Food Chemistry 135 (2012) 127–132

Contents lists available at SciVerse ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

The effect of the subunit composition on the thermostability of collagens from the scales of freshwater fish ☆

Rui Duan [a,*], Junjie Zhang [a], Jing Li [a], Xiaohong Zhong [a], Kunihiko Konno [b], Haibo Wen [a]

[a] School of Marine Science and Technology, Huaihai Institute of Technology, Lianyungang 222005, China
[b] Faculty of fisheries science, Hokkaido University, Hakodate, Hokkaido 041-8611, Japan

ARTICLE INFO

Article history:
Received 31 January 2012
Received in revised form 5 April 2012
Accepted 19 April 2012
Available online 27 April 2012

Keywords:
Scale collagen
Silver carp
Carp
Chromatography
Subunit
Denaturation temperature

ABSTRACT

Collagens were isolated from the scales of carp and silver carp caught both in winter and summer. The denaturation temperatures measured by circular dichroism indicated that the thermostability of summer collagens was higher than that of the winter equivalents. The subunits of scale collagens were studied by column chromatography, which showed that the winter collagens had lower contents of α1 chain, but higher percentages of α2 and α3 chain, than the summer equivalents. It was suggested when season changes from summer to winter, the subunit compositions of scale collagens change accordingly; the content of α1 chain decreased, while contents of α2 and α3 chain increased. Therefore, the seasonal difference in the thermostability of scale collagens from the fish may be attributed to the subunit changes of collagens. This change may substantially affect tissue formation and construction, thus, have dramatic physiological consequences.

1. Introduction

Collagen is the most abundant protein in the living body, comprising approximately 30% of total protein. Fish offal, such as skins, scales, as well as bones, is tissue that is mainly composed of collagen. The physical and chemical properties of aquatic animal collagen are different from those of mammalian collagen; in particular, the denaturation temperatures (T_d) of fish collagens are usually lower than those of mammalian ones. However, many researchers are interested in fish collagens since they are free from BSE (bovine spongiform encephalopathy) and TSE (transmissible spongiform encephalopathy) and will not be forbidden for religious reasons (Morimura et al., 2002).

Many papers have focused on the practical utilisation of marine animals to produce collagen (Piez, 1965; Rigby, 1968). Some concerned collagens from freshwater fish, such as carp (Duan, Zhang, Du, Yao, & Konno, 2009; Kimura, Miyauchi, & Uchida, 1991) and grass carp (Zhang et al., 2007). However, relative lower denaturation temperatures, i.e., lower thermostability, have become one of the main limiting factors for the application of fish collagens, especially for those from marine fish. At present, the explanation for the reduced thermostability of collagens from fish is limited to the imino acid (hydroxyproline and proline) content of the samples. The denaturation temperatures of collagens increase with their imino acid content. Hydroxyproline may stabilise the triple helix by hydrogen-bonded water-bridges, as originally proposed by Ramachandran, Bansal, and Bhatnagar (1973).

Carp and silver carp are the major freshwater fish produced in China. Great quantities of fish scales are produced, but the effective use of scales is minimal. The utilisation of fish processing wastes is of great significance for environmental protection and the production of value-added products to increase revenue for the producers. Fish scales can be employed as an alternative source of collagen. We once reported the seasonal difference in thermostability of collagens from silver carp (Zhang, Duan, & Konno, 2010). However, for the collagens from the two seasons, little difference in the amino acid composition could be found. Until now, very few papers, if any, have reported the other factors related to the stability of fish collagen. In order to increase the present knowledge on the seasonal difference in the thermostability of fish collagen, scale collagens from carp and silver carp caught in winter and summer were isolated and the effects of the subunit components on the thermostability of collagens were studied. More understanding of biochemical properties of scale collagen will contribute to the better utilisation of collagen in the food, as well as other industries.

☆ The paper has not been published previously at any form. It is not under consideration for publication elsewhere, and its publication is approved by all authors.

* Corresponding author. Tel.: +86 13851290162; fax: +86 518 85893890.
E-mail address: duanrui526@yahoo.com.cn (R. Duan).

0308-8146/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.foodchem.2012.04.112

2. Materials and methods

2.1. Raw materials

Live cultured carp and silver carp (average body weight of 850 g) were obtained from a market in Lianyungang, Jiangsu province (February 2, 2009, 3 °C, winter samples and August 1, 2009, 31 °C, summer samples). The scales were removed manually and washed with chilled tap water to get rid of the adhering tissues. The samples were then placed in polyethylene bags and stored at −25 °C until used.

2.2. Chemical reagents

All reagents were of analytical grade and were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan) or Sigma–Aldrich Chemical Co. (St. Louis, MO). The water used was distilled and deionised with a Millipore "Super Q" system.

2.3. Preparation of collagens

The method used for the isolation and purification of acid soluble collagen is according to Nagai et al. (2000), with some modifications (Duan et al., 2009). All procedures were carried out at 4 °C.

Fish scales were extracted with 0.1 M NaOH for 6 h at a sample:alkali solution ratio of 1:8 (w/v) to remove non-collagenous proteins, and washed fully with cold distilled water .The scales were decalcified with 0.5 M Na_2EDTA (pH 7.5) at sample:EDTA solution ratio of 1:10 (w/v) for 24 h, and then washed with cold distilled water. The residue was extracted with 0.5 M acetic acid at sample:acid ratio of 1:2.5 (w/v) for 4 days. The extract was centrifuged at 20,000*g* for 1 h. The supernatant was salted out by adding NaCl to a final concentration of 2.5 M in the presence of 0.05 M tris(hydroxymethyl)aminomethane, pH 7.0. The resultant precipitate was collected by centrifuging at 20,000g for 30 min. The pellet was dissolved in 0.5 M acetic acid, dialysed against 0.1 M acetic acid and distilled water, respectively, and then lyophilised.

Collagens extracted from carp (*Cyprinus carpio*) and silver carp (*Hypophthalmichthys molitrix*) scales in February and August were termed as w-cs and s-cs, w-scs and s-scs, respectively.

2.4. Determination of denaturation temperature measured by circular dichroism (CD)

Collagens dissolved in 0.1 M acetic acid were centrifuged at 50,000g for 20 min at 4 °C. Small aliquots were taken out and transferred to a quartz cuvette (1-mm pathway), and then placed into a polarimeter to record CD spectra. CD spectra of collagen samples at 0.8 mg/ml were recorded at 15 °C on a Jasco J-725 spectropolarimeter (Jasco Inc., Tokyo, Japan). Five scans were averaged for the wavelength 250–190 nm.

The melting curve of collagen was determined by monitoring $[\theta]_{220}$ at the wavelength of a positive extreme at 220 nm. The $[\theta]_{220}$ was recorded while heating the sample from 15 to 45 °C at a rate of 1 °C/min. The transition temperature was determined as the midpoint temperature between native-folded and completely unfolded forms. The analyses were repeated twice. To avoid protein damage by UV irradiation, UV exposure of any sample never exceeded ten minutes.

2.5. SP (Sulphopropyl) Toyopearl column chromatography

The subunit components of scale collagens from carp and silver carp were separated by SP Toyopearl 650 M (Tosoh Co., Tokyo, Japan) column chromatography. According to the methods of Kimura and Ohno (1987), 10 mg collagen was dissolved in 5 ml of 20 mM sodium acetate buffer, pH 4.8, at 4 °C overnight. After centrifugation at 20 °C for 30 min, the collagen was fractionated on a column of SP Toyopearl 650 M (1.5 × 10 cm). Elution was achieved by the starting buffer with a linear gradient of 100–250 mM NaCl over a total volume of 300 ml at a flow rate of 90 ml/h. Fractions of 3 ml were collected and the absorbance was monitored at 220 nm. The fractions were examined by SDS–PAGE.

2.6. SDS–polyacrylamide gel electrophoresis (SDS–PAGE)

SDS–PAGE was performed by the method of Laemmli (1970) using 7.5% separating gel and 4% stacking gel. The collagen samples were dissolved in 0.1 M acetic acid. The protein content of each sample was determined in triplicate by Lowery's method with

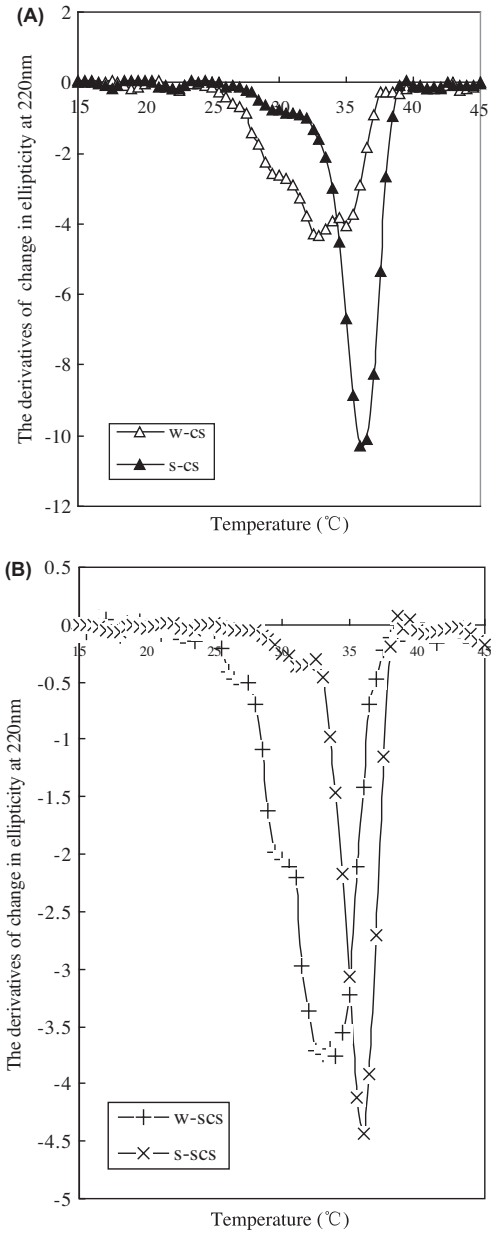

Fig. 1. Thermal unfolding of carp scale collagens expressed as first derivative plots. Changes in ellipticities at 220 nm for scale collagens dissolved in 0.1 M acetic acid upon heating from 15 to 45 °C at a rate of 1 °C/min were compared. Relative changes in the ellipticity by taking the first derivative of the change were shown. (A) w-cs and s-cs. (B) w-scs and s-scs.