# Extraction, Identification and Characterization by Sds-Page Method of Collagen Extracted from *Rhizostoma Pulmo*, a Jellyfish Found in the Black Sea Basin

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## Abstract

Collagen is one of the proteins that plays an important role in many biomedical and cosmetic applications. The present study investigates collagen extracted from the jellyfish Rhizostoma pulmo, a species native to the Black Sea basin. The method of extracting collagen from the marine organism is described. The SDS-PAGE (Sodium Dodecyl Sulfate - Polyacrylamide Gel Electrophoresis) method, an efficient technique for the separation and analysis of proteins according to molecular weight, was used to identify and characterize the protein structure of the extracted collagen. The collagen samples obtained were purified and subjected to electrophoresis, demonstrating a specific band corresponding to type I collagen, the main type of collagen found in marine organisms. The results show a protein structure similar to that of collagen isolated from other marine sources, indicating the high potential of Rhizostoma pulmo as a source of collagen for industrial applications. This research contributes to the development of sustainable methods for the valorization of marine resources from the Black Sea basin in fields such as regenerative, tissue, dermato-cosmetic, nutraceutical and even food medicine.

Keywords: collagen, marine collagen, *Rhizostoma pulmo*, SDS-PAGE, collagen identification

## Introduction

Jellyfish are emerging as a source of collagen from marine invertebrates, which are particularly attractive due to their high proportion of collagen relative to insoluble extracts, lack of calcified tissues, and morphological and functional similarity to

mammalian fibrillar collagen [1]. Collagens can be fibrillar or non-fibrillar depending on their supramolecular structure [2]. The major fibrillar collagens are types I-III, they are found predominantly in mammals and are the main types used in biomedical, cosmetic and nutraceutical applications [3, 4]. Jellyfish collagen has also been used in 2D and 3D *in vitro* models of mesenchymal stem cells, induced pluripotent stem cells, chondrocytes, chondroprogenitors, osteoblasts, fibroblasts and ovarian cancer cell lines [5-7]. Jellyfish have a low proportion of genetic homogeneity between species [8].

As a result of this genetic variation it is unlikely that the peptide sequences of collagens from different jellyfish are identical, and separate characterizations of the collagens derived from each species are required before biomedical applications can be established and exploited for each individual jellyfish species. In addition to the wide range of bioactive peptides and other marine biomolecules that have been reported, marine invertebrate collagen is increasingly being recognized as an alternative to mammalian collagen [9-11].

Collagen analysis is relevant to a number of domains such as tissue engineering, regenerative medicine, drug development and delivery as well as biomechanics. There are currently several methods for identifying and quantifying collagen, most of which lack sensitivity, specificity or full cost- efficiency.

State-of-the-art high-throughput assays can allow highly sensitive parallel processing of multiple samples in a short time with individual subtype specificity [12]. These methods include traditional and antibody-based assays, imaging, mass spectrometry and state-of-the-art proteomic approaches [12].

**Materials and methods**

Materials for collagen extraction. Samples taken in the work are from the organism of the jellyfish *Rhizostoma pulmo*, and the reagents used are 0.5 M acetic acid solution, 0.5 M NaCl solid salt, 0.5 M EDTA, distilled water. The material used for the extraction was a jellyfish from the Black Sea basin with a size of about 35 cm in length and a weight of 1200 g wet tissue. It was collected from the Romanian Black Sea coast.

Materials for the analysis by sodium dodecyl sulfate polyacrylamide gel electrophoresis or SDS-PAGE. The reagents used are: distilled water, Bradford reagent, bovine standard albumin solution 2 mg/ml (BSA), acrylamide bis 40%, SDS 10%, TrisHCl ph 6.8 and 8.8, migration buffer. Materials: powder-free nitrile gloves, 10 and 500 µl pipettes, pipette tips, eppendorf tubes, glass plates, gel comb. Migration tank, electric field generating unit. Sample buffer solution was prepared as follows: 10 mL/4X. 1.5 M TrisHCl, pH = 8.8 15 mM, 10% SDS, 0.5 M EDTA, 7.5% sucrose, 7.5% sucrose, 0 β - mercaptoethanol 0.5%, 1% bromphenol blue were used.

## The Extraction Method Used to Obtain Collagen from Jellyfish is 0.5 M Acetic Acid

The method applied for the extraction of collagen from the species *Rhizostoma pulmo* living in the Black Sea is a biotechnological process of acidic extraction (ASC). Samples from the jellyfish organism were washed, deodorized and demineralized, shredded, treated with EDTA solution to remove unwanted residues. The whole obtained material was placed in 0.5 M acetic acid solution under continuous stirring at 4˚C for 72 h. The obtained mixture was filtered through fabric to remove water insoluble components. Solid NaCl was added to the filtrate at a concentration of 0.9 M, and the precipitate was collected by centrifugation at 4000 rpm for 15 min. After centrifugation, the precipitate was termed acid-soluble collagen (ASC).

**Figure 1** shows the Black Sea Basin jellyfish, *Rhizostoma pulmo* species taken in the work-up which is washed, deodorized and prepared for pretreatment, the second stage of the acid method extraction process.

**Figure 1a.**

**Figure 1b.**

**Figure 1** Washing and preparing the jellyfish *Rhizostoma pulmo* for the pre-treatment step 1a. jellyfish with arms and umbrella after washing 1b. jellyfish harvested after washing and removal of arms.

The steps of the extraction process are shown in **Figure 2** which shows a schematic of the jellyfish collagen extraction process based on the acid method with 0.5M acetic acid.

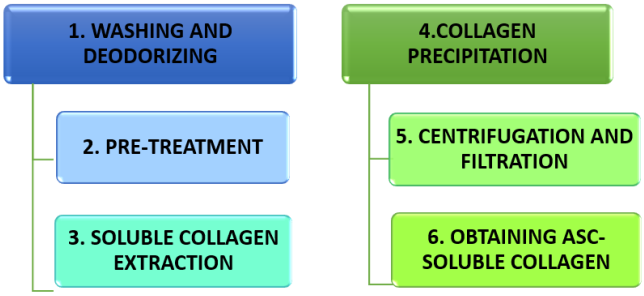

**Figure 2.** Principal steps of collagen extraction by the acid method

**SDS-Page Analysis Method - Gel Electrophage**

Dodecil sodium dodecyl sulfate polyacrylamide gel electrophoresis or SDS-PAGE is a commonly used technique for protein separation and characterization. SDS is an anionic detergent used to produce a uniform loading along the length of proteins that have been linearized. An acrylamide solution is used and bisacrylamide is polymerized. Acrylamide forms linear polymers by itself. Bisacrylamide introduces cross-links between the polyacrylamide chains. The "pore size" is determined by the ratio of acrylamide to bisacrylamide and the concentration of acrylamide.

First the samples are embedded in a gel made of polyacrylamide and then by applying an electric field to the gel, the SDS-coated proteins are then separated. The electric field acts as a driving force, pulling the SDS-coated proteins towards the anode, larger proteins move more slowly than smaller proteins. To identify proteins by size, protein standards of a known size are loaded together with samples, then run under the same conditions.

When proteins and other macromolecules are treated with SDS, they are denatured. The larger the macromolecule, the larger the SDS, so all SDS-treated macromolecules have the same charge-to-mass ratio. The amount bound is proportional to the mass, so for SDS-treated molecules, the attraction per unit mass in an electric field is the same for all molecules, they should have the same rate, if there is no frictional resistance.

SDS electrophoresis is always done in a gel, so the molecules must be drawn through the pores. And the ease of traveling through the pores depends on the diameter of the molecules. Larger molecules are delayed or get stuck and don't move as fast. Because molecules are denatured into random spirals, the diameter depends strictly on the length or molecular weight. The higher the molecular weight, the longer the helix and the slower the molecule moves.

SDS-PAGE is a method based on the migration of a protein, which depends on the ratio of electronegative to electropositive charges in the macromolecule, as well as the shape and size of the molecule. Migration is a characteristic property of a protein at a given pH. Proteins contain electropositive and electronegative charges in their molecule due to the presence of ionizable groups of the constituent amino acids. The laboratory sample is prepared as follows:

The first step is sample degreasing

- Protein precipitation
- Protein extraction in aqueous solution
- Extraction dialysis
- Membrane rupture (cells, microorganisms)
- The last step being protein denaturation at 95°C with LaemmLi reagent.

Finally, make up to 10 mL with distilled $H_2O$. Filter through moistened filter paper. Store in brown colored styrene. Dilute 1:3 in the migrated samples (1 part sample buffer - TP: 3 parts protein solution). The resulting solution is stored at 4°C. The gel electrophoresis equipment used was BIO-RAD Mini-Protean II Cell, shown in **Figure 3**.

**Figure 3.** BIO-RAD Mini-Protean II Cell

*Sample preparation for SDS-Page*

Protein samples are defatted, centrifuged and denatured. Dilute 1:3 in the migrated samples (1 part sample buffer - TP: 3 parts protein solution) and store at 4°C.

*Preparation of the sample buffer solution*

Make up with 3.55 mL deionized distilled water and add 50μL β-mercaptoethanol.

*Preparation of gels*

Prepare the monomer solution by adding all reagents except TEMED and 10% APS. Degas the solution for 15 minutes.

Just before pouring the gels (for 10 mL monomer solution) add: 50 µL APS (ammonium persulfate) 10% and 10 µL TEMED (N,N,N',N'-tetramethylenediamine) to both the separating and concentrating gels. Shake gently to initiate polymerization.

*Preparation of the electrolysis buffer solution (TGS)*

In a 1000 mL volumetric flask, 100 mL 10×TGS was placed in a 1000 mL volumetric flask and made up to the mark with deionized distilled water.

*Protein migration in electric field*

Electrophoresis was carried out at a voltage of 100V in the concentrating gel, then raised to 200V in the migrating gel, which voltage was constant until the end.

*Gel staining*

The gel was washed 3 times for 5 minutes each with 200mL of bidistilled water to remove SDS interfering with staining. Remove the water and add 50mL Bio-Safe Coomassie stain (or as much as needed to cover the gel completely). Shake for 30 minutes at 50 rpm. After 20 minutes the bands are visible and reach maximum staining intensity in one hour. Then place the gel in doubly distilled water for 30 minutes.

*Decolorization of the gels*

The gels were placed in the decolorization solution consisting of 40% methanol and 10%m acetic acid, then kept on the shaker at 50 rpm for 15 hours.

## Results and Discussion

The extraction yield is done with at least 10g of tissue (wet weight) suspended in 10 mL extraction solution/g tissue. The yield for the ASC technique was calculated from the percentage of dry weight of extracted collagen (Mo) compared to the wet weight of jellyfish used were calculated based on the following formula (M):

Yield % = Mo/ M * 100.

The yield obtained from acetic acid extraction was 6.02 mg/g.

## Identification and Characterization by Sds-Page Analysis

The collagen extracted from jellyfish, *Rhizostoma pulmo* species is a polydisperse sample with potential α-chain present at 150 kD and 160 kD as shown in **Figure 4**.

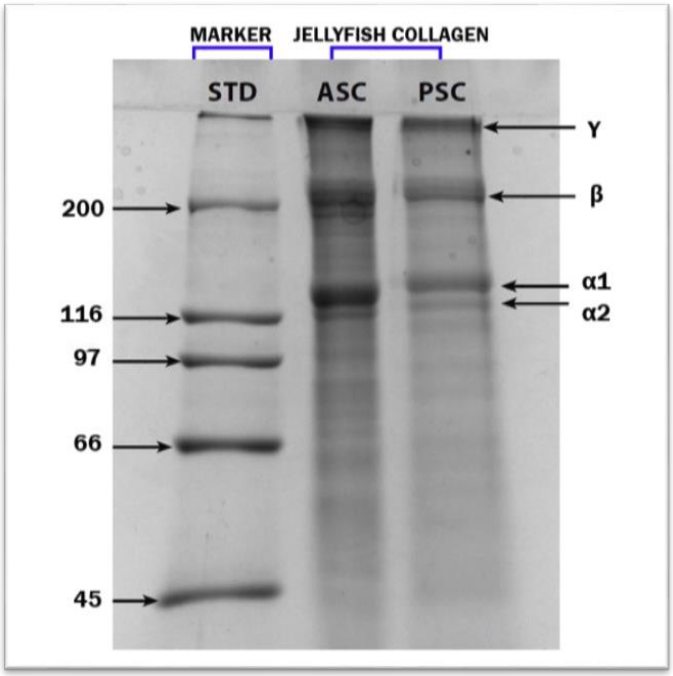


**Figure 4.** SDS-PAGE pattern of collagen extracted from *R. pulmo* by treatment with acetic acid (ASC) and in the presence of pepsin (PSC).

The molecular weights found are slightly higher than those evidenced in SDS-PAGE analysis of collagens extracted from different mammals. An additional, slightly smaller protein band can also be observed, but visible at 90 kD, this is not comparable to any other type I collagen band from mammalian species. It may be either a collagen specific to the jellyfish *R. pulmo*, or it may also be found in other marine invertebrates. The smaller protein band detected also represents an accessory collagen to the main fibrillar collagen chains. The bands at 90 kD are more faintly visible and may be collagen fragments arising during purification.

SDS-PAGE analysis revealed several major protein bands for collagen extracted from jellyfish, which is consistent with previous publications on the molecular weight of collagens extracted from *Rhizostoma pulmo* [13, 14]. It has also been observed to contain several less noticeable bands, which may be due to the presence of degradation products formed during extraction or remaining non-collagenous proteins [15]. In both the (ASC) and (PSC) extraction versions, similar features were evidenced, the electrophoretic pattern is typical of type I collagen, consisting of α chains with two distinct types such as α1 and α2, which vary in their mobility as shown in **Figure 4**.

## Conclusions and future directions

In this article, the extraction of collagen from the jellyfish *Rhizostoma pulmo* collected from the Romanian Black Sea coast was presented. The treatment used in obtaining the collagen is an acidic extraction treatment (ASC). The resulting collagen was further analyzed for protein identification and characterization by SDS-PAGE - gel electrophoresis method. SDS-PAGE-gel electrophoresis gel electrophoresis spectroscopy determinations and analysis were carried out and confirmed the existence of type I collagen in accordance with the literature that mentioned the presence of collagen in this jellyfish species, but from other seas or oceans of the world.

The limitations of this study are related to the equipment, but also to the material under study, jellyfish can and in different stages of development, the season of harvesting may also influence the maturity of jellyfish living in marine waters. The marine collagen present in this jellyfish species is a biomaterial of approximately identical quality to that of marine origin extracted from marine vertebrates, this makes jellyfish collagen a bio-composite from which pharmaceutical, cosmetic, dermatologic or nutraceutical products worthy of future consideration could be made.

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