Lectins of the innate immune system and their relevance to fish health

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Introduction

Aquaculture operations strive to produce large numbers of healthy fish by means that are biologically and economically efficient. Therefore prevention of disease is very important to the industry. In recent years, more effective vaccines have been developed, fish nutrition has improved, and improved fish health management schemes have been developed, all of which have significantly reduced the impact of disease in aquaculture. However, a great deal remains unknown regarding immunity in fish.

The immune system of vertebrates involves both innate and acquired immune responses. Vaccines rely on the acquired (antibody-mediated) immune response, which requires time to develop after exposure to the pathogen. The innate immune response is independent of antibodies and constitutes the first line of defence against infection. Unlike the acquired response, the innate response is rapid and it requires no prior exposure to pathogens. A variety of proteins have direct antibacterial activity or act as opsonins to inactivate pathogens and stimulate their destruction by macrophages or complement (Yano, 1996). This recognition event is the first step in immediate host defence against infection and the basis for innate immunity. Innate immune defence has been reported in vertebrates and a variety of invertebrates. Even in mammals, which have a strong acquired immune response, innate immunity plays a vital role in protection against disease (Epstein et al., 1996; Sumyia and Summerfield, 1997).

Several soluble proteins are effectors in the innate immune response of fish (Alexander and Ingram, 1992). Some have been well characterized, including antimicrobial proteins such as lysozyme and cationic lytic peptides (Yano, 1996; Hancock and Lehrer, 1998). Other important events in innate immune defence include the pattern-based recognition of microbial targets as “non-self” by host lectins and related proteins and their subsequent destruction by complement and/or phagocytic cells (Lu, 1997). The importance and roles of innate immune components such as circulating lectins is well recognized in more derived vertebrates such as mammals (Epstein et al., 1996; Sumyia and...
These proteins recognize non-self cells and enveloped viruses by virtue of their surface carbohydrates and target them for uptake and destruction by phagocytic leukocytes. In fish, which differ from mammals in aspects of the acquired immune response (Kaattari and Piganelli, 1996), lectins and other innate immune effectors may have more important roles. However, characterization of the structures and activities of immune-active lectins in fish has only recently begun.

Lectin overview

Lectins were originally defined as proteins that bind carbohydrates and also agglutinate cells. Based on these criteria, many plant and animal lectins were identified. Although the biological functions of lectins in plants are generally unclear, they are very diverse and have been well studied (Sharon and Lis, 1989). Their in vitro use as carbohydrate recognition and labelling agents has made them valuable tools in life sciences. Our understanding has grown considerably over the last decade as lectins from various animal species have been characterized. The diversity of these lectins has expanded their definition to include any protein containing a non-catalytic carbohydrate-recognition domain (CRD). Monomeric and membrane-bound lectins that cannot agglutinate cells are still effectively lectins at the molecular level and they are now recognized in this way.

Two major classes of lectins, C-type and S-type, are found in animals (Drickamer and Taylor, 1993). S-type lectins are intra- and extracellular, they have no disulphide bonds and they recognize predominantly galactose. C-type lectins are unrelated to the S-type and comprise a large superfamily of membrane and extracellular proteins that share a disulphide-rich Ca$^{2+}$-binding CRD. The classification is based on sequence data and biological function. A large number of animal lectins have been identified only in terms of activity and carbohydrate specificity. In most cases, little is known regarding their biological function or evolutionary classification. Recently, an increasing number of studies have investigated the role of lectins in animals using both immunological and molecular biological techniques (Drickamer and Taylor, 1993). An exciting discovery to emerge was that several C-type lectins play key roles in the immune system. Some are predominant effectors in innate immunity and others were found to have key roles in other aspects of immunity including leukocyte trafficking, cell-cell interaction and other processes (Drickamer and Taylor, 1993; Ni and Tizzard, 1996).

Immune-relevant C-type lectins

The C-type CRDs contain one or two Ca$^{2+}$-binding sites (Weis and Drickamer, 1996). The Ca$^{2+}$-binding site shared by all lectins is also the carbohydrate-binding site (Weis and Drickamer, 1996). The different C-type lectins recognize a variety of carbohydrates. Among these are seven classes of proteins that each have C-type CRDs in different structural arrangements (Drickamer and Taylor, 1993). Many of these proteins are membrane receptors and each type has a different function. However, in every case, the lectin CRD is extracellular. Roles in the innate immune defence system have been ascribed to types III and VII lectins. These proteins are frequently found in blood (vertebrates) or hemolymph (invertebrates) and, in most cases, roles in host defence against pathogens have been demonstrated or postulated.

Type III lectins are named collectins. These proteins form multimers with subunits composed of type I collagen strands near the amino terminal and a C-type CRD at the carboxyl terminal end. The collectins have been identified in mammals and birds, and numerous studies in mammals have shown that their primary function is host defence against pathogens. Proteins in this group, such as the mammalian mannose-binding lectin, bind to the carbohydrate surfaces of pathogens such as viruses and bacteria and opsonize them (Lu, 1997). Experiments using transgenic mice that over-express mannose-binding lectin show that increasing levels of this protein confer greater protection against specific pathogens (Tabona et al., 1995). Moreover, among humans, individuals with mutated forms of mannose-binding lectin have increased susceptibility to infection (Sumyia and Summerfield, 1997).

The type VII lectins are CRDs with no distinguishable associated domains. Proteins in this group include monomers, dimers, and multimeric aggregates of CRDs. Type VII C-type lectins were first found in invertebrates (Takahashi et al., 1985; Giga et al., 1987; Muramoto and Kamiya, 1990; Suzuki et al., 1990). Most of these proteins bind primarily galactose or its derivatives. Some have unknown roles (Takahashi et al., 1985; Giga et al., 1987), while others are thought to act in a host defence mechanism (Muramoto and Kamiya, 1990; Suzuki et al., 1990). One such lectin isolated from the cockroach (Periplaneta americana) binds lipopolysaccharide (Jomori et al., 1990; Jomori and Natori, 1991). Homologous type VII lectins have also been found among vertebrates (Suzuki et al., 1990; Hirabayashi et al., 1991).

Lectins in fish

No direct ortholog of the mannose-binding protein has been identified in fish, although one or more are likely to exist. A number of lectins have been reported from fish (Yano, 1996), but most have been characterized only in terms of agglutination activity and carbohydrate specificity. Some of these may be C-type, but without...
substantial sequence and structural information it is difficult to assign these lectins to a particular class. Among fish, there are no soluble C-type lectins identified by full sequence. However, there is reason to believe that they are present. The presence of lectins from the C-type superfamily throughout the animal kingdom would argue strongly for the presence of these proteins in fish. Moreover, the type II antifreeze proteins found in smelt (Osmerus mordax) and herring (Clupea harengus harengus) belong to the C-type lectin superfamily and closely resemble the type VII lectins (Ewart and Fletcher, 1993). Although these ice-binding proteins do not appear to function as lectins, their presence implies that closely related soluble C-type lectins would have pre-existed in fish (Ewart and Fletcher, 1993).

In an initial attempt to identify soluble C-type lectins in fish, salmonid livers were examined for the presence of type VII C-type lectin transcripts homologous to the smelt antifreeze protein. This was done by Northern blotting using the smelt antifreeze protein cDNA as a probe. The smelt liver showed a strong signal, as expected. However, no signal was detected in salmon or trout livers (Figure 1). Thus, it appears that any salmonid lectin related to the smelt type II antifreeze protein is either divergent from the smelt antifreeze or is expressed only under very specific conditions. This identification method was unsuccessful.

However, using a different approach, a preliminary identification of a C-type soluble lectin was made. A mannose-binding lectin from the blood serum of Atlantic salmon was isolated by mannose-affinity chromatography followed by gel filtration on Sepharose 4B (Pharmacia). Analysis of this lectin by SDS-PAGE under reducing and non-reducing conditions revealed a multimeric structure containing a large number of 17 000 Mr subunits (Figure 2; Ewart et al., 1999). The exact multimer size was difficult to ascertain because the protein formed a ladder-like pattern on non-reducing SDS-PAGE and did not elute as a single peak in analytical gel filtration (Ewart et al., 1999). The banding observed by SDS-PAGE was reminiscent of the rainbow trout ladder lectin (Jensen et al., 1997). The salmon serum lectin was shown to be non-glycosylated and amino acid analysis revealed no unusual compositional features (Ewart et al., 1999). Using ruthenium red staining, the lectin was shown to bind Ca\(^{2+}\) ions, as C-type lectins are known to do. N-terminal sequencing by Edman degradation and confirmation by mass spectrometry of residues within this sequence has led to the identification of this lectin as C-type. In this regard, it is interesting that the limited N-terminal sequences of the trout ladderlectin and the salmon serum lectin can be aligned to show partial sequence identity (Ewart et al., 1999). If cDNA cloning and deduction of complete protein sequences for these two lectins shows them to be homologs, then the trout ladderlectin would necessarily be C-type as well.

Immune-active fish lectins
Recognition of bacteria is a reported property of several fish lectins. A galactose-binding lectin with bacterial recognition activity was identified in coho salmon (Onchorhynchus kisutch) eggs, but it does not appear to be an opsonin (Yousif et al., 1994, 1995). Biochemical characterization and sequencing has not yet been reported. The salmon serum lectin was also shown to bind Aeromonas salmonicida and Vibrio anguillarum in experiments employing biotinylated lectin, followed by detection using streptavidin-conjugated antibodies (Ewart et al., 1999). A number of lectins were identified in rainbow trout serum by Ca\(^{2+}\)-dependent binding to A. salmonicida lipopolysaccharide (Hoover et al., 1998). One corresponded to the trout ladder lectin (Jensen

![Figure 1. Northern blot of total RNA from smelt and salmonid livers. Samples are 1, Rainbow trout, January; 2, Smelt, February; 3, Atlantic salmon, December; 4, Atlantic salmon, July. Duplicate loadings (3 and 15 μg) were made on a formaldehyde gel for each sample and sample loading accuracy was ascertained by ethidium bromide staining of the gel prior to RNA transfer to the nylon membrane. The blot was hybridized with a \(^{32}\)P labelled probe made from a smelt antifreeze protein cDNA by the random primers labelling method and then washed at low stringency. Exposure shown is 2 h. Overnight and 2-d exposures did not reveal further bands in the salmonid RNA lanes but did reveal a larger sized transcript in the smelt sample (not shown).](image-url)
et al., 1997). Another was an unidentified lectin and the limited N-terminal sequence did not resemble any other known proteins. However, it showed a subunit Mr of 37 000 and formed dimers, tetramers, and pentamers.

It has been suggested that the collectins might foster infection by intracellular pathogens by allowing their passage into phagocytes (Ezekowitz, 1998). The salmon serum lectin was shown to have the same properties using analogous lectins in mammals, including the mannose-binding lectin.

Figure 2. Analysis of Atlantic salmon serum mannose-binding lectin by SDS-PAGE. (a) Proteins eluted from mannose-agarose beads using mannose and run on a 15% acrylamide SDS-PAGE gel run under reducing conditions. (b) Pure mannose-binding lectin obtained after Sepharose 4B chromatography and run on a 15% SDS-PAGE gel run under reducing conditions. (c) Pure non-reduced mannose-binding lectin run on a 5% SDS-PAGE gel under non-reducing conditions. Arrowheads indicate to the lectin monomer on panels (a) and (b) and the smallest lectin oligomer on panel (c). (Reprinted from Comparative Biochemistry and Physiology C, 123, Ottinger et al., 1998, with permission from Elsevier Science.)

Figure 3. Effect of salmon serum lectin on phagocytosis of Aeromonas salmonicida by salmon macrophages. Heat-killed A. salmonicida were incubated for 2 h at room temperature with a range of concentrations of the salmon serum lectin. Lectin-opsonized and non-opsonized bacteria were then incubated for 3 h at 17°C with Atlantic salmon macrophages, after which they were fixed, permeabilized, stained with FITC, and counterstained with bis-benzimide. Values shown are means ± s.e. of macrophages that contained a minimum of five FITC-labelled bacteria. Results significantly different (p<0.05) from control are indicated by an asterisk (*). For each condition, n=10. (Reprinted from Comparative Biochemistry and Physiology C, 123, Ottinger et al., Enhancement of anti-Aeromonas salmonicida activity in Atlantic salmon (Salmo salar) macrophages by a mannose-binding lectin, 1999, pp. 53–59, with permission from Elsevier Science.)

Possibilities for fish health programmes

Opsonizing lectins present in fish serum should be useful in fish health, both in terms of gaining a fuller understanding of the immune system and of the development of applications for disease prevention. Functionally analogous lectins in mammals, including the mannose-binding lectin, play the initial role in host defence by recognizing pathogens as “non-self” and subsequently enhancing their destruction and presentation to phagocytic cells of the immune system. The mammalian mannose-binding lectin can opsonize yeasts, bacteria, and enveloped viruses, such as HIV and influenza (Turner, 1996). The salmon serum lectin was shown to have the same properties using A. salmonicida as a model target. In the case of the mammalian mannose-binding lectin, the opsonizing role is itself crucial for resistance to infectious disease. Humans with opsonizing defects were found to have mutations in genes encoding this protein, and these same individuals were unusually susceptible to infectious diseases (Super et al., 1989; Turner et al., 1993). It is reasonable to expect that the salmon lectin and possibly one or more of the new lectins recently identified in rainbow trout would have similar importance.

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serum lectin is not expected to have this role, not at least in the case of *A. salmonicida*. In addition to opsonizing the bacteria, the salmon lectin enhanced their killing by macrophages (Ottinger et al., 1999). This finding specifically contradicts the above hypothesis.

The biological roles of lectins in the sera of salmonid species will require further study if they are to be properly defined. At the molecular level, the carbohydrate ligands on *A. salmonicida* and other pathogens have not been identified. In addition, the repertoire of pathogens recognized by the lectins has not been investigated. A key question to address is whether any of the soluble lectins bind to the infectious salmon anemia virus and other serious viral and bacterial pathogens outside of the *Vibrio* and *Aeromonas* genera. In addition, it would be valuable to identify the lectin receptors on macrophages and other cells that mediate the recognition of lectins that have opsonized pathogens. Finally, it will be important to determine how the different soluble lectins are regulated and their implications in salmonid biology. Do levels in sera vary with season, development, infection, or other parameters? Are there substantial individual or population-level variation in lectin levels? Do higher circulating levels of any of these lectins lead to increased disease resistance? Answers to these questions will determine whether the lectin can indeed be useful in enhancing fish disease resistance. If higher levels bring about a greater degree of disease resistance in salmon, as was found for mannos-binding lectin in transgenic mice (Tabona et al., 1995), then the potential for use in aquaculture would be excellent. Selection of individuals or populations with the highest levels could allow breeding of more disease-resistant fish. Another possibility might be the development of transgenic fish expressing higher levels of these proteins.

Conclusions

Research aimed at producing more disease-resistant fish is of great value to the aquaculture industry. Since pathogens are present in aquatic environments, fish with enhanced disease resistance would be excellent candidates for culture.

Acquired (antibody-mediated) immunity is reduced at low temperature, even in eurythermal fish. However, preliminary work suggests that components of innate immunity in fish may be less affected by temperature and, in some cases, may even increase at lower temperatures (Magnadottir et al., 1999). If these findings are extended by further studies on more components of the innate immune system, then enhancement of innate immunity would be the route of choice in generating greater disease resistance in cold ocean species such as salmon. Ideally, this work would be coordinated with other research aimed at enhancing fish disease resistance, including over-expression of antimicrobial peptides, modulation of antibody production, or other processes involved in innate or acquired immunity, nutritional enhancement of disease resistance, and breeding of disease-resistant strains for cultured species.

Decreased susceptibility of salmon to diseases will lead to greater productivity and economic gains for the aquaculture industry. It would also allow further reduction in the use of antimicrobial agents.

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