Japanese flounder immunoglobulin D and M cDNA

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INTRODUCTION

B lymphocytes (B cells), which express immunoglobulins on their surfaces, are usually produced in response to a microbial infection. Antibody-mediated responses of host cells fight invading pathogens either immediately, at the site of pathogen entry, or later, when the infection spreads to the blood and/or organs. Antibodies act directly on the invading pathogen.

Previously, several fish Immunoglobulin (Ig) cDNAs and genes have been cloned and characterized.¹⁻⁸⁾ Two types of immunoglobulin heavy chains, IgM and IgD are present in teleostei.^{2-5,7)} Little is known about the mechanisms regulating gene expression of the immunoglobulin heavy chain gene in teleosts. So far, these mechanisms have been only partially characterised in channel catfish.^{9,10)} Characterization of the regulation mechanism of gene expression and the expression pattern of immunoglobulin genes are useful for understanding the biology of fish B cells. In addition, in the development of vaccines, the immunoglobulin gene can be useful for evaluating candidate antigens. The detection and quantification of the mRNAs of immunoglobulin genes would be easier than detection and quantification of proteins. In addition, it is possible to detect variations in the immunoglobulin variable region after vaccination if the DNA sequences of the immunoglobulin gene is available.

Japanese flounder is a member of the superorder Acanthopterygii, as well as a member of one of the most recently diverged orders, Pleuronectiformes. Therefore characterization of the immunoglobulins of Japanese flounder can help to elucidate the evolution and diversity of the fish immune system. However, virtually no information is presently available on these molecules. Tokuda *et al.*¹¹⁾ has used an antibody against serum immunoglobulin to characterise Japanese flounder lymphocytes. In this study, we cloned and sequenced the full lengths of Japanese flounder IgM and IgD cDNAs.

MATERIALS AND METHODS

The cloned Japanese flounder peripheral blood leukocyte (PBL) cDNA library used in this study has been previously reported.¹²⁾ Partial cDNA fragments of IgM and IgD¹²⁾ were used as DNA probes to screen the cDNA library in order to isolate full-length IgD and IgM cDNAs. Hybridisation was conducted as previously reported.¹³⁾ cDNA clones were sequenced using ThermoSequenase (Amersham-Pharmacia, U.S.A.) with M13 forward and/or M13 reverse primers and an automated DNA sequencer LC4200 (Li-Cor, U.S.A.). Each determined sequence was compared with all sequences available in DDBJ/EMBL/GenBank using the BLAST ver.2.0. The nucleotide sequences and predicted amino acid sequences were edited and analysed with Genetyx-Mac (SDC Software Development, Japan).

RESULTS AND DISCUSSION

The IgM cDNA (DDBJ accession No. AB052744) consisted of 1,875 bp encoding 578 amino acid residues, the IgD cDNA (DDBJ accession No. AB052658) consisted of 3,240 bp encoding 998 amino acid residues. We found only one type of nucleotide sequence of the constant region of IgM and IgD cDNA although we sequenced several clones of IgM and IgD cDNAs. The constant region of IgM consists of μ 1 to μ 4, whilst the constant region of IgD consists and μ 1, δ 1 to δ 7, and TM regions. These domain structures of Japanese flounder IgM and IgD are identical to those of several other teleost IgM and IgD,¹⁻⁶⁾ although the structure of Atlantic cod IgD is different.⁷⁾ The positions of cysteine residues involved in disulfide bonds are well conserved in the Japanese flounder immunoglobulin heavy chains. The amino acid sequence of the constant region of Japanese flounder IgM shares 34 to 50 % identity to that of previously reported teleost IgMs (Table 1).

Table 1 Percentage identities of amino acid sequences of fish immunoglobulin IgM heavy chain constant region

	2	3	4	5	6	7
1 <i>Paralichthis olivaceus</i>	50.0	37.2	33.9	41.1	41.3	33.6
2 <i>Trematomus bernacchii</i>		38.8	33.3	39.2	40.6	31.9
3 <i>Gadus morhua</i>			30.4	38.6	38.3	30.9
4 <i>Elops saurus</i>				40.5	39.6	35.7
5 <i>Salmo salar</i>					90.6	40
6 <i>Oncorhynchus mykiss</i>						39.4
7 <i>Ictalurus punctatus</i>						

Sequences were obtained from the DDBJ/EMBL/GenBank database.

The amino acid sequence of the constant region of Japanese flounder IgD shares 40 to 53 % identity to that of previously reported teleost IgDs (Table 2). These amino acid sequence identities of the fish immunoglobulin heavy chain constant region are low when compared to those of mammals. The amino acid sequences of human immunoglobulin IgM heavy chain constant region is more than 60% identical to the constant regions of other mammals such as mouse, cow, and dolphin. In addition, immunoglobulin IgM heavy chain constant region of chicken is 36.8% identical to that of human and 26.7% identical to that of Japanese flounder. These results indicate that the divergence of fish immunoglobulin heavy chains is higher than that of mammals. A possible reason for the large divergence of fish immunoglobulin is that fish must adapt to several different environmental conditions and are exposed to a wider variety of pathogens.

Table 2 Percentage identities and similarities of amino acid sequences of fish immunoglobulin IgD heavy chain constant region

	2	3
1 <i>Paralichthis olivaceus</i>	52.7	39.6
2 <i>Salmo salar</i>		44.8
3 <i>Ictalurus punctatus</i>		

Sequences were obtained from the DDBJ/EMBL/GenBank database.

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