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A New Kind of Biomaterials—Bullfrog Skin Collagen

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Abstract: Pepsin-soluble collagen was prepared from bullfrog skin and partially characterized. This study revealed interesting differences, such as molecular weight, amino acid composition, denaturation temperature (T_d), in the frog skin collagen when compared to the known vertebrate collagens. This study gives hints that bullfrog skin can be a potential, safe alternative source of collagen from cattle for use in various fields.

Keywords: Bullfrog skin, collagen, denaturation temperature.

Collagen is a natural material with good biological compatibility, well characterized low antigenicity. Therefore, collagen has attracted great interest as biomaterials in medical use, such as drug delivery, tissue engineering, and so on[1-2].

The use of cattle as the main source for collagen has to be reconsidered because of the risks of BSE (bovine spongiform enceohalopathy) and TSE (transmissible spongiform enceohalopathy). So, an alternative safe collagen should be searched. In this paper, bullfrog skin was firstly used to extract collagen. Bullfrog is a kind of amphibians, it has no threat of BSE and can be taken as a safe collagen source. The preparation and characterization of collagen from bullfrog skin are described.

Experimental

Collagen from bullfrog skin was prepared according to the method of Eostein[3]. The yield of bullfrog skin collagen was determined based on the dry weight.

Protein samples were analyzed by SDS-PAGE according to Laemmli[4] using 5% resolving gels in a electrophoresis instrument. A collagen sample was hydrolyzed in 6 mol/L hydrochloric acid at 110°C for 22 h. The hydrolysates were analyzed on a Hitachi 835-50 amino acid analyzer. Referred to the method of Pitchumani *et al*[5]. T_d was determined as the temperature at which the change in viscosity was half completed. The IR spectrum of the pepsin-soluble bullfrog skin collagen was recorded using FTIR (Nicolet 200SXV) spectroscopy.

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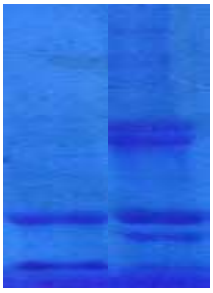
Results and Discussion

The collagen of bullfrog skin was easily solubilized by limited pepsin proteolysis. The yield of pepsin-soluble collagen was 12.6% on a dry weight basis.

As **Figure 1** shows, the electrophoretic profile of bullfrog skin collagen was significantly different from that of pig species, showing the high molecular weight components were scarce. Especially, the β chain of bullfrog skin collagen was disappear. It may be contributed to the digestion of pepsin. On the other hand, bullfrog skin collagen showed only a single α_1 (I) band.

Figure 1 Electrophoretic analysis of the bullfrog collagen



(A) (B)

(A) bullfrog skin collagen; (B) pig skin collagen. Pig skin collagen was used as mobility markers for α -chains and β -chains.

The amino acid compositions of pepsin-soluble collagen of bullfrog skin are presented in **Table 1**. It was observed that the bullfrog skin collagen had a little lower glycine content (300). The more hydroxylysine (11) of the bullfrog skin collagen reflected a little higher amount of carbonhydrate than that of the calf skin collagen. As shown in the **Table**, bullfrog skin collagen has cystine residues, while calf skin collagen has none. Hydroxyproline is derived from proline by post-translational hydroxylation mediated by prolylhydroxylase. The proline to hydroxyproline ratio (0.48) of bullfrog skin collagen was far below than that (0.78) of calf skin collagen, which suggests the crosslinking and stability of bullfrog collagen are lower.

Thermal denaturation profile of bullfrog has provided useful clues to understand the thermal stability of collagen in relation to enviroment and imino acid content. It was calculated from thermal denaturation curve that T_d of pepsin-soluble bullfrog skin collagen was 30.3°C (**Figure 2**). This was about 6°C lower than that of pig skin collagen[7]. T_d is proportional to the hydroxyproline content[8]. The denaturation temperature is known to increase with an increase of amino acid residues. According to the amino acid analysis of bullfrog skin collagen, the content of hydroxyproline is 5.36%, far below that of calf skin collagen (~10%).

The amide A band is associated with the N-H stretching frequency. A free N-H stretching vibration occurs in the range of 3400~3440 cm^{-1}, and when the NH group of peptide is involved in a hydrogen bond, the position is shifted to lower frequency, usually 3300 cm^{-1}. The amide A band of bullfrog skin collagen was found at 3335 cm^{-1},

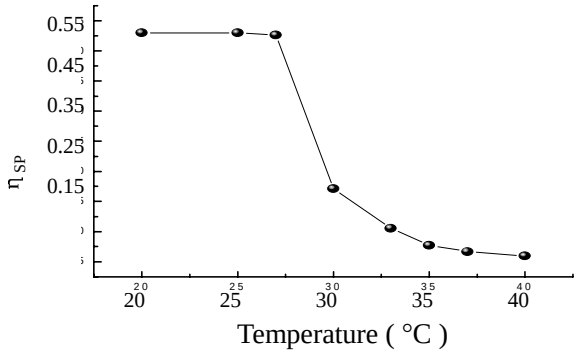
suggesting the presence of its involvement in hydrogen bonding.

The helical structure of the collagen was confirmed from the IR absorption ratio between 1235 (amide III) and 1450 cm^{-1} bands, which was approximately equal to one for all preparations[9].

Table 1 Amino acid compositions of the pepsin-soluble collagen of bullfrog skin, compared with calf skin collagen[6] (results are expressed as residues/ 1000 residues)

Amino acid	Bullfrog skin collagen	calf skin collagen
Hydroxyproline	54	94
Glycine	300	330
Hydroxylysine	11	7
Aspartic acid	53	53
Leucine	27	23
Threonine	23	18
Tyrosine	5	3
Serine	53	39
Phenylalanine	18	3
Glutamic acid	80	80
Lysine	33	26
Alanine	119	119
Histidine	6	5
Cystine	8	--
Valine	25	21
Arginine	56	50
Methionine	5	6
Proline	113	121
Ileucine	11	11

Figure 2 Thermal denaturation curve of pepsin-soluble bullfrog skin collagen solution



Thermal denaturation curve of pepsin-soluble bullfrog skin collagen solution is measured by viscosity in 0.5 mol/L acetic acid. Collagen cocentration: 0.1%.

Conclusion

In conclusion, a great quantity of collagen could be prepared from bullfrog skin by pepsin treatment process. The bullfrog is one of the best delicious foods so that the amount of bullfrog consumed is great. However, the bullfrog skin was taken as waste without any utilization. Bullfrog is living in water that it has no threat of BSE or TSE.

For these reasons, bullfrog skin is a potential alternative source of the collagen from cattle. This study is only a preliminary report for exploiting collagen from underutilized resources.

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