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ISOLATION AND PURIFICATION OF COLLAGEN FROM MARINE SPONGE *DENDRILLA MEMBRANOSA*

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ABSTRACT

Collagen is a protein and is widely used in medical applications. Collagen plays an important role in the formation of tissues and organs, and is involved in various functional expressions of cells. Nowadays collagen is isolated from calf, porcine tissue etc. As an alternative, collagen can be isolated from marine sponge. The aim of the present work is to be isolated and partially characterized the collagen from the marine sponge, *Dendrilla membranosa*. The isolated collagen was estimated by using Braford assay and partially purified by using gel filtration on a Sepharose 4B column. These purified collagens were conformed by SDS PAGE. The isolated material was dispersed in the following solvents at room temperature to investigate their solvent action: acetone, methanol, ethanol 96%, ethyl acetate, double-distilled water, citric acid and hydrochloric acid 37%. Now a days commercially available collagen in the market are expensive and are not affordable to the common people. The present information on collagens from marine sponge *Dendrilla membranosa* easily isolated and could assist the future attempts to unravel the therapeutically important, safer collagens from marine sponges for their use in biomedical field.

KEYWORDS: Collagen, *Dendrilla membranosa*, SDS PAGE, gel filtration.

1. INTRODUCTION

Collagens are the predominant fibrous proteins in animals.[1] They constitute a major structural component in the extracellular matrix of all multicellular animals, including

sponges, invertebrates, and vertebrates. Collagen constitutes one to two percent of muscle tissue, and accounts for 6% of the weight of strong, tendinous muscles. The most abundant and well-characterized collagens are found in axially banded fibrils with a 670A0 repeat.[2] Collagen exhibits biodegradability and weak antigenicity.[3] Marine Sponges are the predominant source of collagen, a mass of cells formed of a porous skeleton made of collagen fibres and/or spongin[4] Apart from the secondary metabolites, tough bundles of collagen called natural collagen fibres have also been isolated from different marine sponges and with great applications in pharmaceutical technology, cosmetics and nutrition and a high potency in tissue regeneration, especially after injuries.[5] The importance of safer marine originated collagen and its applications are increasing day by day. Thus industrial use of bovine collagen is mandatory to avoid the risks of BSE (bovine spongiform encephalopathy) and TSE (transmissible spongiform encephalopathy).[5] The present research aimed to isolate and purify the collagen from the marine sponge, *Dendrilla membranosa*.

2. MATERIALS AND METHODS

2.2 Collection of sponge

Sponges were carefully collected from the waters of Vizhigam, Kerala, India. The sponges were gently removed from the substratum and placed in plastic bags underwater, then transferred into large containers of aerated seawater for transport to the laboratory for species identification.

2.2 Bradford Protein Assay Procedure

Prepare six standard solutions (1 mL each) containing 0, 250, 500, 1000, 1500 and 2000 µg/mL BSA. Set the spectrophotometer to collect the spectra over a wavelength range from 400 to 700 nm and over an absorbance range of 0 to 2 Absorbance units, and to overlay the collected spectra. Use a 4 mL plastic cuvette filled with distilled water to blank the spectrophotometer over this wavelength range. Empty the plastic cuvette into a test tube and shake out any remaining liquid. Then add; 2.0 mL Assay reagent 0.04 mL of protein standard solution, starting with the lowest protein concentration and working up, or one of the samples to be assayed. Cover with parafilm and gently invert several times to mix. Record the absorbance spectrum of the sample from 400 to 700 nm, and note the absorbance at 595 nm. Repeat the steps above for each of the protein standards and for the samples to be assayed. Examine the spectra of the standards and samples.

2.3 Isolation of sponge collagen

Collagen was isolated by following the method of Diehl-Seifer et al. (1985).[6] The methanol-conserved sponge materials were washed three times with tap water and distilled water, finally cut into small pieces and homogenized using homogenizer (Remi, RQ-127A, India). 250 ml of 100 mM Tris–HCl buffer (pH 9.5; 10 mM EDTA; 8 M urea; 100 mM β-mercaptoethanol) were added. The pH of the resulting dark-coloured dispersion was raised from pH 7 to 9 with dilute NaOH. After 24 h of continuous stirring at room temperature, the viscous extract was centrifuged (5000 × *g* for 5 min at 2°C) (Remi C- 24 BL, India). The pellet was discarded and collagen was precipitated from the supernatant by adjusting the pH to 4 with glacial CH3COOH and collected by centrifugation (20,000 × *g*, for 30 min at 2°C). The pellet was resuspended in distilled water, centrifuged (20,000 × g, for 30 min at 2°C) and freeze-dried.

2.4 Purification of crude collagen

The collagen was purified with little modification of the protocol followed by Saravanan et al. (2009),[7] through Sephadex G-50 column chromatography.

2.5 Electrophoresis (SDS-PAGE)

The purity of this collagen preparation was assessed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) by the Laemmli,[8] system with an 10% resolving gel. The gel was allowed to set for 45 min. Distilled water was pipetted over the gel to prevent oxygen inhibition of the polymerisation reaction. The 4% stacking gel was then prepared on top of the resolving gel. The comb was placed carefully to prevent formation of air bubbles. This gel was allowed to set for 30 min. Samples were diluted prior to electrophoresis using sample buffer to give a final protein concentration of 1 µg/ml. Samples were boiled at 100˚C for 5 min. Gels were run at 25 mA per gel for 45 min. On completion of electrophoresis, gels were fixed and stained with Coomassie Blue R-250 for 1 h. The gel was then transferred to destained solution and subsequently photographed.

2.6 Solubility of sponge collagen

The isolated material was dispersed in the following solvents at room temperature to investigate their solvent action: acetone, methanol, ethanol 96%, ethyl acetate, double-distilled water, citric acid and hydrochloric acid 37%.

3. RESULTS

3.1 Species Identification

Species was identified as *Dendrilla membranosa* (class: demospongiae; order: dendroceratida, family: darwinellidae) at Vizhinjam Research Centre of Central Marine Fisheries Research Institute (ICAR), Vizhinjam, Thiruvananthapuram, India.

3.2 Bradford assay

The concentration of protein estimate by using Bradford method. Pippete aliquots of standard solution of protein 0,0.2,0.4,0.6,0.8,1.0ml an transferred in numbered test tubes. After the Bradford assay, read the absorbance of blue colour at 595nm and determine result with the help of standard curve (table 1).

Table 1: Concentration of a protein estimate by Bradford assay.

Sample	OD at 595 nm	Concentration of a protein In mg/g of sample
Dendrilla membranosa extract	1.248	1.123

3.3 Isolation and purification of sponge collagen

Collagens were isolated and facilitated there lease of intertwined insoluble collagen like filaments from the sponge matrix. In addition, 6 Murea has been used to retain non filamentous collagens along with the soluble and insoluble collagens in the subsequent extraction and purification procedures.

The collagen was purified through Sephadex G-50 column chromatography. The column (1.5x50 cm) was eluted with 0.1, 0.2, 0.4 and 0.6 M phosphate buffer saline (pH 7.4) with a flow rate of 0.33 ml/min and the fractions were collected. The active fractions were pooled and loaded in a dialysis membrane and dialyzed against double distilled water at 4°C for 12 h and freeze-dried.

3.3 Electrophoresis (SDS-PAGE) of purified collagens

The purified collagens from *D.membranosa*, when electrophoresed, showed specific band above 80 kDa. (Figure 1). In the present study, the isolated crude collagen showed three bands with 40, 53 and 59 kDa molecular weight; whereas the purified collagen recorded only a single band with 80 kDa molecular weight in SDS-PAGE.

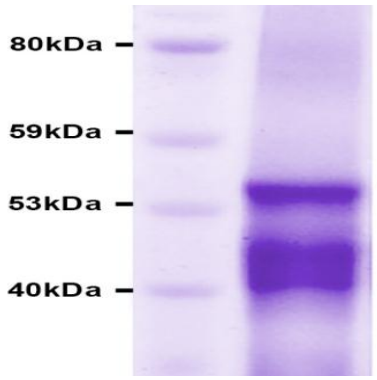

Figure 1: Electrophoresis (SDS-PAGE) of purified collagens.

3.4 Solubility of sponge collagen

The isolated material was dispersed in the following solvents: acetone, methanol, ethanol 96%, ethyl acetate, double-distilled water, citric acid and hydrochloric acid 37%. Acetic acids showed high solubility compared to other solvents.

4. DISCUSSIONS

The existence of collagen in marine as well as freshwater sponges was first proved electron microscopically in 1980s.[9] Garrone et al.[9] Investigate the fine structure and physicochemical properties of collagen of the marine sponge *Dendrilla membranosa*. Collagen arrangements and rearrangements play a major role in the adhesion and tissue growth in animals.[10] Although the presence of sponge collagen has been histologically proved, the characteristic insolubility and mineralization causes methodological problems while isolating and studying the collagen from the collected sponges.[11] In this studies, collagen was isolated and partially characterized from the marine sponge, *Dendrilla membranosa*. The isolated collagen was estimated by using Braford assay and partially purified by using gel filtration on a Sepharose 4B column. These purified collagens were conformed by SDS PAGE. The isolated material was dispersed in the following solvents at room temperature to investigate their solvent action: acetone, methanol, ethanol 96%, ethyl acetate, double-distilled water, citric acid and hydrochloric acid 37%. Marine demosponges were chosen for the present study because of their potentiality of producing novel collagens towards various medicinal and cosmeceutical applications.[5] The presence of immunocompatible collagens in marine sponges could form a basis for the followers to understand the form and type of the collagens so that specific genes involved in the production of safer collagens can be targeted.

5. CONCLUSION

Collagen has various advantages as a biomaterial and is widely used as carrier systems for delivery of drug, protein and gene. Because of its superior biocompatibility and low immunogenicity, collagen is still the protein of choice for biomaterials preparation. In this study, could ascertain the benefits of sponge collagen to mankind in the future course of the study.

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