J. of Applied Tissue Engineering
December 2014; 1(1): 18-21

Isolation and Purification of Collagen from the Skin of Black Pomfret (Parastromateus Niger) for Tissue Engineering Purpose

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Received: 13 December 2013/ Accepted: 15 February 2014

Abstract:

Collagen is one of the main biomaterials with tissue engineering application. It plays an important role in designing and providing scaffold to support the interactions between cells and scaffolds aiming for high attachment, proliferation and differentiation. Currently research proceed to extraction collagen from the skin Black pomfret (*Parastromateusniger*), one of the most important and economical species of the Oman sea and Persian gulf. Collagen is the most abundant protein in vertebrates; have a biomedical matters, pharmacutical, tissue engineering, industrial and biotechnological application. Samples taken from the fishery market and carried out to laboratory. Acid soluble collagen (ASC) isolated by acidic methods at the 4℃ for 5 day. Then, collagen precipitates with NaCl and purification to 0.1M acetic acid solution and distilled water. The results showed that collagen this fish is highest value of 13.6% on a dry weight basis . The SDS-PAGE pattern show the ASC producted from this species have hetrotrimer structure and made of two chains; $\alpha_1$and α_2 . So collagen provides is considered type I collagen. Moreover UV-spectra exhibited this collagen have a absorbance 220nm. Therefore black pomfret fish can be use for alternative collagen source. It may also be subsequently increase the commercial value of fish.

Key Words: black pomfret, skin, Collagen, Biomaterial

Introduction

The collagens are a family of fibrous proteins found in all multicellular animals, 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 such as skins, bones, cartilage, tendons, ligaments, blood vessels, teeth, cornea, and all other organs of vertebrates [1]. 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 [2]. Recently, due to extension mad cow disease and Limitations associated with the extraction collagen from mammals, scientist is looking suitable replace for safe collagen production. They introduced whole fish and their byproduct Due to lack of Risk disease transmission and pathogenesis, easy availability, Lack of religious limited and high collagen content as safe collagen source. The black pomfret, Parastromateusniger (Bloch, 1795), belong to the family of Carangidae, exist in the Oman Sea and Persian gulf. These species are midwater pelagic that occur over muddy bottoms in coastal areas and distributed in the Indian Ocean to the western Pacific Ocean, All black pomfrets are wild-caught, usually by trawling or gillnetting [3,4]. It was the most important commercial fishes the southern coast of Iran. The annual catch this fish is about 3,000 tons [5].

Therefore with caught many of these fish can be hoped to extraction economically collagen from this fish. The aim of

this study is isolation and purification acid soluble collagen from black pomfretfish.

Materials and methods

Materials

fresh skin black pomfret fish (*Parastromateusniger*) purchased from the fishery market, then carried to laboratory and used within one month after death. First in the laboratory washed samples with topic water then cutting the scale by scissor minced to small pieces (0.5 × 0.5 cm). Then washed with cold distilled water. After mixture skin samples were packed in polyethylene bags and kept at -20 ℃ until used.

Chemicals

B- mercaptoethanol (BME), pepsin from porcine gastric mucosa (Ec 3.4.23.1) powdered; 0.7 FIP/mg dry matter, acetic acid, Bothyle alcohol (Butanol), tris (Hydroxymethyl) aminomethane, sodium dodecyl sulfate (SDS), coomassie Brilliant blue R-250, all these materials obtained from Merck and sigma Aldrich company.

Alkali-extraction of non-collagenous proteins

All operation were performed by Nagai and Suzuki methods [6], at 4℃ with a slight modification. First fish samples were washed with distilled water for once more time. Then the sample was added 10 volume(v/w) of 0.1M NaOH. The suspension was stirred overnight for 2 days. The final precipitation was washed thoroughly with distilled water until neutral pH of wash water was obtained. the solution was changed every 8 h.

Removal fat from tissue

Fat was removed in 10% (v/v) Butyl alcohol solution in ratio of 1:10 (w/v) for 24 h with a gentle stirring and changed solution every 8 h. Defatted tissues was thoroughly washed with cold distilled water.

Preparation of acid soluble collagen (ASC) from pomfret fish skin

To extract the collagen, the prepared tissues was soaked in 0.5M acetic acid with a sample: solution ratio of 1:15 (w/v) for continuously 3 days with gentle stirring using shaker device model (GFL, 3005). The mixture was filtered through two layers of cheese cloth. The supernatant was collected and kept at 4℃. The residue was re-extracted in the same manner. Both supernatant obtained were combined and added with NaCl to obtain a concentration of 0.9M. This solution stayed for 30 minutes. Then added with NaCl to obtain a final concentration of 2.6M in 0.05 M tris(hydroxymethylaminomethane), pH 7.0. The resultant precipitate was collected by centrifuging at 10,000g for 20 min, using a refrigerated centrifuge (Sigma, 3-30K). The pellet was dissolved in some volumes of 0.5M acetic acid. The solution obtained was dialyzed against 10 volumes of 0.1M acetic acid in a dialysis bag(dialysis tubing, D116, D117) for 24h, with a change of dialysis solution every 2h, subsequently, the solution was dialyzed with some volumes of distilled water. The changes of dialysis water were performed until neutral pH was obtained. The dialyzate was freeze-dried and referred to as acid-solubilized collagen (ASC).

Sodium dodecylsulfate-polyacrylamide gel electrophoresis

SDS - polyacrylamide gel electrophoresis was performed by the method of laemmli (1970) [7], using 7.5% gel containing with 10% SDS at pH8.8. protein samples containing 50 µl dialysis collagen,10 µl SDS10% and 3 µl 2- mercaptoethanol were heated in boiling water for 5–10 minutes. then added to that 50 µl glycerol 20% and bromophenolblue 0.005%.the gel was stained for protein with coomassie brilliant blue R-250.

UV absorption spectrum

The UV-Vis absorption spectrum of ASC collagen was performed using a Shimadzu spectrophotometer in the range wavelengths of 220 - 350 nm. purified collagen dissolved in 0.5 M acetic acid were to reach 0.5 mg / ml concentration then 200 µl from concentrate solution dissolved in 800 µl of 0.5 M acetic acid. Then the value of the homogenized solution was placed into a quartz cell to determine the absorption wavelength.

Result and Discussion

Collagen

Skin tissue collagen was prepared from black pomfret (Parastromateusniger) fish. Collagen of these fishes was not completely solubilized with 0.5M acetic acid for 72h. So the residues were re-extracted with the same solution for a further 2 days. All residues were then solubilized and highly viscous solutions were obtained. The collagen was precipitated by the addition of NaCl to a final concentration of 0.9M in 0.5M acetic acid and of 2.6M in 0.05 M tris(hydroxymethyl) aminomethane, pH 7.0. The yields of these collagens were in on the basis of lyophilized dry weight, respectively. Different tissues collagen is generally difficult to purify due to the presence of non-collagenous proteins. But preliminary treatment with alkali solution is shown to remove most of the non-collagenous proteins without the effect on collagen. For skin tissue, the extractability of acid-soluble collagen in black pomfret fish was 13.6% on a dry weight basis. This is quite similar from some marine fishes. Yield of collagen black pomfret was higher than the collagen of other marine fish bones, muscle, scale and invertebrate. For example, muscle collagen Salmogairdnerii, 0.47 %; Scomber japonicas, 0.50%; Cyprinuscarpio, 0.60% [8]. Bone and scale Cyprinuscarpio, 1.06%, 1.35% respectively [9]. Some of aquatic invertebrate example ASC Neritacrepidularia is 1.78% [10]. On the contrary, acid solubilized collagen obtain from black pomfret fish was low relation to extracted collagen from marine vertebrate such as mink whale skin, marine and fresh water fish skin. This indicated

that the skin tissue addition of black pomfret can be a use source of collagen.

Electrophoresis

The acid–soluble collagen from the skin tissue of black pomfret fishes were examined by SDS-PAGE using a 7.5 % resolving gel (fig.1). This showed that ASC existed as trimers consisting of two distinct α chains (α1 + α2). There were different positions in mobility in the α region (lan3,4 and lan5). The electrophoretic mobility position of α1 was different from that of α2 position and α2 chain move more space. This demonstrated that the molecular weight of the α2 was smaller than the α1. Based on electrophoretic mobility and subunit composition, it was suggested that collagens from skin tissue black pomfret fish were type I collagens and were composed of two α1 and one α2 chains.

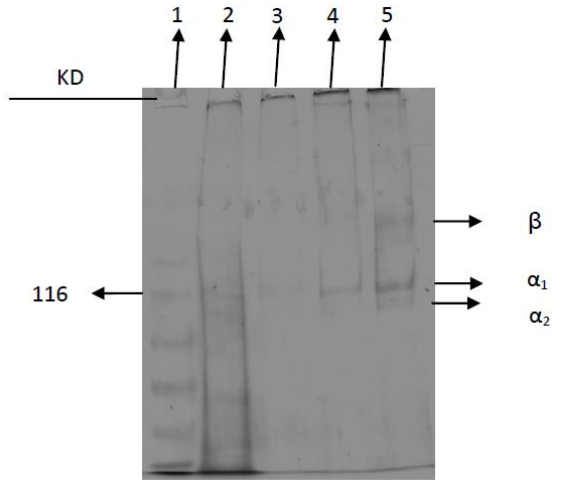

Figure 1 : SDS-PAGE patterns of acid-soluble collagen from Black pomfret skin,1- Marker protein; 2- Gelatin; 3,4,5- ASC

The results were in agreement with the previous report; Senaratne et al.,2006; Zhang et al.,2009; Yan et al.,2008; Wang et al.,2007; Duan et al.,2009; Jongjareonrak et al.,2005; Ogawa et al.,2004; Singh et al.,2010 [9,11,12,13,14,15,16,17]. A great amount of β chain can be observed in the pattern of all these collagen(lan5).

UV-Vis Spectra

Absorption wavelength of the black pomfret fish collagen was observed At 220 nm (Fig. 2), closer to the absorption of other fish collagen such as Nile tilapia and walleye pollock skin collagen [13,18,]. Most proteins have a maximum ultraviolet absorption at 280 nm. Phenylalanine, tryptophane and tyrosine have absorption bands be- tween 250 and 290 nm[13,19]. While the absorption wavelength this collagen was less, Which may be due to the groups C=O, -COOH, CONH2 in polypeptides chains of collagen[18].Thus that is consistent with the characteristics of a collagen.

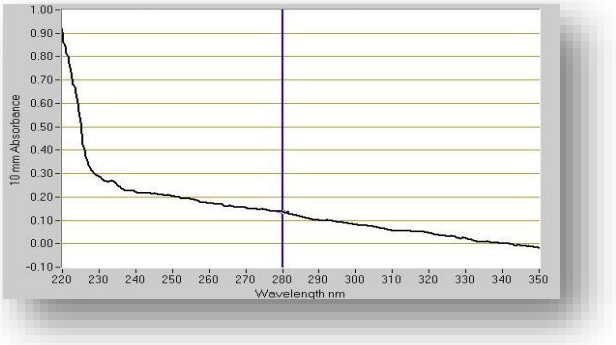

Figure 2 : UV- Spectra of acid soluble collagen from black pomfret skin

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