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# Functional Diversity of Complement C4 Isotypes in Bony Fish Complement

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(硬骨魚類における補体成分 C4 アイソタイプの機能的多様性)

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## Thesis Summary

The complement is a major humoral system of innate immunity. The complement system consists of a group of proteins in the body fluid and membrane proteins. These proteins help the immune system fight off invading pathogens, such as viruses and bacteria, and other foreign substances. There are three activation pathways of complement: the classical pathway, the alternative pathway, and the lectin pathway. In the classical and lectin pathways of mammalian complement, complement component C4 plays an important role by tagging the target.

The complement C4 is a member of the thioester-containing protein family. The thioester site plays a crucial role endowing C4 with an ability of covalent binding to the target. This binding reaction is catalyzed by a histidine (H) residue, which is located at about 100 residues C-terminal from the thioester site, to accelerate its binding to hydroxy-group rich in carbohydrates of microbial targets. In several fish species, an additional C4 isotype, in which the catalytic H is replaced by an aspartic acid (D), has been reported to show binding specificity towards amino-group, relatively rich in proteins. The catalytic site difference has been inferred to impacts its pathogen-binding specificity, affecting the tagging function of C4. However, the functional differentiation of the C4-1, the atypical isotypes, has been still to be clarified with experimental evidence. Thus, evolutionary implication of the divergence of two isotypic lineages, H-type (C4-1) and D-type (C4-2), remains unclear.

In the present study, antibodies to detect the C4-1 and C4-2 isotypes of a model animal, common carp (*Cyprinus carpio*) were established using recombinant domains of carp C4-1 and C4-2 and also using synthetic peptides representing the two isotypes.

Using the established specific antibodies, the involvement of common carp C4-1 isotype in the classical and lectin pathways was investigated using purified components responsible for proteolytic activation of C4 in the two pathways. The western blotting results showed that carp C4-1 isotype was cleaved into its active fragment C4-1B by both C1s and MASP, serine proteases in the classical and lectin pathways, respectively. The results suggest that the C4-1 isotype participates in both the classical and the lectin pathways of complement activation.

Binding specificities of C4-1 and C4-2 to model targets and natural microbial targets were analyzed by immunoassays using anti-C4-1 and anti-C4-2. In contrast to the specificity of the thioester site-mediated covalent binding, predicted from the catalytic site difference, C4-1 and C4-2 showed similar binding spectra against. The results suggest that the thioester-catalytic site is not a primary determinant of the binding of C4 isotypes to natural target and that both C4-1 and C4-2 equally participate in the complement activation in bony fish.