The lysozyme gene in fish

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INTRODUCTION

Lysozyme (muramidase, EC.3.2.1.17) is a widely distributed enzyme, which are located in the serum, mucus and many other tissues of fish and higher vertebrates. It catalyzes the hydrolysis of bacterial cell walls, and acts as a non-specific innate immunity molecule against the invasion of bacterial pathogens.^{1,2)} According to their amino acid sequences, lysozyme are classified into 5 types: chicken-type lysozyme (c-type), goose-type lysozyme (g-type), plant lysozyme, bacterial lysozyme and T4 phage lysozyme (phage-type).^{2,4,5)} In fish, only cDNA sequences of two c-type lysozymes from rainbow trout have previously been reported.⁶⁾ Recently, we have cloned and characterized the c-type and g-type lysozyme genes from Japanese flounder, *Paralichthys olivaceus*.^{3,7,8)} This paper summarizes their gene structure, gene evolution, and gene expression and activity.

FLOUNDER C-TYPE LYSOZYME GENE

The fish c-type lysozyme gene sequence from Japanese flounder,³⁾ consists of 3,617 bp, including 4 exons and 3 introns (Fig. 1). Structural comparison of the c-type lysozyme genes from other vertebrates and Japanese flounder indicate a correspondence in the positions of the exons and catalytic residues (Glu 35 and Asp 52). Hikima *et al.*³⁾ have found 45 novel repeated sequences (approx. 1.4 kb in length) in intron 2 of the flounder c-type lysozyme gene. These repeated sequences were not observed in other vertebrate c-type lysozyme genes, or in other regions of the flounder lysozyme gene.

Fig. 1 Structure of the flounder c-type lysozyme gene.

The rainbow trout c-type lysozyme gene is expressed in the liver and kidney,⁶⁾ while the Japanese flounder c-type lysozyme gene is expressed in the head kidney, posterior kidney, spleen, brain and ovary.⁷⁾ Furthermore, in a Japanese flounder injected with *Edwardsiella tarda*, (a known pathogen of Japanese flounder) and examined after 72 hours, c-type lysozyme mRNA levels in the head kidney, spleen

and ovary were greatly increased.⁷⁾

FLOUNDER G-TYPE LYSOZYME GENE

The g-type lysozyme gene has also been isolated from Japanese flounder, which is 1,252 bp in length, encoding for 195 amino acid residues.⁸⁾ The Japanese flounder g-type lysozyme gene is about 2.3 kb longer than the chicken g-type lysozyme gene, this appears to be due to structural differences between the two genes; a 5 exon/4 intron structure in the flounder gene and a 6 exon/5 intron structure in the chicken gene (Fig. 2).^{8,9)} This difference in length also is partly due to the shortness of the flounder introns relative to the corresponding introns in the chicken gene. In addition, there is a structural differences in exon 1 and intron 1 of the flounder gene relative to the chicken. The 5' upstream region of the flounder gene must have undergone unknown evolutionary changes, e.g. a recombination, insertion or deletion at the time after higher vertebrates diverged from fish.

Fig. 2 Comparison of the structure of the Japanese flounder and chicken g-type lysozyme genes.

The Japanese flounder g-type gene is expressed extensively in the head kidney, posterior kidney, spleen, skin, muscle, heart and brain, and also in the liver, intestine, ovary and whole blood to a lesser extent. Furthermore, gene expression in the heart, intestine and whole blood increased following injection with *E. tarda*.⁸⁾ In contrast, the chicken g-type gene is expressed only in the bone marrow and lung.⁹⁾

THE DIVERSITY OF FISH LYSOZYME

The c-type, g-type, and phage-type lysozymes were clustered correctly in the phylogenetic analysis (Fig. 3). It has been proposed that the Japanese flounder g-type lysozyme is closer to the phage-type lysozyme when molecular evolutionary events are considered.

Potentially the phage-type lysozyme diverged to the flounder g-type lysozyme through the recombination, insertion or deletion of a gene within the 5' upstream region and that this led to formation of the avian g-type lysozyme.⁸⁾ Grütter *et al.*¹⁰⁾ hypothesized that the molecular evolution of lysozyme had a common precursor which was like the c-type lysozyme that this gave rise to the g-type lysozyme that led to the present phage-type lysozyme, suggesting that the g-type lysozyme evolved from a phage-type lysozyme, and both of these from a common "c-type like" precursors. However, other authors have proposed that the phage-type and the common precursor of c-type-like lysozyme converged to form the flounder g-type lysozyme. This possibility is based on the similarity between the three dimensional structure of the g-type lysozyme and that of the c-type and phage-type lysozymes.^{2,11)} Further analysis, particularly of g-type lysozyme from a variety of organisms is required to clarify this point.

Fig. 3 Phylogenetic analysis of Japanese flounder lysozyme.

LYSOZYME ACTIVITY OF JAPANESE FLOUNDER

It is known that fish lysozyme possess the lytic activity against *Micrococcus lysodeikticus*, a Gram-positive bacteria.^{2,12)} Rainbow trout lysozyme (type I) has been found to possess substantial antibacterial activity to seven bacterial strains of Gram-negative species. However, hen egg lysozyme was bactericidal only against one species of nonpathogenic bacteria.¹³⁾ These results suggest that fish lysozyme, in contrast to other higher vertebrate lysozymes, has antibacterial activity not only against Gram-positive bacteria, but also against Gram-negative bacteria. Recombinant c-type and g-type lysozymes of Japanese flounder have been shown to have high bacteriolytic activity against *Pasteurella piscicida* and *Vibrio anguillarum*, but low

activity against *E. tarda* and β -*Streptococcus* sp, which are pathogenic agents of Japanese flounder.^{8,14)} These results indicate that fish lysozyme possess a high potential for bacteriocidal or bacteriolytic activity against nonpathogenic Gram-positive and Gram-negative bacteria, however, they appear to possess a low potential against pathogenic agents.

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