MOLECULAR CLONING AND ANALYSIS OF A SALMON SERUM C-TYPE LECTIN

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EXTENDED ABSTRACT ONLY – DO NOT CITE

Innate immunity involves the direct recognition and destruction of a pathogen without prior exposure. Fish have a relatively variable capacity for antibody-mediated immunity (reviewed elsewhere). Therefore, the role of innate immunity, including humoral events such as complement activation and cellular systems such as phagocytosis by macrophages, is expected to be very important in fish, particularly under conditions in which antibody-mediated immunity might be compromised. Innate immunity hinges on the recognition of non-self cells and this frequently occurs by means of the distinct carbohydrate patterns on their surfaces. In chicken and in several mammals, C-type (Ca^{2+}-dependent) lectins can play this role. For example, the human mannose-binding lectin plays a key role in resistance to common diseases and lectin deficiency is linked to increased susceptibility to infection (Turner and Hamvas, 2000).

To determine whether similar immune-active lectins might be present in fish, mannose-binding proteins were purified from serum of healthy Atlantic salmon (*Salmo salar*) and the most abundant protein was characterised. This multimeric serum lectin was found to bind common salmon pathogens and was shown to opsonise one of these as well (Ewart et al., 1999; Ottinger et al., 1999).

cDNA clones encoding this lectin were sought in order to better study its structure and expression. Internal protein sequence was required in order to

produce oligonucleotide primers for the lectin. Proteolysis followed by mass spectrometry sequencing gave peptide sequences suitable for oligonucleotide design. Since the mammalian and chicken lectins were produced in liver, our initial attempts at cloning the fish lectin focused on that tissue. No clones were isolated. However, using the oligonucleotides, a portion of a gene for the lectin was amplified from genomic DNA using the polymerase chain reaction (PCR). This allowed the design of new PCR primers for lectin expression analysis. Reverse-transcriptase PCR using these primers showed kidney to be the site of synthesis, and not liver. A 641 base pair lectin cDNA was then cloned from kidney and fully sequenced. Screening for this cDNA clone using high-fidelity PCR revealed the presence of several similar cDNAs with minor sequence variations. This implied the presence of a lectin multi-gene family, later confirmed by Southern blotting. The 173-amino-acid sequence of the lectin confirmed its homology with the C-type lectin superfamily. The sequence is most similar to the subfamilies of lectins that bind galactose and related sugars. Furthermore, this lectin lacks the collagen triple helix “stalk” that is characteristic of the immune-active mannose-binding lectins in mammals and birds. In contrast to the galactose-binding lectins, however, the salmon serum lectin contains the Glu-Pro-Asn motif of mannose-binding C-type lectins. Carbohydrate binding-inhibition assays show binding to mannose and related sugars including glucose, N-acetylglucosamine and others. The lectin is secreted into the blood and Western blotting using phage-display antibodies show that it forms a complex multimer in blood plasma, consistent with its opsonizing role.

Studies are underway to determine the mechanism of oligomerisation in this lectin and future work will involve its binding to pathogen and immune cell surface ligands. Further collaborative work is planned to determine whether expression of this protein is responsive to infection in the fish. With this knowledge, it will be feasible to evaluate this protein for use in fish health monitoring and biotechnology for disease prevention.

References

Ewart, K.V., Johnson, S.C. and Ross, N.W. 1999. Identification of a novel pathogen-binding lectin in salmon serum. Comp. Biochem. Physiol. C 123: 53-59.

Ottinger, C.A., Johnson, S.C., Ewart, K.V., Brown L.L., and Ross, N.W. 1999. Enhancement of anti-*Aeromonas salmonicida* activity in Atlantic

salmon (*Salmo salar*) macrophages by a mannose-binding lectin. Comp. Biochem. Physiol. C 123: 9-15.

Turner, M.W. and Hamvas, R.M. 2000. Mannose-binding lectin: structure, function, genetics and disease associations. Rev. Immunogenet. .2: 305-322.

Acknowledgements

This work was supported by NRC IMB and by an NSERC research grant to KVE.