EXTRACTION AND PURIFICATION OF COLLAGEN 1 FROM RAT TAIL BY FPLC METHOD

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Abstract:
Collagen was extracted from Rat tail source and purified in acetic acid solution at 4^0 Celsius for 24hours.The rat tail source collagen consists mainly the amino acids like, glycine, proline, hydroxyproline and glutamic acid. Electrophoresis pattern demonstrated two distinct α- chains (α1 and α2) and β chain, indicating that collagen is a major component of rat tail,which is perform using FPLC Method, with the use of standard. we characterise the type 1 with the peaks in monitor later, and confirms the process by SDS Page study. The study demonstrated that rat tail collagen extraction and isolation method is possible as commercial alternative biomaterial.

Keywords: collagen, FPLC, Rat tail

1.Introduction

Type I collagen has extensive properties which is mainly used in biomedical studies[1] and its use is widely documented. It is a natural material with good clinical compatibility and low antigenicity It is found in skin, bone, tendon, ligament, and cornea of animals. The main sources of type I collagen for biomedical use are from animal skin and tendon[2]. Collagen from different species has its own unique chemical, physical, and biological properties. Rat-tail tendon is among the original sources of type I collagen extracts[3]..

Moreover Collagen has attracted great interest[4] as a biomaterial in various medical and clinical uses and as a substrate for tissue engineering[5]. It has been widely employed as a surgical dressing, surgical suture, haemostatic material , and membranes for guided tissue regeneration (GTR) or guided bone regeneration (GBR) in dental science[6], wound healing and biocompatibility studies

The extraction protocol of type I collagen from Rat tail tendon is widely documented. Up to now, there are limited numbers of comparative study on the species-related properties of collagen. This study is related to obtain the basic knowledge on collagen properties, bio compatibility and purification by The fast protein liquid chromatography (FPLC) method and its is use in purification of proteins. Previous studies has showed that FPLC was used to separate the plasma, urinary proteins[7], and beta-thalassemia[8] these studies used to find a good source of type I collagen[9] that can be developed in Future research

2.Materials and Methods

2.1 Isolation of collagen

Rat tails were brought and immerse tails in 70% Ethyl alcohol to remove debris. Then Remove skin from rat tail, separated tendon[11] from tail by using scalpel and forceps. The Collected tendon soaked in PBS and Washed 3times in PBS. Then Sterilize in 70% ETOH for > 1 hour in order to Remove as much ETH as possible from the tendon and transfer to a sterile bottle with a stir bar. Dissolve the Resultant tendon by adding about 75 ml (per tail) of 0.1% acetic acid to the tendon and stir for 2 days at 4^0 C. Monetarily Checking the state of the collagen solution periodically. If it is too viscous such that the stirring bar is not operating, added more 0.1% acetic acid. (Note: desired consistency ~ a bit thicker than Elmer's Glue.) Then Centrifuged[12] resulting viscous solution at 10,000 rpm for 60 min at 4^0 C. Resulting supernatant is collagen[13] stock solution. We Determined the concentration using BioRad DC protein assay and Stored at 4^0 C

2.2 FPLC analysis

Samples of purified collagen were chromatographed[14] on a Mono Q anion exchange column using the fast protein liquid chromatography (FPLC) apparatus of AKTA Prime plus. A 1mg sample was applied in 20 mM Tris buffer, 1 *mM* CaCI,, pH 8.5 and was eluted in the column with two successive linear gradients of NaCl. The column elutes at a rate of 1ml per min and 1 ml fractions were collected. One ml of 100 *mM* Tris, 1 *mM* CaCI,. pH 7.0 was adjusted to each collected fraction prior to the assay of collagen activity[15].

2.3 SDS

Collagen extracts from source were subjected to sodium dodecyl sulfate polyacrylamide[16] gel electrophoresis (SDS-PAGE) analysis using 6% of separating gels with the standard collagen bought from sigma Aldrich India. The gel was then stained overnight with 0.017%m(w/v) Coomassine blue R-250 (Bio-Rad) in 38.8% methanol and 6.8% acetic acid. Subsequently, each gel was destained with 5% methanol and 5% acetic acid for 48 hours[17]

3. Results

Purified collagen was analyzed using FPLC with standard collagen protein (Bought from sigma Aldrich).In analysis we got elution points at different point of time so, we analyse the curves with standard collagen by using graph(Fig 1). For our convininces we note down values of elution points and divided the X-axis points as A,B,C.D,E,F,G and standard collagen, with Y-Axis as Time. The Elution point at D is similar to the standard collagen elution time, then to find the resultant elution characterization of proteins has to be done.

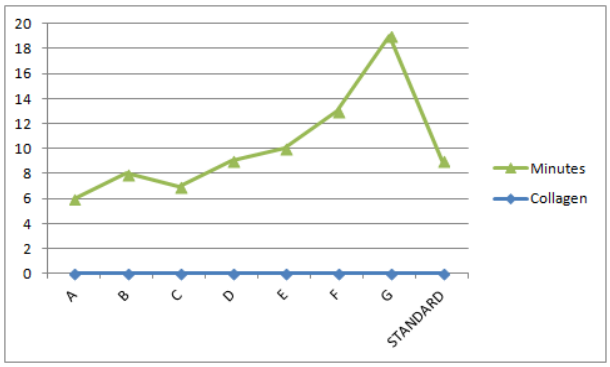
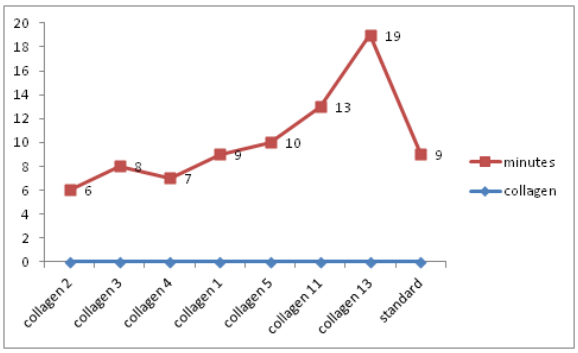


Fig.1 The FPLC Analysis of collagen in which the standard curve and D point elution time is similar

3.1 Characterisation of proteins

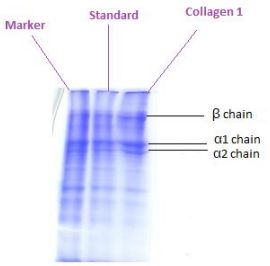
The protein profile was dominated by a major peak eluting at the enzymatic activity associated, with this protein peak was characteristic and exhibited a preference for the substrate Hexapeptide. A small amount of a Type 2 protein eluted at 7-8 minutes as indicated by the activity against Hexapeptide. FPLC analysis of purified collagen resolved a number of protein peaks. In parallel to collagen four proteins eluted at 7,8,11 min. The enzymatic activities of the proteins eluting at 7, 8 and 11th min was characteristic of a type 3,4,5 collagen. The protein eluted at 9th minute appeared to be a Class I collagen protein which peak is greatly suitable to standard solution, and the protein eluted at 10 min had no measurable activity against any substrate. Two protein peaks eluted at 13 and 19 min that had enzymatic activities characteristic of the type11,12 collagen. However, the amount of Class l collagen suitable to standard peak at 9 minute is collagen 1 and we extracted the peak of that collagen 1 protein and checked with amino acid composition, and SDS PAGE electrophoresis method is performed with standard collagen.



The characterisation of proteins at elution of points

3.2 SDS PAGE

The SDS–PAGE patterns of purified and standard protein patterns determined in SDS Page method. collagen contained two distinct α and β chain. The two bands which had showed similar length on electrophoresis plate Therefore, the results indicated that type I collagen is a major component of rat tail collagen.. These results showed collagen1 were successfully removed from rat tail collagen. Furthermore, the results also proved that the collagen isolated throughout the purification processes.



Coloagen 1 protein is runned with marker and standard collagen in SDS, the collagen 1 bands are similar to the standard collagen

4. Discussion

Rat tail collagen are an economically and technologically feasible substrate to extract collagen. The rat tail collagen had similar pattern of amino acid composition, molecular weight and structural properties when compared to the commercial collagens. The thermal properties were also similar to the commercial collagen at higher and lower temperatures. This study demonstrated that rat tail collagen using FPLC isolation method is possible as commercial alternative ingredient.

5.References

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