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ISOLATION OF TYPE I COLLAGEN FROM OUTER SKIN WASTE OF LITTLE SQUID (*LOLIOLUS UYII*)

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ABSTRACT

The acid and pepsin sloubilized collagen were isolated from little squid and characterized. Initial extraction of *Loliolus uyii* acetic acid yielded 10.54% of collagen, subsequent digestion of residue with pepsin 31.16, on a dry weight basis. While, the total protein of ASC and PSC little squid was determined by Bradford method which contains 64.27µg/ml, 18.93µg/ml respectively. The FT-IR spectrum showed that ASC and PSC are helpful in predication and confirmation of secondary structure of proteins. This report indicates that *Loliolus uyii* outer skin might be useful as a new source of collagen.

INTRODUCTION

Collagen is the major insoluble fibrous protein in the extra cellular matrix and in connective tissue as well as this constitutes approximately 30% of the total body protein. It has potential use in a wide range of applications in the pharmaceutical, cosmetic, biomedical, and food industries[1]. Collagen regarded as one of the most useful biomaterials, mainly due to its biocompatibility, non-toxicity, well-documented structural, physical, chemical, and immunological properties[2]. In principle, more than 10,000 types of triple-stranded collagen molecules could be assembled in the body from various combinations of the 25 but only 15 types of collagen molecules have been identified. Until now, type I- V and IX collagens present mainly in connective tissues. Type I collagen represents over 90 percent of our total collagen and is composed of three very long protein chains; it is also widespread all over the body.

In general, fish's muscle contains 0.2-2.2% collagen in the case of Teleosts and upto10% in elasmobranches[3] for association with swimming movement and meat texture[4]. In Particular, type I and V like collagens have been identified in fish's intramuscular connective tissue. Recent research has also shown about 70% of the squid skin dry matter is collagen[5]. In the present study, isolation and partial characterization of collagen from outer skin waste of little squid for to use little squid collagen as an alternative source of collagen from industrial purposes and subsequently it may increase the economical value of the little squid.

MATERIALS AND METHODS

Sample collection

Little squid were collected from Cuddalore landing centre and the animals were kept in to the laboratory at -20ºC. The outer skins were removed, cut into small pieces and stored at -4ºC until used.

Preparation of collagen from the outer skin

Outer skins treated with 0.1M NaOH to remove non-collagenous proteins for 3 d, then washed with distilled water and lyophilized. Lyophilized skins were treated with 0.5 M acetic acid for 3 d, and the extract was centrifuged at 5000 rpm for 1 h. The residue was re-extracted with the same solution for 2 d, and centrifuged under the same conditions. Then the acid-solubilized collagen (ASC) in the supernatant was salted out by adding NaCl to a final concentration 0.8M followed by precipitation of collagen by addition of 2.3M final concentration of NaCl (pH 7.5). The resultant precipitates was obtained by centrifugation at 5000 rpm for 1 h and dissolved in 0.5M acetic acid and dialyzed against 0.1M acetic acid, distilled water and then lyophilized.

After acetic acid extractions, the insoluble fraction was suspended in 0.5 M acetic acid and digested with 10% (w/v) (Sigma) pepsin at 4ºC for 48 h. The pepsin solubilized collagen was

centrifuged (PSC) at 5000 rpm for 1 h and the supernatant were dialyzed against 0.02M Na_2HPO_4 (pH 7.2) for 3 d, changing the solution once a day. The precipitates obtained by centrifugation at 5000 rpm for 1 h were dissolved in 0.5M acetic acid and salted out by adding 0.8 M final concentration of NaCl and followed by precipitation of collagen by further adding to final concentration of 2.3M NaCl in 0.05 M Tris-HCl (pH 7.5). The resultant precipitate was obtained by centrifugation at 5000 rpm for 1 h and dissolved in 0.5M acetic acid. Then dialyzed with 0.1M acetic acid and distilled water in the same way as for ASC preparation, and lyophilized.

Estimation of collagen protein

In little squid, the amount of collagen protein was estimated by Bradford method[6] with BSA used as standard.

SDS Polyacrylamide gel electrophoresis (SDS- PAGE)

SDS-PAGE was performed by following the slight modification method of Laemmli's, using the 1.5M Tris HCl buffer (pH6.8) containing 10% SDS and 11.14% 2-mercaptoethanol, 40% glycerol and 0.02% bromophenol blue with 10% separating gel and 4% stacking gel. After electrophoresis the gels were stained with Coomassie Brilliant Blue R-250 (Sigma, St. Louis, MO) and de-stained with methanol / acetic acid / water 5:2:5 (v/v/v). Gels were stained with 0.1% Coomassie Brilliant Blue R-250 dissolved in methanol, acetic acid, and water (5:2:5), and then de-stained in 15% methanol and 7.5% acetic acid.

Fourier Transform- Infra Red Spectrum Analysis

FT-IR absorption of ASC and PSC of little squid relied on at Bio red FT-IR – 40 model, USA. Sample (10 mg) was mixed with100 mg of KBr, and compared to prepare as a salt disc (10mm) diameter for reading spectrum further by using KBr for pelleted forms of samples. FT-IR spectrum of ASC and PSC was obtained and the effective peaked were assigned with that of standard collagen.

RESULT AND DISCUSSION

The present investigation is the first reporting of isolation and partial characterization of collagen from outer skin waste of little squid (*Loliolus uyii*). The yield of ASC was very low and its value about 2% or (10.54%) on the basis of lyophilized dry weight. On the other hand, PSC was perfectly solubilized from the residue from the acetic acid extraction and the yield of pepsin-solubilized collagen was very high, about 35% or (31.16%) on the basis of lyophilized dry weight. This result was higher to that of jelly fish (25.2 – 35.2 %), *Crassostrea gigas* (11.1%)[7], *Sepia lycidas* (34.5%)[8] and *Gadus morhua* (21.5%). This suggests that the squid outer skin was potential source of natural collagen.

Electrophoresis analysis

The little squid collagens were examined by SDS-PAGE using a 10% gel. The PSC was comprised only a single type α chain such as α1. But ASC was shown to comprise two types of α chain (α1, α2). It was found that these collagens had a chain composition of $(\alpha1)_2\alpha2$ heterotrimers, which was different to those of *Ommastrephes bartrami*[9], *Todarodes pacificus* skin[10]. Moreover, great amount of β chains, inter and intra molecular cross linked components were also found in squid skin collagen. However, the acid soluble collagen contain higher amount of protein (64.27µg/ml) than the pepsin solubilized collagen (18.93µg/ml) respectively.

FT-IR analysis

The presence of secondary protein structure was assigned with standard collagen spectral range. From the FT-IR results, absorption of ASC and PSC corresponding to amide split at 1654 cm^{-1} and 1653 cm^{-1} and in prominent absorption at 3441 cm^{-1} and 3368 cm^{-1} characteristic of collagens were identified. 1236 cm^{-1} and 1238 cm^{-1} range corresponds to the C-H stretching and N–H stretching. From the spectral data was helpful in predication and confirmed of secondary structure of proteins of isolated collagen from little squid.

CONCLUSION

In the present study, little squid outer skin waste was found to provide higher amount of type I collagen. In SDS-PAGE, indicated the present of this collagen in ASC and PSC and it was further confirmed by FT-IR test. Among, ASC and PSC α chains (α1 and α2) are present in same level but β chains present in ASC at higher level. Future work is required to evaluate mechanisms of action of extra active constituent.

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