Characterization of Top Shell Muscle Collagen Comprising Three Identical $\alpha 1$ Chains

Shigeru KIMURA* and Hiroyuki TANAKA*

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The foot muscle collagen of top shell *Turbo cornutus* of the class Gastropoda was isolated by limited pepsin digestion of the muscular tissue, followed by selective salt precipitation at 0.40 M NaCl. Characterization of the isolated muscle collagen has suggested that it comprised three identical $\alpha 1$ chains and may correspond to Type I trimer of vertebrate collagens.

We have recently reported that the arm muscle collagen of octopus belonging to the class Cephalopoda was composed of two $\alpha 1$ chains and one $\alpha 2$ chain. Thus, the combined results demonstrated the interclass differences in chain composition of molluscan muscle collagens.

Molluscs, as well as fish, include a number of species important as seafood resources and their myofibrillar proteins have been extensively studied from the viewpoints of muscle contraction and food processing. On the other hand, little is known about the biochemical properties of collagen which is relatively abundant in molluscan muscles.¹⁻³⁾

We have recently reported the occurrence in octopus arm muscle of a protein homologous to Type I collagen with structure of $(\alpha 1)_2\alpha 2$.⁴⁾ This is in contrast with our previous finding that the foot muscle of abalone contains a large amount of collagen comprising only a single type of α chain.⁵⁾ In order to extend comparative studies of molluscan muscle collagen, the present paper deals with the isolation and characterization of collagen from a gastropod, top shell. As a result, we obtained evidence for the occurrence of top shell muscle collagen with the structure of $(\alpha 1)_3$.

Materials and Methods

Samples of foot muscles weighting 50 g were obtained from 9 individuals of the top shell *Turbo cornutus*. They were cut into small pieces and sequentially extracted with 0.5 M sodium acetate and 0.5 M acetic acid for 3 days; a significant amount of the foot muscle collagen was not brought into solution. Thus, the residual muscular tissues were subjected to limited proteolysis with pepsin (Sigma, 2 $\times$). Briefly, the muscular tissues were suspended in 0.5 M acetic acid to which 100 mg of pepsin was added and then in-

cubated for 2 days at 3°C while actively stirring. After centrifugation at 10,000 $\times g$ for 1 h, the solubilized collagen in the supernatant was precipitated by addition of 4 M NaCl to a final concentration of 0.40 M. The resulting precipitate was immersed overnight in 0.02 M Na_2HPO_4 and washed extensively in order to inactivate and remove the pepsin. The collagen precipitate was redissolved in 0.5 M acetic acid, centrifuged, and lyophilized. The yield of purified collagen was about 400 mg. For comparison, the arm muscle collagen and α chains from octopus *Octopus vulgaris* were prepared as described previously.⁴⁾

SDS-polyacrylamide gel electrophoresis, CM-cellulose chromatography, Sepharose CL-4B molecular sieve chromatography, CNBr-peptide mapping, and amino acid and hydroxylysine-linked carbohydrate analyses of the isolated foot muscle collagen and/or its α chain were performed essentially as described previously.⁶⁾

Results

The muscular tissue of top shell foot was estimated by its hydroxyproline assay to be extremely rich in collagen, which amounted to approximately 33% of the total tissue protein. The yield of purified collagen in the present experiment was about 15% of the total tissue collagen, though the majority of foot muscle collagen was solubilized by prolonged pepsin digestion. When examined by SDS-gel electrophoresis (Fig. 1), the pepsin-solubilized collagen was found to contain a single type of α component together with con-

* Laboratory of Biochemistry, Tokyo University of Fisheries, Konan-4, Minato, Tokyo 108, Japan (木村 茂・田中廣行: 東京水産大学生物化学研究室).

Fig. 1. SDS-polyacrylamide gel electrophoresis of molluscan muscle collagens on 3.5% gels containing 3.5 M urea. A: Top shell, B: Octopus.

siderable amounts of crosslinked components. This α chain was indistinguishable in its mobility from an $\alpha 1$ chain of octopus muscle collagen. CM-cellulose chromatography of the top shell muscle collagen (Fig. 2) and SDS-gel electrophoresis of its chromatographic fractions (Fig. 3) also confirmed the occurrence of only one type of α chain. In order to isolate the top shell $\alpha 1$ chain, the collagen preparation was dissolved in 0.05 M Tris-HCl, pH 7.2, containing 4 M urea and subjected to molecular sieve chromatography on Sepharose CL-4B. As shown in Fig. 4, isolation

Fig. 2. CM-cellulose chromatography of top shell muscle collagen. A column, 1.5×10 cm, was equilibrated with 0.01 M sodium acetate buffer, pH 4.8, containing 4 M urea and maintained at 30°C . Samples were eluted with a linear gradient of 0 to 0.11 M NaCl over a total volume of 800 ml at a flow rate of 120 ml/h.

Fig. 3. SDS-gel electrophoresis of several fractions recovered following CM-cellulose chromatography of top shell muscle collagen. Gels 1-4 are for the fractions shown in Fig. 2.

Fig. 4. Molecular sieve chromatography on Sepharose CL-4B of top shell muscle collagen. A column, 1.9×160 cm, was equilibrated and eluted with 0.05 M Tris-HCl, pH 7.5, containing 4 M urea at a flow rate of 11 ml/h at 27°C . The arrow indicates the elution position of calf skin $\alpha 1$ chain.

of the $\alpha 1$ chain corresponding to a molecular weight of 100,000 was successfully achieved.

The analytical results of amino acids and hydroxylysine-linked carbohydrate are listed in Table 1 which clearly indicates the identity, within experimental error, of the original muscle collagen with its constituent $\alpha 1$ chain. Moreover, the top shell $\alpha 1$ exhibited many of the compositional features previously observed for the octopus $\alpha 1$.^{4,6} A distinctive property of these molluscan $\alpha 1$ chains was a relatively high content of hydroxylysine and its glycoside.

Table 1. Amino acid composition of top shell muscle collagen (residues/1,000 residues)

	collagen	$\alpha 1$
4-Hyp	87	87
Asp	62	58
Thr	24	26
Ser	47	47
Glu	106	104
Pro	95	98
Gly	336	342
Ala	78	75
Val	16	18
Met	14	15
Ile	9	10
Leu	27	27
Tyr	8	6
Phe	6	6
Hyl	14	14
Lys	8	8
His	3	2
Arg	60	57
Glc-Gal-Hyl	7	8

Fig. 5. SDS-gel electrophoresis of CNBr-peptides derived from top shell muscle collagen and its α chain. Samples were digested with CNBr and the resulting peptides were resolved on 10% polyacrylamide gels in the absence of urea. The $\alpha 1$ chain of octopus muscle collagen was digested in parallel with the top shell collagen and its $\alpha 1$ chain. A: Top shell collagen, B: Top shell $\alpha 1$ chain, C: Octopus $\alpha 1$ chain.

The isolated $\alpha 1$ chain was then characterized with respect to its CNBr-peptide map. Fig. 5

suggests that the top shell $\alpha 1$ is very susceptible to the conditions of CNBr digestion, compared to the octopus $\alpha 1$, yielding a considerable number of fuzzy bands. However, it is possible to say that comparison of the top shell $\alpha 1$ with the octopus $\alpha 1$ revealed marked difference in peptide positions. On the other hand, the whole molecule of the top shell collagen was very similar in its CNBr-peptide map to its $\alpha 1$ chain (Fig. 5-A and B), confirming that top shell muscle collagen is made up of three identical $\alpha 1$ chains.

Discussion

Electrophoretic and chromatographic behaviours of the top shell muscle collagen have clearly demonstrated the occurrence of only one type of α chain. In addition, this α chain, $\alpha 1$, bears a close resemblance in amino acid composition and CNBr-peptide map to the original collagen. These composite results led us to the conclusion that the top shell muscle collagen comprised three identical $\alpha 1$ chains and may correspond to Type I trimer of vertebrate species.⁷⁾ This is consistent with our previous observation for abalone muscle collagen.⁸⁾ Thus, muscle collagens of the class Gastropoda may have a common structure of $(\alpha 1)_3$. These gastropod muscle collagens, however, are distinct from the arm muscle collagen from octopus of the class Cephalopoda; it is homologous to Type I collagen with a structure of $(\alpha 1)_2\alpha 2$.⁴⁾ On the other hand, it appears that one of the features of these molluscan collagens is their precipitation at acidic pH; they were selectively precipitated at 0.40–0.45 M NaCl, whereas vertebrate Type I collagen and Type I trimer collagen precipitated at about 0.90 M NaCl.⁹⁾

The present finding that the interclass difference in chain composition of collagens is observed for molluscan muscular tissues is of particular interest from the viewpoints of evolution and differentiation of muscle collagen, because such a difference has not yet been reported for vertebrate muscular tissues. Studies are now being carried out in our laboratory to examine the possible presence of a protein homologous to Type I collagen in other tissues of top shell.

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