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Research Article

ISOLATION AND CHARACTERIZATION OF ACID AND PEPSIN - SOLUBILISED COLLAGEN FROM THE MUSCLE OF MANTIS SHRIMP (*ORATOSQUILLA NEPA*)

H. M. PREETHA MINI JOSE[1], P. MURUGESAN[1]*, M.ARUMUGAM[1], K. MAHESH KUMAR[2]

[1]Centre of Advanced Study in Marine Biology, Faculty of Marine Sciences, Annamalai University, Parangipettai 608502, [2]Madras Christian College, Department of Marine Studies and Coastal Resource Management, Tambaram, Chennai 600073, Tamil Nadu, India.
Email: pmurugesaan74@gmail.com

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ABSTRACT

Introduction: Mantis shrimp (*Oratosquilla nepa*) muscle obtained from by-catch resources was used as an alternative source for mammalian collagen.

Objectives: The aim of the study was to isolate the Acid soluble collagen (ASC) and Pepsin soluble collagen (PSC) from body muscles of Mantis shrimp *Oratosquilla nepa*.

Methods: Body muscles of *O. nepa* the exoskeleton was removed, the muscles were cut into small pieces (0.3-0.5 cm) and stored at -4°C until used. *O. nepa*was extracted with 0.1M NaOH to remove non-collagenous protein for 3 days. Then, the deproteinised muscles was washed with distilled water and lyophilized for further analysis.

Results: The net yield of ASC and PSC was provisionally estimated as 42% and 23% respectively in dry weight basis. The molecular masses of the ASC and PSC subunits (α1, α2) were about 105 kDa and 96 kDa. According to the FT-IR spectrum, both ASC and PSC results showed the chemical shifts of amid regions were characterized as Type I collagen.

Conclusion:These finding shows the great potential of Mantis shrimp muscle collagen as a new source biomedical materials, food andnutraceutical industries.

Keywords: Mantis shrimp; Collagen; SDS-PAGE; FT-IR spectroscopy. Acid solublised collagen (ASC), Pepsin solublised collagen(PSC).

INTRODUCTION

Collagen is one of the long, fibrous structural proteins whose function is quite different from those of globular proteins such as enzymes. Collagen is distinct from other proteins wherein molecule comprises three polypeptide chains which form a unique triple helical structure. Collagen is abundant protein in animal tissues and constitutes approximately 30% of total body protein [1]. It has a wide range of applications in leather and film industries, pharmaceutical, cosmetics and biomedical materials, and food industry [2]. The natural collagen applicable for wound dressings, vitreous implants and carriers for drug delivery, edible casings [3].

Marine natural products have recently attracted industrial applications not only as a source of pharmaceutical products but also for their beneficial effects on human health. There is a tremendous increase in the number of marine substances and natural products derived from marine organisms finding commercial applications in industries every year. The main sources of collagen for industrial use have been limited to land-based animals, such as bovine or porcine skin and bone. However, the outbreak of bovine spongiform encephalopathy (BSE) and foot-and-mouth disease (FMD) crisis in recent decades have raised concerns among consumers over collagen and collagen-derived products of land-animal origin [4]. In addition, the collagen extracted from porcine is not suitable for use as a component in some foods due to social and cultural concerns. Therefore, alternative source of collagen should be explored. Researchers have found that the skin, bone, scale, fin and cartilage of freshwater and marine fishes proved to be the good source of the same. Apart from these, the type I collagen derived from the scallops mantle; Ascidian muscle layer and the pearl oyster adductor muscle were also good source of collagen [5,6,7].

Mantis shrimps are exploited in several parts of the world with the most extensive fisheries being for *Squilla mantis* in the Mediterranean, *Oratosquilla oratoria* in Japan and *Oratosquilla nepa* in India [8,9]. In India they are being only used for fishmeal, poultry feeds, and fertilisers and are also consumed as the meat which is reported to possess medicinal value[8].Queensland and Moreton Bay appears to be the major harvester of mantis shrimps.

The present paper deals with the isolation and characterization of acid and pepsin solubilised collagen from muscles of mantis shrimp (*Oratosquilla nepa*).

MATERIALS AND METHODS

Sample collection

The animals were collected from the landingsin Parangipettai (*Lat.*11° 29 'N; *Long.* 79° 46' E), south east coast of India. The collected animals were kept in ice box and stored in laboratory at -20°C. The exoskeleton was removed, the muscle were cut into small pieces (0.3-0.5 cm) and stored at -4°C until used.

Preparation of collagen sample

The muscle of *O. nepa*was extracted with 0.1M NaOH to remove non-collagenous protein for 3 days. Then, the deproteinised muscles was washed with distilled water and lyophilized for further analysis.

Isolation of Acid Soluble Collagen

The lyophilized muscle was treated with 0.5M acetic acid (1:3 w/v) for 3 days and centrifuged the extracts at 5000 rpm for 30 minutes at 4°C. The supernatant was collected and the residue was re-extracted with the same solution for 2 days and centrifuged under the same conditions. Each solution was mixed and salted out by adding NaCl to a final concentration of 2.3 M at neutral pH 7.5. The resultant precipitates was obtained and redissolved in 0.5 M acetic acid. Finally, the resultant solution was dialyzed with acetic acid and distilled water for 3 days and lyophilized. The dried matter was referred to as Acid soluble collagen (ASC).

Isolation of Pepsin Soluble Collagen

Insoluble residue obtained after acid extraction was used for the extraction of pepsin (Sigma, India) soluble collagen. Residue was thoroughly washed with distilled water and solubilized in 0.5 M acetic acid containing 0.1% (w/v) pepsin for three days with

continuous stirring. The mixture was centrifuged at 5000 rpm for 60 min at 4°C. Supernatant of the extract was salted-out by the addition of NaCl to give a final concentration of 2.3 M at neutral pH 7.5. Resulting precipitate was obtained by centrifugation at 5000 rpm for 30 min at 4°C. Then, the precipitate was dissolved in 0.5 M acetic acid and the resulting solution was dialyzed against 0.1 M acetic acid and distilled water for 3 days. The resulting dialysates was freeze-dried and considered as Pepsin soluble collagen (PSC).

Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

SDS-PAGE was performed on gradient separating gel of 10% polyacrylamide using a 4% stacking gel as described earlier by [10].The collagen sample was mixed with 1.5 M TrisHCl buffer (pH6.8) containing 10% SDS and 11.14% 2-mercaptoethanol, 40% glycerol and 0.02% bromo-phenol blue, heated at 100°C for 2 min and electrophoresed at 50 v in vertical slab gels. Samples of ASC, PSC, and standards were loaded on each well. Gels were stained with 0.1% Coomassie Brilliant Blue R-250 in methanol / acetic acid / water 5:2:5 v/v/v) and de-stained in 75% methanol, 25% acetic acid.

Fourier Transform – InfraRed Spectroscopy (FT-IR)

Amide band patterning of collagen sample was analyzed using FTIR (Bio-Rad, FTIR 40 model, USA) spectroscopy. About 0.5mg lyophilized collagen sample and 100mg potassium bromide (KBr) was ground together under drying condition. The spectrum was obtained with 32 scans per sample ranging from 4000 to 400 cm. The resulting spectral data was analyzed using ORIGIN 8.0 software (Thermo-Nicolet, USA).

RESULTS AND DISCUSSION

Isolation and yield of collagen from mantis shrimp muscles

Acid-solubilized collagen and Pepsin-solubilized collagen were isolated from *O. nepa* muscle with yields of 42% and 23% (dry weight basis) shown in Table 1, respectively. The *O. nepa* muscle was not completely solubilized by 0.5 M acetic acid extraction. On the other hand, while treating the residue with pepsin in 0.5 M acetic acid, PSC was completely solubilized. Figure 1 shows presents of ASC and PSC obtained were as fibril nature of whitish and grayish colour, which might be due to the ommochrome pigment, remained in the collagen sample. The yield of ASC was also higher than that of PSC on the dry weight basis**.**

The net yield of *Oratosquilla nepa* collagen was estimated as 65% on the dry weight basis which was higher than that of other sources like Jelly fish (25.2-35.2%) (11), Ocellate puffer fish (44.7%) [12]. The extractable yield of *O. nepa* muscles collagens was much higher than that of squid skin (52.6%) (13), brownstripe red snapper (13.7%) [14]and bigeye snapper (7.5%) [15]. The *O. nepa*muscle was not completely solubilised by 0.5 M acetic acid extraction. This result was in agreed with [4]; [15] who reported the incomplete solubilisation of bigeye snapper (*Priacanthus marcracanthus*) and brownstripe red snapper (*Lutjanus vitta*) skin in 0.5 M acetic acid.

Fig. 1: Lyophilized collagen of ASC and PSC

Table 1: ASC and PSC collagen yield obtained from *O. nepa*

Mantis shrimp species	Source	%ASC yield	%PSC yield
Oratosquilla nepa	Body muscle	42%	23%

Electrophoresis (SDS- PAGE)

Figure 2 shows Electrophoretic pattern of ASC and PSC showed that the two different α chains such as α1 and α2. Based on the mobility of the protein, both ASC and PSC had the similar electrophoretic pattern as that of type I collagen with two distinct α chains. The electrophoretic patterns of α chains and molecular weight of both ASC and PSC were consists of 105 kDa for α1 chain and 96 kDa for α2 chain, respectively. The band intensity of α1-chain was 2-fold higher than that of α2-chain are consist of both ASC and PSC. Moreover, considerable amount of inter and intra molecular cross linked compound β chains (Dimers) was obtained in ASC and PSC.

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) analysis confirmed that the isolated collagen *O. nepa* muscles as a type I collagen. Both ASC and PSC consisted of α1- and α2-chains at a ratio of approximately 2:1. High-molecular-weight components (High MW components), including β chain (dimmers) and γ chain (trimers) components, as well as their cross-linked molecules, were also observed in both fractions. Therefore, both ASC and PSC should most likely to be classified as type I collagen. Similarly, the electrophoretic patterns of type I collagen from the skin of brownstripe red snapper was reported [4], squid (*Ommastrephes bartrami*) skin collagen [13], and marine eel fish (*Evenchelys macrura*) [16]. High MW cross-linked molecules in collagen increase with animal age [15]and starving fish has more cross-linked collagen than those that are well fed [17].

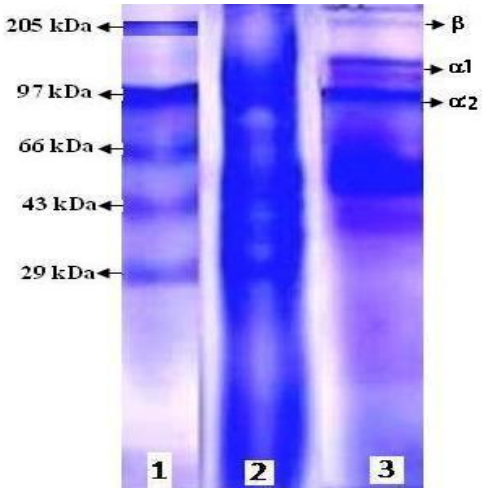

Fig. 2: SDS– Polyacrylamide gel electrophoresis of ASC and PSC from Mantis shrimp muscles non-reducing conditions. Lane 1: Standard Protein Marker; Lane 2: ASC, Lane 3: PSC.

FT-IR Analysis

Figure 3 shows FTIR spectra of ASC and PSC from the mantis shrimp muscles exhibited the characteristic peaks of Amide I, II, III as well as amide A and B bands are shown in Figure 2. The amide A and amide B position of ASC & PSC was found at 3400 & 3392 $cm^{-1}$and 2926 &2920 cm^{-1}, respectively. The sharp amide I, II, and III band of ASC & PSC was observed at 1645 & 1652 cm^{-1},1542 &1540 cm^{-1}, and 1245 &1242 cm^{-1}, respectively (Table 1). The spectral data was useful in predication and confirmation of secondary structure of collagen.

The FT-IR regions of amides I, II and III are known to be directly related with the nature of a polypeptide. The presence of Amide A band (3400-3440 cm^{-1}) is related to N-H stretching vibrations. Amide I band (1600-1660 cm^{-1}) is associated with stretching vibrations of carbonyl groups in peptides, being the most important factor for investigating the secondary structure of a protein. Amide II (~ 1550 cm^{-1}) is associated with NH bonding and CN stretching Amide III (1320 – 1220 cm^{-1}) is related to CN stretching and NH and it is involved with the triple helical structure of collagen. The present study revealed that the amide regions of A, B, I, II and III. The vibrational region of ASC was observed in amide A (3400 cm^{-1}), amide B (2926 cm^{-1}), amide I (1645 cm^{-1}) amide II (1542 cm^{-1}) and amide II (1245 cm^{-1}). The amide region of PSC was observed in amide A (3392 cm^{-1}), amide B (2920 cm^{-1}), amide I (1652 cm^{-1}), amide II (1540 cm^{-1}), and amide III (1242 cm^{-1}). The same amide band was observed in PSC of grass carp (*C. Idellus*) viz., Amide A (3,323 cm^{-1}), amide B (3,084cm^{-1}), Amide I (1,658cm^{-1}), Amide II (1,552cm^{-1}) and amide III (1,238cm^{-1}) [18]. This was similar to the other sources which include Whale (19), Shark [20], Nile Perch [1] and marine eel fish [16].Thus, both ASC and PSC showed a similar secondary structure of the protein. The presence of FT-IR signals were also confirmed that the isolated mantis shrimp collagen is Type I collagen nature.

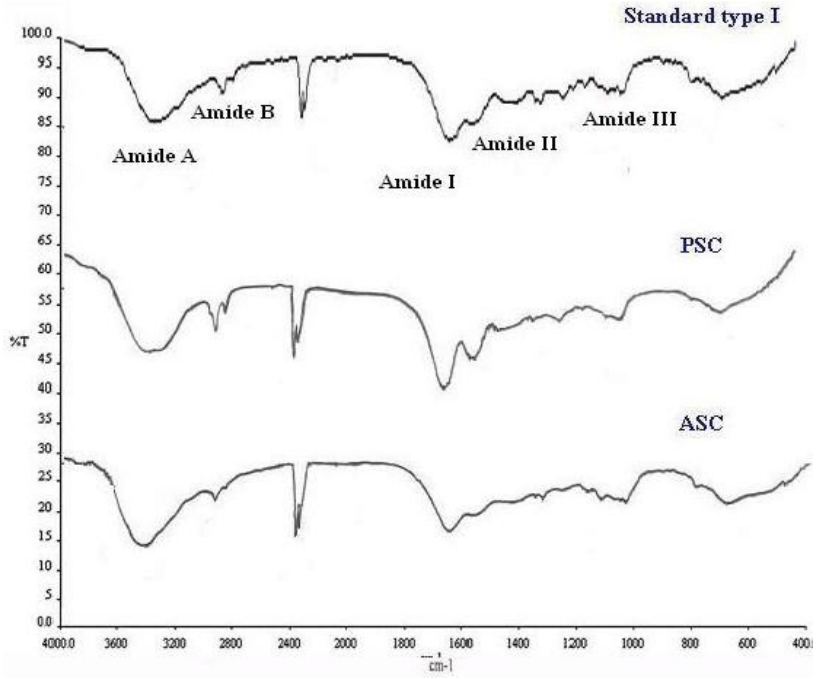

Fig. 3: Fourier transforms infrared spectra of standard type I collagen; acid soluble collagen (ASC) and pepsin soluble collagen (PSC) from the Mantis shrimp muscles

CONCLUSION

Collagen is the most abundant and unique protein in vertebrates, and has a wide range of applications in healthy food, cosmetics, biomaterial and pharmaceutical industries. Presently there are at least 19 variants of collagen. Type I collagen is found in all connective tissues, especially in mammals. Collagen from aquatic source has received much attention from many countries, although there are many reports available in skin and bone collagen from marine organisms. Therefore, the present result of *O. nepa* collagen was confirmed as type I collagen based on the results of SDS-PAGE and FT-IR analysis. Since, the mantis shrimp (Squilla) forms a major by catch resources in India and regularly utilized as feed ingredients in aquaculture industries, the new concept "wealth from the waste", could be used as a alternative source for biomedical applications and mammalian collagen especially type I.

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