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Isolation and characterization of collagen from rhizostomous jellyfish (*Rhopilema asamushi*)

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Abstract

As a part of the study into the potential development of unused and under-used resources, collagen was isolated from the mesogloea of the rhizostomous jellyfish, *Rhopilema asamushi*, by limited pepsin digestion and characterized. The yield of this collagen was high (35.2% on a dry weight basis). The primary structure was very similar to that of pepsin-solubilized collagen from edible jellyfish mesogloea, but it was different from those of the collagen from edible jellyfish exumbrella and the acid-soluble collagen from its mesogloea. The denaturation temperature (T_d) of 28.8°C. This collagen contained a large amount of a fourth subunit that was provisionally designated α4. This collagen may have the chain composition of an α1α2α3α4 heterotetramer.

Keywords: Rhizostomous jellyfish; *Rhopilema asamushi*; Mesogloea; Collagen; α Chain composition

1. Introduction

The industrial use of collagen has been expanded and now includes foods, cosmetics and biomedical materials (Nimni, 1988). Although there are many studies about collagen in marine vertebrates and invertebrates, the main sources of industrial collagen are limited to those from bovine and pig skins. Among marine invertebrates, some jellyfish are used in Chinese food owing to their unique textures. For example the rhizostomous jellyfish and the esculent jellyfish are used for food. Although the umbrella (mesogloea) of the jellyfish *Aurelia coerulea* was shown to contain a collagenous protein (Tanikawa, 1971), little is known about the chemical properties of jellyfish mesogloea in general. If a significant amount of collagen can be obtained from them, jellyfish could have potential as an important source of collagen. In the present paper, we describe the partial characterization of a collagen obtained by limited pepsin digestion from rhizostomous jellyfish mesogloea.

2. Materials and methods

2.1. Sample

Rhizostomous jellyfish, *Rhopilema asamushi*, was caught in Senzaki Bay, Nagato City, Yamaguchi Prefecture, Japan, cooled in ice and transported to the laboratory. The mesogloea was excised, washed with distilled water and extracted with 0.1M NaOH. The insoluble mesogloea was then lyophilized.

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2.2. Isolation of mesogloea collagen

The lyophilized mesogloea was suspended in 0.5M acetic acid and acid-soluble proteins were extracted with 0.5M acetic acid for 3 days. The insoluble matter was lyophilized.The insoluble mesogloea was suspended in 0.5M acetic acid and was digested with 10% (w/v) pepsin (EC 3.4.23.1; 2× crystallized, Sigma, USA) for 48 h at 4°C. The pepsin-solubilized collagen was centrifuged at 20 000×g for 1 h and the supernatant was dialyzed against 0.02M Na_2HPO_4 (pH 7.2) for 3 days. The resultant precipitate obtained by centrifugation at 20 000×g for 1 h, was dissolved in 0.5M acetic acid and was salted out by adding NaCl to a final concentration of 1.0M. The resultant precipitate was obtained by centrifugation at 20 000×g for 1 h and dissolved in 0.5 M acetic acid, dialyzed against 0.1 M acetic acid, and then lyophilized.

2.3. Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE)

SDS–PAGE was performed by the method of Weber and Osborn (1969). The collagen sample was dissolved in 0.02 M sodium phosphate (pH 7.2) containing 1% SDS and 3.5 M urea. Electrophoresis was performed on 3.5% gels in 0.1 M phosphate buffer (pH 7.2) containing 3.5 M urea and 0.1% SDS. After electrophoresis, protein bands were stained with 0.25% (w/v) Coomassie Brilliant Blue R250.

2.4. Peptide mapping

Two hundred micrograms of collagen sample were dissolved in 0.1 M sodium phosphate buffer (pH 7.2) containing 0.5% SDS. The sample was heated for 5 min at 100°C. The digestion was carried out for 30 min at 37°C by 5μg lysyl endopeptidase from Achromobacter lyticus (Wako Pure Chemicals, Japan). After adding SDS to a final concentration of 2%, proteolysis was stopped by boiling for 5 min. SDS–PAGE was performed by the method of Laemmli (1970) using 15% gels.

2.5. CM–Toyopearl 650M chromatography

A collagen sample was dissolved in 5 ml of 0.02 M sodium acetate buffer (pH 4.8) containing 6 M urea at 4°C and was denatured for 30 min at 45°C. The denatured collagen was applied to a CM-Toyopearl 650M column (1.0×6.0 cm; Tosoh Co., Japan) previously equilibrated with the same buffer. Elution was achieved with a linear gradient of 0–0.15 M NaCl in the same buffer at a flow rate of 0.65 ml/min. Absorbance at 230 nm was used to monitor the column chromatography.

2.6. Determination of denaturation temperature

Five ml of 0.03% collagen solution in 0.1 M acetic acid was used for viscosity measurements. Its measurement was done by using a Canon–Fenske type *viscometer* with an average shear gradient of 400 s^{-1}. The thermal determination curve was obtained by measuring solution viscosity at several temperatures from 10 to 50°C; the temperature was raised stepwise and maintained for 30 min. The denaturation temperature, T_d, was determined as the temperature at which the change in viscosity was half completed. Each point is the mean of triplicate determinations.

3. Results and discussion

3.1. Isolation of mesogloea collagen

The mesogloea collagen of rhizostomous jellyfish was easily solubilized by limited pepsin proteolysis.The pepsin-solubilized collagen was precipitated in 1.0 M NaCl at acid pH. Unfortunately, this collagen preparation could not be fractionated by salt precipitation at neutral pH. The yield of this collagen was 35.2% on the basis of the lyophilized dry weight. The value is high compared with other jellyfish (Miura & Kimura, 1985). The collagen sample obtained was examined by SDS-PAGE and was found to contain two different α chains, α1 and

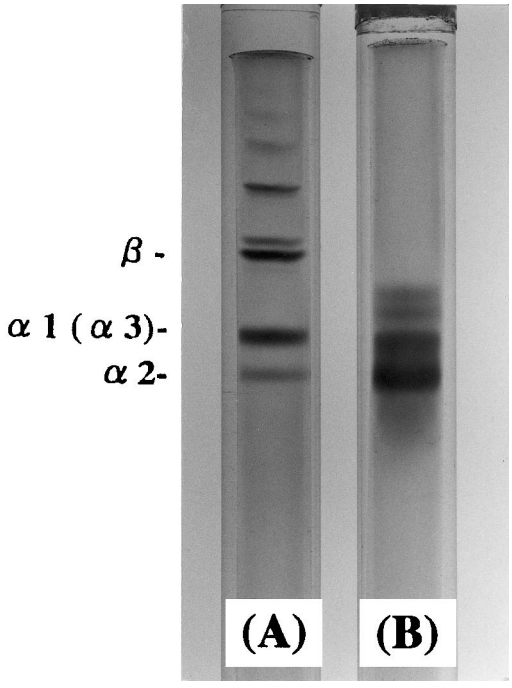

Fig. 1. SDS–polyacrylamide gel electrophoresis of porcine skin type I collagen and rhizostomous jellyfish mesogloea collagen on 3.5% gels containing 3.5 M urea. (A), porcine skin; (B), rhizostomous jellyfish mesogloea.

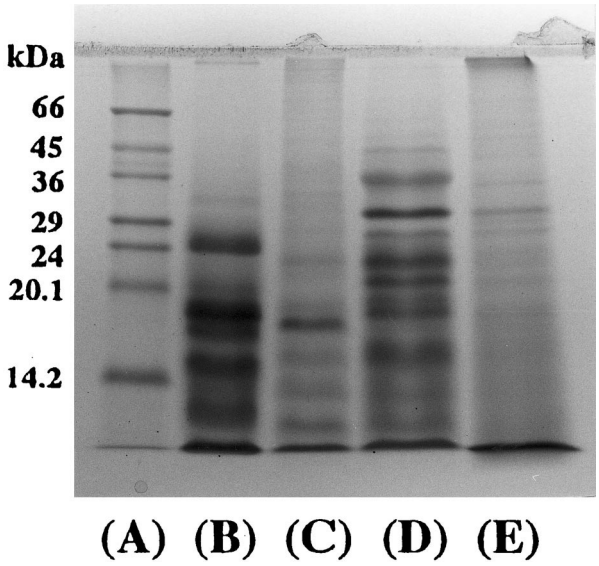

Fig 2. Peptide mapping of lysyl endopeptidase digests from rhizostomous jellyfish mesogloea collagens. (A), molecular weight marker; (B), edible jellyfish exumbrella pepsin-solubilized collagen; (C), edible jellyfish mesogloea acid-soluble collagen; (D), edible jellyfish mesogloea pepsin-solubilized collagen; (E), rhizostomous jellyfish mesogloea pepsin-solubilized collagen.

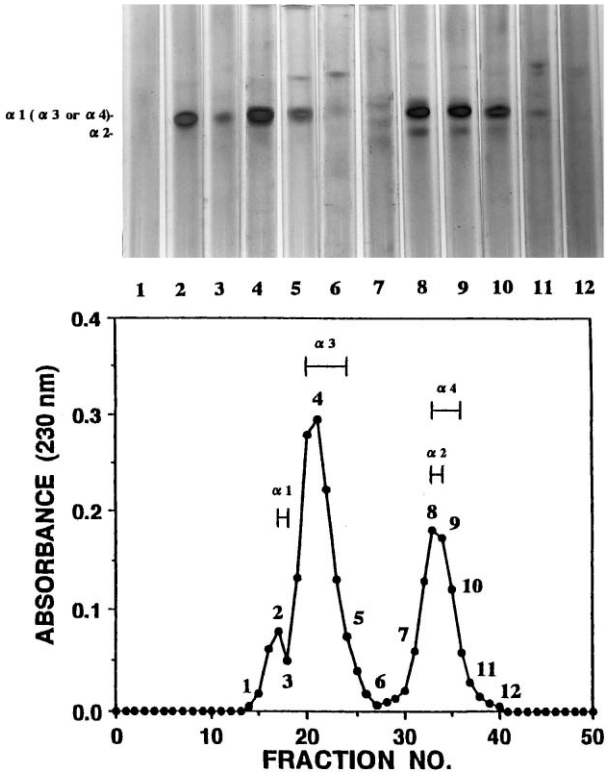

Fig. 3. CM–Toyopearl 650M column chromatography of denatured rhizostomous jellyfish mesogloea collagen. A 1.0×6.0 cm column of CM-Toyopearl 650M was equilibrated with 0.02 M sodium acetate buffer (pH 4.8) containing 6M urea, and maintained at 31°C. The soluble collagen (10 mg) was dissolved in 5 ml of the same buffer, denatured for 30 min at 45°C and then eluted from the column with a linear gradient of 0 to 0.15 M NaCl at a flow rate of 0.65 ml/min. The fractions indicated by the numbers were examined by SDS–PAGE.

α2 (Fig. 1). In addition, there were more than two additional components above the α1 band.

3.2. Peptide mapping

The denatured collagen samples, digested by lysyl endopeptidase were examined by SDS–PAGE, in order for the primary structure to be compared easily with those of other jellyfish samples. As a result, the electrophoretic pattern of rhizostomous jellyfish mesogloea pepsin-solubilized collagen was similar to that of edible jellyfish mesogloea pepsin-solubilized collagen, but was different from those of edible jellyfish exumbrella collagen (Nagai et al., 1999) and its mesogloea acid-soluble collagen (Fig. 2).

3.3. CM–Toyopearl 650M chromatography

The denatured collagen was examined by CM–Toyopearl 650M column chromatography. As shown in Fig. 3, it was separated into three fractions containing an α chain as a major component. This result suggests that this collagen consists of three α chains. That is, these chains were α1 (fraction numbers 2 and 3), α3 (fraction numbers 4 and 5) and α2 (fraction numbers 8 and 9) in the order of their elution positions (Piez, 1965). To confirm each chain, several fractions as indicated by the numbers were analyzed by SDS–PAGE. Interestingly, this collagen contained a large amount of a fourth subunit that was provisionally designated α4 (fraction numbers 8 to 11). Kimura, Ohno, Miyauchi and Uchida (1987) reported that, among some teleosts, only eel skin collagen was quite unique and its collagen contained a fourth subunit α4.

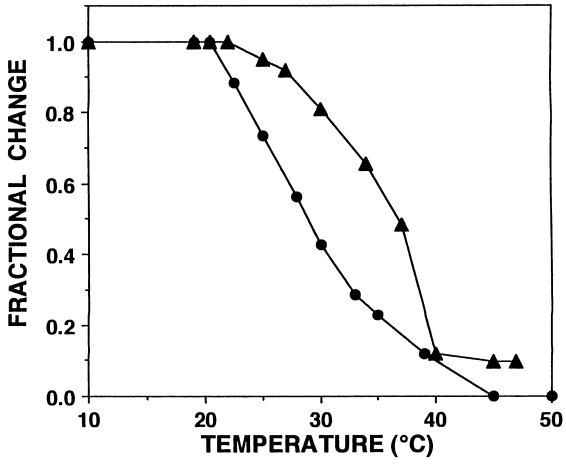

Fig. 4. Thermal denaturation curve of rhizostomous jellyfish mesogloea collagen solution as measured by viscosity in 0.1 M acetic acid. The incubation time at each temperature was 30 min. Collagen concentration: 0.03%; ●, rhizostomous jellyfish mesogloea collagen; ▲, porcine skin collagen.

3.4. Thermal stability of mesogloea collagen

The thermal denaturation curves are shown in Fig. 4. For comparison, the curve of porcine skin collagen is shown. The rhizostomous jellyfish mesogloea collagen had a denaturation temperature (T_d) of 28.8°C which was about 10°C lower than the Td of porcine skin collagen. In general, it is known that T_d of the inside portion (dark and ordinary muscles) is higher than the T_d of the skin in fish (Kimura, Zhu, Matsui, Shijoh & Takamizawa, 1988). From this finding, it would be expected that the T_d of rhizostomous jellyfish mesogloea collagen would be higher than that of the skins (such as exumbrella and subumbrella).

4. Conclusions

The collagen was extracted from rhizostomous jellyfish mesogloea by limited pepsin digestion and characterized. It was found that a large amount of collagen was obtained from rhizostomous jellyfish mesogloea, although the yield was lower than that from edible jellyfish exumbrella collagen (Nagai et al., 1999). The T_d of this collagen, however, was higher by about 2.8°C than that of edible jellyfish exumbrella (Nagai et al., 1999). If we can use these collagens properly according to their characteristics, the industrial use of jellyfish collagen could be extended in various fields.

The existence of an α4 chain was confirmed in rhizostomous jellyfish mesogloea pepsin-solubilized collagen. At present, with the exception of the report by Kimura et al. (1987), the existence of an α4 chain is unknown, to our knowledge. Moreover, we do not know the significance of the α4 chain developmentally.

It is planned to analyze the rhizostomous jellyfish mesogloea collagen in chemical detail and to compare some of its properties, particularly its subunit composition, with that of other jellyfish collagens, such as those from esculent jellyfish and hydrozoan jellyfish.

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