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学 位 論 文 要 旨
Dissertation Abstract

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学位論文題目：
Title of Dissertation

Studies on the adaptations to deep sea environment of type I collagens of *Coryphaenoides yaquinae* and other species
（シンカイヨロイダラを中心とした魚類 I 型コラーゲンの環境適応に関する研究）

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Limited information is available for the primary structure, biochemical and mechanical properties of deep-sea fish proteins in connective tissue, such as collagen, which is exposed to low temperature, high static pressure and mechanical stress in the deep sea.

Collagen, which is one of the fibrillar proteins is thought to support the maintenance of structural integrity, provide shape, texture, and resilience. Type I collagen is the most abundant collagen type in vertebrates. This molecule is generally composed of two α1 / 3 (col1a1 / col1a3) chains and one α2 (col1a2) chain. The triple-helix domains in type I procollagen were characterized by the formula $[Gly\text{-}X\text{-}Y]_{338}$, which represents 338 uninterrupted Gly-X-Y repeats. Here, X and Y can be any two amino acid residues, but they are frequently Pro and its post-translationally modified form, 4-hydroxyproline (Hyp), respectively.

Previous studies have suggested that poikilothermic fish collagen adapts to lower water temperatures by altering its amino acid composition, especially by replacing more Pro or Hyp with Ser. It has also been suggested that deep-sea fish myosin and actin are adapted to high pressure by changing the amino acid composition of the molecule.

However, a comprehensive analysis of the correlation between Pro, Hyp and Ser content and biochemical and mechanical properties for collagens from deep sea fish species is lacking. Increasing knowledge on the structure, biochemical and mechanical properties of deep sea fish collagen is considered to help understanding the adaptation mechanism of the organism to the environment.

Here, in Chapter I, Deep sea macrouridaes inhabiting depths of 500-5,800 m; from *Coryphaenoides acrolepis C. armatus* and *C. yaquinae* to gain insights into the adaptation of fish physiology to deep sea environments. There was no significant difference in the proximate composition between all three *Coryphaenoides* and

shallow-sea cods, while aspartic acid was prominently abundant in amino acids, and polyunsaturated fatty acid (PUFA) were also abundant in three deep sea fishes.

In Chapter II, acid-soluble collagen (ASC) was extracted from the skin of eleven fish species at various physiological temperature (T_p: 0.3 - 30.0 °C). Structural features were analyzed in detail using Circular Dichroism (CD) and compared with their biochemical characteristics. Further, combining the resultant data with that of a previous study, analysis of ASCs from 23 fish species, demonstrated a positive correlation (r = 0.74, $p < 0.01$) between the T_p / T_d and ratio of positive peak intensity to negative peak intensity (R_{pn}). Interestingly, cold-water fish ASCs contain significantly higher levels of Ser as shown by the data in R_{pn} value ($p < 0.01$). These results suggest that unlocalized substitution of Ser for imino acids might be favourable in the cold-water environments to ensure flexibility by reducing imino acid rings and maintaining stability of the triple helix with hydroxyl group of Ser in the fibrous collagen, which require elongated and regularly repeated sequences.

In Chapter III, it was shown that *C. yaquinae* type I collagen has a characteristic structure. In the triple helix region, the Ser content was the highest (72 aa/ 1014 aa) comparing other fishes. Additionally, a pressurization test conducted at 5 °C showed that the CD spectrum pattern did not change much in *C. yaquinae* before and after pressurization. It was suggested that it is less susceptible to the effects of high pressure. When ASCs are exposed to low temperature and pressure in vitro, *C. yaquinae* ASC self-assemble even at 40 MPa, which does not self-assemble in *Gadus chalcogrammus* inhabiting shallower waters. Furthermore, the results of mechanical tests of ASC-polymerized fibrils showed that *C. yaquinae* collagen fibrils showed lower Reduced elastic modulus (Er = 1.0 ± 0.3 GPa) than those of mammals (Er = 3.0 ± 1.0 GPa) in Atomic Force Microscope analysis.

In conclusion, present study demonstrated that type I collagen from *C. yaquinae* which lives in deep-sea environments has a large amount of Ser sporadically in the triple helix region compared to that of other fish species, which potentially resulted from low temperature adaptation of *C. yaquinae*. At the same time, it could lead to structual "flexibility" of helical structure, and was also suggested that the structure makes the self-assembly process of this molecule less susceptible to pressure, and that the resulting fibrils are more "soft" than those of other species.