A LECTIN IN THE SERUM OF THE DOGFISH SHARK *(SQUALUS ACANTHIAS)* BINDS AND PRECIPITATES NEOGLYCOPROTEINS

Carolyn R. Newton and Thomas A. Stibitz
Department of Biology, Kalamazoo College, Kalamazoo, MI 49006

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). Similar proteins may play a role in the defense mechanisms of other vertebrates. We are continuing to characterize a soluble mannose-binding lectin (MBL) found in the serum of the spiny dogfish.

Our previous sugar binding studies with MBL (Newton et al., Bull. MDIBL 34: 89-91, 1995) showed that MBL has a similar selectivity for simple sugars as MBP, having the strongest affinity for L-fucose and D-mannose. More recently, our studies of MBL binding to mannosylated bovine serum albumin (BSA) (Newton et al., Bull. MDIBL 37: 108-110, 1998), suggested 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). Such multiple binding sites would allow the precipitation of glycoproteins under the appropriate conditions. In the present study, we used the Ouchterlony double diffusion technique to examine the ability of MBL to interact with two neoglycoproteins, mannosylated BSA (man-BSA) and fucosylated BSA (fuc-BSA). If MBL has multiple binding sites, it should form a latticework, and eventually a visible precipitate, with the neoglycoprotein.

MBL was isolated from the serum of the spiny dogfish by affinity chromatography on a mannose-Sepharose 6B column and gave a single band at 140 kD following SDS-PAGE. Plates were prepared for Ouchterlony double diffusion by adding 5 ml of 1% agarose in phosphate-buffered saline (PBS) to 6 cm petri dishes to form a thin layer. Once the agarose was firm, wells were punched in it and filled with protein solutions as indicated in Fig. 1. After 48 hours of incubation in a humid chamber at room temperature (20-25° C), precipitation bands were visible. Unprecipitated protein was removed by soaking the plates in a chloride-borate wash solution (0.4% NaCl, 0.4% boric acid) for two days and the gels were stained with Coomassie blue. Both fuc-BSA and man-BSA formed a single band of precipitate with MBL; a ten-fold higher concentration of man-BSA was required, perhaps reflecting its lower affinity for MBL. The fact that a precipitate was formed in either case provides further confirmation that shark serum MBL has multiple carbohydrate recognition domains.

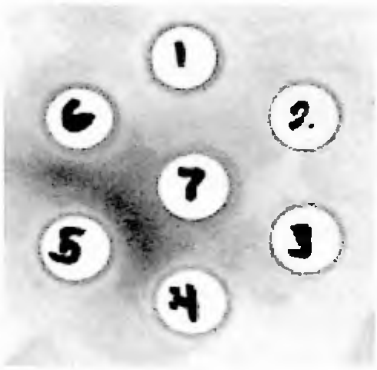

Plate 1

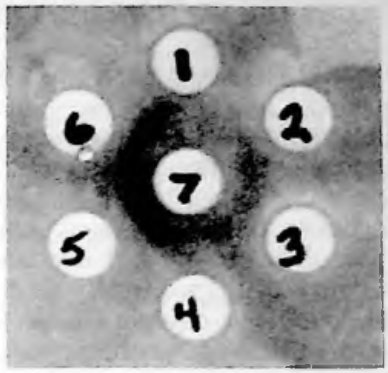

Plate 2

Figure 1. Plate 1: wells 1-3, PBS; well 4, 2 mg/ml BSA; well 5, 10 mg/ml man-BSA; well 6, 1.1 mg/ml MBL; well 7 5 mg/ml MBL. Plate 2: well 1, PBS; well 2, 2 mg/ml BSA; well 3, 2 mg/ml man-BSA; well 4, 1 mg/ml man-BSA; well 5, 2 mg/ml fuc-BSA; well 6, 1 mg/ml fuc-BSA, well 7, 3 mg/ml MBL.

This work was supported in part by a Kalamazoo College Faculty-Student Project Grant (TAS).