ml serum, over 10-fold greater than that found in humans (Taylor and Summerfield, op. cit., 1987). The subunit molecular weight in shark MBL is 124 kD whether reduced or nonreduced, while the reduced subunit molecular weight for humans is about 30 kD (Summerfield and Taylor, op. cit., 1986). Both proteins oligomerize to form large native structures, 525 kD for shark MBL, and 500-600 kD for mammalian MBP (Lee, et al., J. Biol. Chem. 266: 4810-4815, 1991). Perhaps most significantly, the MBL from shark serum cannot be eluted from the mannose-Sepharose column with 10 mM EDTA while the MBP from mammalian serum is eluted under these conditions (Taylor and Summerfield, op cit., 1987). We are currently examining the sugar specificity of MBL. Mammalian MBP show a strong affinity for mannose, N-acetylglucosamine, and fucose (Summerfield and Taylor, op cit. 1986). In future studies we plan to investigate the functional role of MBL to determine if these lectins can enhance phagocytosis.

This work was supported in part by a grant from the Maine Science and Technology Commission EPSCoR Program, the MDIBL Blum/Halsey Scholar Award, the CMTS (ESO3828-08), and the Maine EPSCor Merits Program Award to L. L'Italien.