EFFECT OF UREA ON SUGAR SPECIFICITY AND CALCIUM INDEPENDENCE OF A GLYCOPROTEIN-BINDING LECTIN IN THE SERUM OF THE DOGFISH SHARK *(SQUALUS ACANTHIAS)*

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We are continuing to characterize a soluble mannose-binding lectin (MBL) found in the serum of the spiny dogfish. In human serum, soluble mannose-binding proteins (MBP) are believed to act as primitive immune molecules, serving as opsonins and initiating the complement cascade (Super et al., Clin. Exp. Immun. 79: 144-150, 1990). MBL in dogfish serum may, therefore, play a role in the defense mechanisms of the shark. MBL differs from MBP in its larger subunit size and its calcium independence (Newton et al., Bull. MDIBL 33: 12-14, 1994)

Our previous sugar binding studies with MBL (Newton et al., Bull. Mount Desert Island Biol. Lab. 34:89-91, 1995) showed that MBL has a similar selectivity for simple sugars as MBP, but those studies were not done under conditions that approximate the physiological environment within the shark. Urea serves as an osmolyte in marine elasmobranchs at concentrations up to 400-500 mM, and at these concentrations can affect the properties of enzymes and other proteins, although in many cases these effects are counteracted by the methylamines also present in high concentrations in sharks (Hochachka and Somero, in *Biochemical Adaptation*, pp. 305-353, Princeton University Press, 1984). In this study the binding medium contained the concentrations of urea, TMAO (trimethylamine N-oxide), and salt typically found in shark Ringer's solution (268 mM NaCl, 6 mM KCl, 350 mM urea, 70 mM TMAO, 20 mM $NaHCO_3$, pH 7.6). We also investigated the effect of adding the divalent cations present in shark Ringer's solution (3 mM $MgCl_2$, 5 mM $CaCl_2$). Ringer's solution without calcium and magnesium contained 2 mM EDTA to chelate free divalent cations.

MBL was isolated from the serum of the spiny dogfish by affinity chromatography on a mannose-Sepharose 6B column. Sugar selectivity and affinity were 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. A comparison of MBL binding in the presence of free mannose or L-fucose is shown in Fig. 1. As we have noted before (Newton et al., op. cit., 1995), MBL shows a much higher affinity for L-fucose than for mannose. Samples in the presence of 350 mM urea and 70 mM TMAO or in the absence of both of these reagents show very similar inhibition profiles. This physiological concentration of urea appears to have no marked effect on the binding properties of MBL. Similarly, when MBL binding to mannose-Sepharose beads is measured in the presence and absence of both urea and Ca^{+2} (Fig. 2), no differences are evident. MBL is independent of Ca^{+2} in its sugar binding properties. This lack of Ca^{+2} dependency in MBL binding is very interesting, since in animal MBP, the calcium bound to the MBL actually participates in the formation of the sugar binding site. The carbohydrate-recognition domain in rat serum MBP, for example, has two Ca^{+2} coordinated with the equatorial hydroxyl groups at C3 and C4 of the terminal mannose residue as well as with the conserved amino acid residues, asparagine and glutamic acid (Drickamer and Taylor, Annu. Rev. Cell Biol. 9: 237-264, 1993). L-fucose has a high affinity for MBP because its C2 and C3 hydroxyl groups may be oriented in the same way as the C3 and C4 hydroxyl groups of mannose. Shark MBL has the same monosaccharide specificity as MBP, but Ca^{+2} appears not to be required to form the binding site. MBL may therefore represent a new class of lectins in which binding to the carbohydrate-recognition domain is stabilized solely through interactions between the hydroxyl groups and specific amino acids residues.

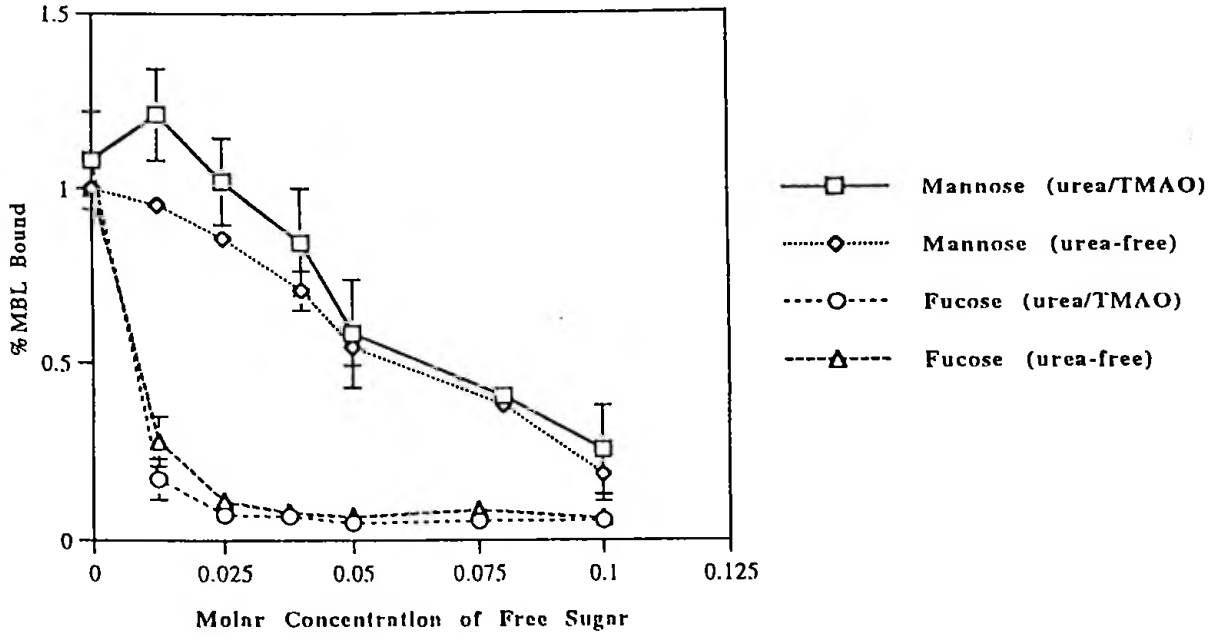

Fig. 1 Effect of urea/TMAO on sugar affinity of MBL. Bars represent mean +/- Std. Dev. (n=3)

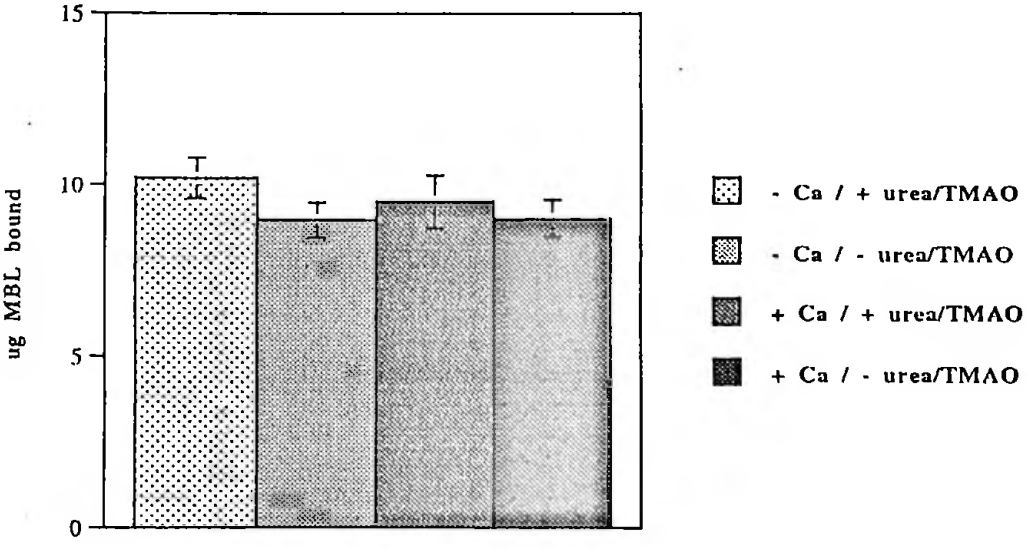

Fig. 2 MBL bound to mannose-Sepharose +/- Ca and +/- urea/TMAO. Bars represent mean +/- Std. Dev. (n=3)

To investigate the ability of MBL to bind to glycoproteins as well as mannose-Sepharose beads and simple sugars, we compared protein binding to mannose-Sepharose in the presence and absence of mannosylated BSA (man-BSA), a neoglycoprotein containing 20-30 moles of mannose per mole of albumin. Although we expected that man-BSA would inhibit the binding of MBL to mannose-Sepharose by blocking the binding sites of MBL, we consistently obtained results showing that more total protein was bound to the beads in the presence of man-BSA (Fig. 3). This 34 % increase was not due to nonspecific binding of man-BSA to the beads which was found to be negligible (0.65 ug). The results we obtained can be explained if MBL is able to bind to man-BSA and mannose-Sepharose simultaneously, thereby linking man-BSA to the Sepharose beads. We therefore conclude that MBL has multiple binding sites for mannose, similar to the multiple carbohydrate recognition domains found in MBP obtained from humans (Drickamer et al., J. Biol. Chem. 261: 6878-6887, 1986).

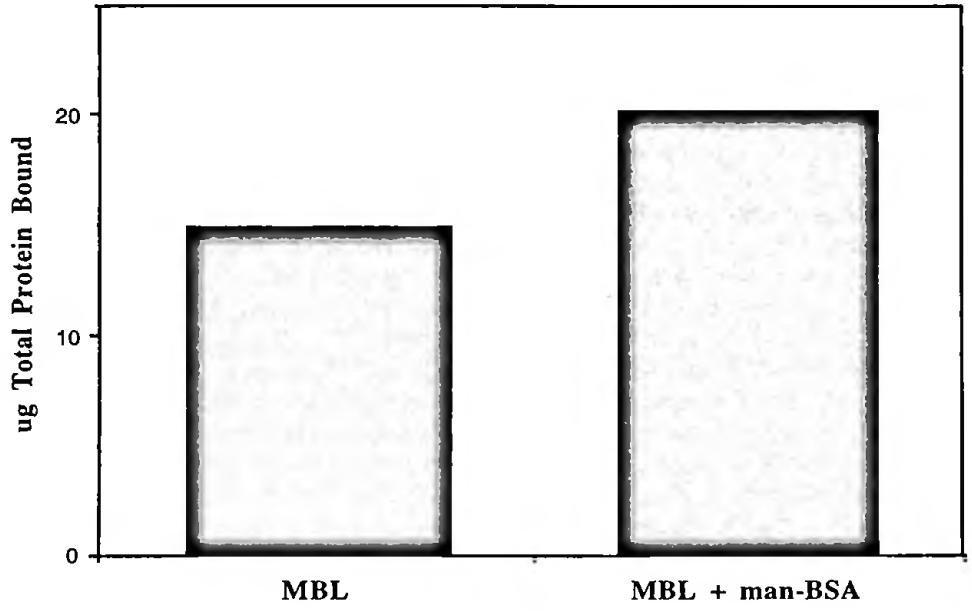

Fig. 3. Binding of MBL +/- Mannose-BSA to Mannose-Sepharose

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