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Biochemical properties of acid-soluble collagens extracted from the skins of underutilised fishes

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Abstract

Acid-soluble collagens (ASCs) were extracted from the skins of several underutilised fishes, namely dusky spinefoot (*Siganus fuscescens*), sea chub (*Kyphosus bigibbus*), eagle ray (*Myliobatis tobijei*), red stingray (*Dasyatis akajei*) and yantai stingray (*Dasyatis laevigata*). The yields of the ASCs from skins of dusky spinefoot and sea chub were about 3.4–3.9%, and from ray species were about 5.3–5.7%, on a dry weight basis. According to the electrophoretic pattern, ASCs consisted of two different α-chains (α1 and α2) and were classified as type I collagen. However, the molecular weights of α2-chain for ray species were lower than those of bony fishes. ASC from ray species contained a higher content of imino acids than those from dusky spinefoot and sea chub. The denaturation temperatures (T_d) of ray species were about 33 °C, which was about 5 °C higher than those of dusky spinefoot and sea chub. The high T_d of ray species suggested the possibility of its utilisation as a substitute for mammalian collagen.

Keywords: Collagen; Underutilised fish; Ray; Skin; Denaturation temperature

1. Introduction

The collagens are a family of fibrous proteins found in all multicellular animals, and they represent about 25% of their total proteins and are the predominant protein of the extracellular matrix. They comprise the major structural element of all connective tissues and are also found in the interstitial tissue of virtually all organs, where they contribute to the stability of tissues and organs, and maintain their structural integrity (Gelse, Poschl & Aigner, 2003). So far, it has been established that even though the identified collagens have common characteristics, they include more than 20 different types, varying considerably in their complexity and diversity of their structure. Due to their unique chemical features, collagens have been utilised in various fields of industry, such as leathers and film, beauty and cosmetics, biomedical and pharmaceutical applications, materials and food (Kittiphattanabawon, Benjakul, Visessanguan, Nagai, & Tanaka, 2005; Morimura et al., 2002; Swatscheka, Schattona, Kellermannd, Muller, & Kreuterc, 2002). In addition, as the denatured collagens, most commonly known as gelatins, they also find important applications in the food and the biomedical industries. Generally, in the collagen industry, skins and bones of mammals, cattle and pig, etc., have been used as raw materials. Nevertheless, after the outbreaks of bovine spongiform encephalopathy and foot-and-mouth disease, it has been of great interest to find collagens from alternative sources, such as those that can be obtained from the aquatic environment (Senaratne, Park, & Kim, 2006).

The use of fish collagen, instead of mammalian collagen, has numerous rewards. During food processing, fish solids

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waste constitute 50–70% of the original raw material, depending on the processes used and types of products (Jongjareonrak, Benjakul, Visessanguan, Nagai, & Tanaka, 2005). Also fish collagen could be used by the Islamic and Hindu nations, which cannot use mammalian collagen, owing to religious constraints.

In Ariake Sound, fishes such as dusky spinefoot, sea chub and ray species have been considered of low economic importance because of their characteristic bad odour and taste, and unpleasant appearance. Furthermore, among those fishes, dusky spinefoot, sea chub and eagle ray, have recently increased their population, and as a result, have been regarded as the cause of whitening of seaweed (a phenomenon called "isoyake" in Japan) and feeding damage to valuable shellfish. For this reason, nowadays, great quantities of those fishes have been caught by Nagasaki prefecture; however, the cost of disposal or management has become a problem, and prompt solutions are required. There are few instances of the industry taking advantage of this important resource of fish collagen. With this and the above considerations in mind, we obtained and studied the acid-soluble collagen materials from underutilised fish, in an effort to evaluate their potential utilisation.

2. Materials and methods

2.1. Fish skin preparation

Dusky spinefoot (*Siganus fuscescens*), sea chub (*Kyphosus bigibbus*), eagle ray (*Myliobatis tobijei*), red stingray (*Dasyatis akajei*) and yantai stingray (*Dasyatis laevigata*) were caught in Ariake Sound, Nagasaki, Japan. The skins were removed, cut into small pieces (1×1 cm^2) with scissors, and kept at −30 °C until used.

2.2. Preparation of acid-soluble collagens

Collagens were extracted according to the method of Yoshinaka, Sato, and Ikeda (1976), with a slight modification. All procedures to prepare acid-soluble collagen (ASC) were carried out at 4 °C. The cleaned skins were extracted with 20 volumes (v/w) of 50 mM acetic acid for 3 days with stirring. The extract was centrifuged at 9000*g* for 1 h by high speed refrigerated centrifuge (KUBOTA 7930, KUBOTA Co., Tokyo, Japan), and the supernatant was salted out, by adding NaCl, to give a final concentration of 10%. The resulting precipitates were collected by centrifugation at 9000*g* for 1 h. The precipitate was dissolved in 20 volumes (v/w) of 50 mM acetic acid and then any insoluble material was removed by centrifugation at 9000*g* for 1 h. The supernatant was salted out by adding NaCl to 20%, and the resulting precipitates were separated by centrifugation as described above. The precipitate was dissolved in 5 volumes (v/w) of 50 mM acetic acid. After centrifuging at 9000*g* for 1 h, the supernatant was dialysed against 50 volumes (v/v) of 0.01 M Na_2HPO_4 for 3 days with a change of solution twice per day. The precipitate was obtained by centrifugation at 9000*g* for 1 h, and then lyophilised.

2.3. SDS-polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was performed by the method of Laemmli (1970), using 6% separating gel and 3% stacking gel. The samples, dissolved in 50 mM acetic acid, were mixed with the sample buffer (20 mM Tris–HCl buffer (pH 8.0) containing 8 M urea and 2% (w/v) SDS), using the sample/sample buffer ratio of 1:1 (v/v), and heated in boiling water for 5 min. The prepared samples (5–10 μg) were applied to each well and separated at 20 mA/gel. Type I collagen from tiger puffer (*Takifugu rubripes*) was used as a standard of α-chains and β-component of collagen mobilities (Mizuta, Fujisawa, Nishimoto, & Yoshinaka, 2005). After electrophoresis, protein bands were stained using the collagen staining method, as described by Fairbanks, Steck, and Wallach (1971). The gel was initially stained by soaking in 10% acetic acid containing 0.05% CBB R-250 and 25% 2-propanol overnight at room temperature. The gel was then stained in 10% acetic acid containing 0.005% CBB R-250 and 10% 2-propanol. After 6–9 h, the gel was soaked in 10% acetic acid containing 0.002% CBB R-250 overnight. Then the background of the gel was extensively destained with 10% acetic acid.

2.4. Amino acid composition

Twenty milligrams of the lyophilised samples of ASC were hydrolysed in 6 N HCl at 110 °C for 22 h under vacuum pressure. The hydrolysates, neutralised with 4 N and 0.1 N NaOH, were applied to an automated amino acid analyser (ALC 1000, Shimadzu Seisakusho Co. Ltd., Kyoto, Japan), and the amino acids were quantified with respect to the known standards. Cysteine (Cys) and methionine (Met) were analysed by the procedure of Moore (1963).

2.5. Determination of denaturation temperature

Collagen samples were prepared by the method of Kittiphattanabawon et al. (2005) with a slight modification. The freeze-dried collagen samples were rehydrated in 50 mM acetic acid solution with a sample and solution ratio of 1:40 (w/v). The mixture was allowed to stand for 2 days at 4 °C. Calorimetric measurements were performed by differential scanning calorimetry (DSC) (DSC 120, Seiko Instruments Inc., Tokyo, Japan). The instrument was calibrated for temperature and enthalpy using indium and tin as standards. The samples (5–10 mg) were weighed accurately into aluminum pans and sealed. The samples were scanned over the range of 20–50 °C with a heating rate of 1 °C/min using ice water as the cooling medium. An empty sealed pan was used as the reference. The denaturation temperature (T_d) was estimated from the thermogram. The change of enthalpy (ΔH) was determined from the peak area and expressed in mJ/mg sample material.