主論文の要約

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学 位 論 文 題 目

Studies on the properties of sturgeon collagen as potential biomaterials

（チョウザメコラーゲンに関する研究 ― 生物材料への応用の可能性）

As one of the major proteins in animals, collagen accounts for approximately 30% of total proteins. In recent years, collagen has been widely used in many industries such as food and cosmetics. Additionally, because of its low antigenic activity, cell adhesion properties, biocompatibility and biodegradability, collagen-based biomaterials have been broadly applied in the field of medical research as well as tissue engineering. Till now, the main sources of industrial collagen are those from the skins and bones of porcine or bovine origin. However, outbreaks of zoonosis, such as bovine spongiform encephalopathy (BSE), have developed anxieties among users about collagen originated from land animals. Instead, fish collagen has received increasing attention as its advantages of low cost because of the high abundance of collagen-containing fish offal, as well as avoiding zoonosis and religious objections. Nevertheless, probably because it has a lower denaturation temperature (below 35℃) than mammalian collagen, there are only a few reports about the biomaterial utilization of fish collagen. Actually, collagen molecules self-assemble into fibrils *in vitro* when collagen solution is adjusted to an appropriate temperature, pH, and ionic strength. After fibril-formation, the low denaturation temperature of fish collagen in some species can be improved to more than 40℃, and therefore it is possible for utilization as a biomaterial for humans. Thus fish collagen is a promising source for biomaterials application. However, studies about the fibril-forming abilities and fibril properties (fibril morphology and denaturation temperature etc.) of fish collagens, which directly affect the processing characteristics and functionality of the biomaterials, are scarce.

Sturgeon is highly valued as a food fish, especially famous for its caviar. However, culture cost of sturgeon is higher than other fishes, as the required long time culture for obtaining caviar. Additionally, the lack of utilization of other fish parts also constrains the development of sturgeon

culture. The objective of this study is to extract and characterize the collagens from Bester sturgeon *Huso huso* × *Acipenser ruthenus* and Amur sturgeon *Acipenser schrenckii*, species possessing considerable economic and ecological value in China and Japan, with special reference for their biomaterial and tissue-engineering application. Moreover, another objective of this study is to reveal the primary structure of proα chains of type I collagen in Amur sturgeon, and discuss its predictable characteristics and functions.

Firstly, collagens purified from Bester sturgeon (0.76 m, 2.0 kg) organs were characterised biochemically, and their fibril-forming abilities and fibril morphologies formed *in vitro* were clarified. Yields of collagens were 0.08 g, 6.01 g, 0.69 g, 4.40 g, 0.11 g, 0.46 g, and 0.002 g (dry weight) from scales, skin, muscle, swim bladder, digestive tract, notochord and snout cartilage, respectively. Using SDS–PAGE and amino acid composition analyses, collagens from scales, skin, muscle, swim bladder and digestive tract were characterised as type I, and collagens from the notochord and snout cartilage as type II. All the collagens had glycine as the major amino acid, and were rich in alanine, proline and hydroxyproline. These data suggest that the type I collagens obtained from skin (SC) and swim bladder (SBC) are enough to be industrialized. In addition, notochord type II collagen (NC) is a valuable resource, since type II collagen in the market is scarce. Denaturation temperatures of the collagens, measured using circular dichroism (CD), were 29.6, 26.8, 29.0, 32.9, 31.6 and 36.3 ℃ in scales, skin, muscle, swim bladder, digestive tract, and notochord, respectively. For fibril formation, swim bladder and skin collagen showed a more rapid rate of increase in turbidity, a shorter time to attain the maximum turbidity, and formed thicker fibrils compared with porcine tendon type I collagen. Especially, the big fusiform fibril structures of swim bladder collagen suggest its special utility for enhancing mechanical strength of collagen-based biomaterials.

Similar to Bester sturgeon, from an Amur sturgeon (0.67 m, 1.22 kg), 6.0 g of SC, 4.1 g of SBC, and 0.4 g of NC could be purified. Using SDS-PAGE and amino acid composition analyses, SC and SBC were characterized as type I and NC as type II. All the collagens had glycine as the major amino acid, and were rich in alanine, proline and hydroxyproline. Denaturation temperatures of SC, SBC and NC were calculated as 28.5, 30.5 and 33.5 ℃, respectively. SC and SBC had higher fibril-forming ability compared with porcine type I collagen, as described in Bester sturgeon. Characteristic fusiform fibril structures of SBC were also observed. The major glycosides of Amur

sturgeon collagens were galactose and glucose, the same as those in mammalian collagens, and the contents were highest in NC, lowest in SBC, and SC in between. Such differences in glycosides contents were speculated to explain different fibril formation speed of Amur sturgeon collagens in part. Additionally, the morphology of SC and SBC fibrils formed *in vitro* was found to be similar to *in vivo*. The maximum transition temperature (Tm) of re-assembled fibrils formed in the buffer solution containing NaCl at 0 and 140 mM was 34.4 ℃ and 38.9 ℃ in SC, and 40.1 ℃ and 40.7 ℃ in SBC, respectively, suggesting the possible biomedical application to human beings, because of the Tms higher than human body temperature. These characteristic features suggest the potentials of sturgeon collagens application in the biomedical industries.

To characterize type I procollagen α1 and α2 chains in Amur sturgeon (Ascol1a1 and Ascol1a2) on the molecular level, cDNAs encoding both chains were cloned and sequenced. The ORF regions were 4371b coding 1457 amino acids in *ascol1a1* and 4071b coding 1357 amino acids in *ascol1a2*. The amino acid sequence of Ascol1a1 and Ascol1a2 showed 82% and 76% of homogeneity to goldfish, rainbow trout and zebrafish Col1a1 and Col1a2, 80% and 71% to cattle and human COL1A1 and COL1A2, respectively. On the other hand, Ascol1a1 showed only 54% of homogeneity to Ascol1a2. By phylogenetic analysis, a clear separation of Ascol1a1 and Ascol1a2 was suggested. Ascol1a1 and Ascol1a2 appeared in an initial position in monophyletic group with teleost fish in the phylogenetic tree reconstructed based on full deduced amino acids sequences by Minimum-Evolution method. For the primary structure, deduced amino acid sequence of Ascol1a1 and Ascol1a2 included signal peptide, N- and C-propeptides, telopeptides, and triple-helical domain consisting of uninterrupted Gly-X-Y triplets. Sequences expected to share basic functions of fibrillar collagen were conserved in Ascol1a1 and Ascol1a2; such as Lys-Gly-His-Arg and Lys-Gly-Leu-Arg (a substrate for cross linking of fibrils), Gly-Phe-Hyp-Gly-Glu-Arg (a collagen-specific, possible integrin-binding site), Arg-Gly-Asp (a possible cell-binding site) and the others were all well conserved. In amino acid compositions of Ascol1a1 and Ascol1a2, glycine was most abundant, proline and alanine followed, which was consistent with biochemical characterisation. On the other hand, difference in amino acid composition between Ascol1a1 and Ascol1a2 was found, which suggest the possibility of different amino acid composition of type I collagen having different composition of Ascol1a1 and Ascol1a2. Actually, gene expression of *ascol1a1* and *ascol1a2* was analyzed by quantitative real-time PCR, and organ-specific expression

ratio of *ascol1a1* and *ascol1a2* was found. Thus, the organ-specific amino acid compositions in type I collagens were suggested to be resulted from organ-specific composition of Ascol1a1 and Ascol1a2. This is in consistent with the result of biochemical analysis of amino acid composition. In addition, gene expression of *ascol1a2* is much higher than *ascol1a1* in swim bladder, which is different with the other organs. This may be related to the different biochemical character of swim bladder collagen such as fibril forming ability.

In conclusion, this study assessed the biochemical nature of collagens purified from Bester and Amur sturgeon, and clarified primary structure of proα chains of type I collagen in Amur sturgeon. Especially, for the purpose of collagen used in biomaterials, details of the fibril-forming ability and morphology of fibrils formed *in vitro* and *in vivo* were clarified. Sturgeon collagens have relatively higher thermal stabilities compared with other fish species. Moreover, the higher fibril-forming ability with unique higher-order structures and better thermal stability of type I collagen fibrils was found. These suggest high potential of sturgeon collagens applied in biomaterials. In addition, this study provides basic information about primary structure of sturgeon type I collagen, which is beneficial to promote the safe and effective utility of sturgeon collagen resource as well as to accumulate the knowledge of basic biology. As most studies on fish collagen deal with the biochemical nature for utilization as food, and there are only a few studies proposing its use as a biomaterial, this study gives the basic data which will help to open a new field of industrial use for fish collagen, and may increase the economic value of sturgeon to accelerate aquaculture development of this fish.