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学位論文全文に代わる要約
Extended Summary in Lieu of Dissertation

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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）

学位論文要約：
Dissertation Summary

ABSTRACT

The area of the ocean is equivalent to about 70 % of the total area of the earth, of which the so-called "deep sea" deeper than 200 m accounts for almost 95 % of the volume of the ocean. Despite the fact that the deep sea occupies such a size, most of the marine organisms are distributed shallower than 200 m. The reason for this is the special environment in the deep sea, such as low temperature and high pressure.

Generally, the water has more heat capacity than the atmosphere, considering that the biochemical character of proteins in poikilothermic animals including fish is more sensitively influenced by the physiological temperature (T_p). However, fish species in warm or cold-water are living without hindrance, thus, it is expected that the proteins of fishes have structure, functions, and properties that adapt to T_p.

Another major feature of the deep sea environment is high static pressure. On land, the atmospheric pressure drops by about 0.0001 MPa (1 atm = 1 kg / cm^2) for every 10 m rise. On the other hand, in the marine environment, the water pressure increases by about 0.1 MPa for every 10 m dive. Therefore, for example, at a depth of 6,000 m, organisms are constantly exposed to high water pressure of about 60 MPa (600 kg per 1 cm^2).

However, limited information has been accumulated for the primary structure, biochemical and mechanical properties of deep-sea fish proteins especially in connective tissue, such as collagen, which is exposed to cold, high static pressure and mechanical stress in the deep-sea. Collagen is thought to support the maintenance of structural integrity, provide texture, shape, and resilience, widely distributed in various tissues of multicellular organisms, including the skin, cartilage and so force. The biological function of collagen is mainly in its mechanical properties. In recent years, there have been studies on the mechanical properties of collagen fibrils studied by Atomic Force Microscope (AFM). However, they have been focused at mammals such as the bovine achilles tendon and the rat tail tendon, and there are few examples of fish-derived collagen fibrils.

Type I collagen is a main component of the extracellular matrix and is the most abundant collagen type in vertebrates. This molecule is generally constituted by two α1 / 3 (col1a1 / col1a3) chains and one α2 (col1a2) chain. Each chain contains the signal peptide, N- and C-terminal propeptides and telopeptides, and a triple-helix domain. Both in vivo and in vitro, type I collagen molecules self-assemble into fibrillar structures, which may entangle and / or cross-link to form visco-elastic gels with widely different mechanical properties and network structures.

The triple-helix domains in type I procollagen were characterized by the formula $[Gly\text{-}X\text{-}Y]_{338}$, which represents 338 uninterrupted glycine (Gly)-X-Y repeats. Here, X and Y can be any two amino acid residues, however in vertebrates they are frequently proline (Pro) and its post-translationally modified form, 4-hydroxyproline (Hyp), respectively. Pro and Hyp contain imino rings that confer conformational stability, imposing rigid constraints on rotational movement about the N-Cα bond of the back bone. This gives collagen great tensile strength and rigidity.

In most of the collagen research of fish, it had been focused on the imino acids, such as Pro and Hyp, involving in the stability of the structure. On the other hand, Rigby (1967) reported that the correlation between thermal stability and amount of serine (Ser), which is amino acid without cyclic structure and with an OH group, of various Ice-fish skin collagens inhabiting the cold-water (-1～+3 °C) and its T_p.

However, limited knowledge has been acquired on the basic biochemical character composing the tissue of deep sea fish. In addition, a comprehensive analysis of the correlation between Pro, Hyp and Ser content in collagen and biochemical and mechanical properties for collagens from deep-sea fish species is still lacking although it is predictable that fish collagen has an adapted structure and property to each habitat environment such as temperature and static pressure.

The three species of deep-sea cod fish, *Coryphaenoides acrolepis*, *C. armatus* and *C. yaquinae*, targeted in this study had no significantly difference for the proximate compositions compared to shallow-sea cods such as *Gadus chalcogrammus* and *G. macrocephalus*. Aspartic acid (Asp) was the most abundant of all *Coryphaeonides* in muscle amino acid composition, and Gly, and alanine (Ala) were followed. A similar tendency was observed in the liver of each fish species. On the other hand, glutamic acid (Glu) and Gly were the most predominant amino acids in muscle of shallow sea cods, followed by Asp. All three *Coryphaenoides* contained a large amount of polyunsaturated fatty acid (PUFA) in muscle as compared with shallow sea cods, while the polyunsaturated fatty acid to saturated fatty acid (PUFA / SFA) ratio of the formers was lower than the laters. In tissue observation, the network structure between muscle fibers in the connective tissue (endomysium), which mainly consist of type I collagen, was stainned in the three types of *Coryphaenoides*. These results suggested that these basic compositions differ depending on the habitat depth.

In Chapter II, to grasp the comprehensive profiles of the triple helix of fish collagens from various temperature regions, Type I collagen was isolated from the skin of eleven marine teleosts and analyzed by using Circular dichroism (CD) spectrum including its denaturation process. The contents of Ser in acid soluble collagen (ASC) from warm water fish were averaged at 44 ± 8 residues per 1,000 residues, while in that of cold water fish at 71 ± 6 residues. In general the ratio of positive peak intensity to negative peak intensity (R_{pn}) in the spectrum pattern increased with T_d and imino acid content in warm water fish while it decreased with T_d and Ser in cold water fish which followed the data obtained in the model peptide using R_{pn}. Considering the properties of Pro, Hyp and Ser, present data suggests that, decrease of imino acid and increase of Ser allows the flexibility to the triple helical collagen, while maintaining stability with seryl hydroxyl group driven hydrogen bonds especially in cold water fish.

In Chapter III, we investigated the primary structure, biochemical, physicochemical properties, self-assembly dynamics of molecule and mechanical properties of fibril of *C. yaquinae* type I collagen, which inhabits the deepest of the three *Coryphaenoides*. The primary structures of all three subunits (α1, 2 and 3 chains) in this type I collagen were analyzed. *C. yaquinae* was the highest content of Ser in the triple helix region among the data available in fish collagen which are not localized but sporadically found in the molecule. Considering the results of Chapter II, it was suggested that the triple helix of type I collagen from *C. yaquinae* is extremely flexible. Additionally, ASC was extracted from this species and various experiments were conducted. The T_d and R_{pn} of ASC from *C. yaquinae* was lower than other species, and this value was negatively correlated with Ser content in ASC as shown in Chapter II. Additionally, a pressurization test and mechanical test of ASC were examined, and compared with other species.

Together with the data shown in Chapter II, these facts suggested that *C. yaquinae* ASC followed the structural and thermostability of cold-water fish which contains high Ser contents and low imino acids. The CD spectrum analysis under pressurized conditions suggested that the helical structure of ASC from *C. yaquinae* with higher Ser content was less affected by pressure than that of *G. chalcogrammus*.

The fibrous protein, collagen is typical extracellular protein. Unlike the localized active sites, fibrous proteins containing repeated sequence require extended regular amino acid repeats that impart stability and flexibility of the entire molecule. Therefore, it is likely that fibrous proteins, such as collagen, with different functions and structures, adapt to the deep sea environment using the distinct mechanism from globular proteins which has the hot spot for the substitution in the molecule.

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