ELSEVIER

Process Biochemistry 42 (2007) 542–546

Process Biochemistry

www.elsevier.com/locate/procbio

Separation of β_{22} dimer from bovine bone collagen

An-Jun Liu, Ying Chen, Yong-Li Jia, You-Zhi Liu, Wen-Hang Wang, Guo-Rong Zhang *

Tianjin Key Laboratory of Food Nutrition and Safety, College of Food Engineering and Biotechnology, Tianjin University of Science and Technology, Tianjin 300457, China

Received 14 June 2006; received in revised form 15 September 2006; accepted 4 October 2006

Abstract

The precise role of the α_2-chain in collagen type I is of considerable scientific interest. Our recent studies demonstrated that the most noticeable difference between type I collagens, which were obtained from bovine hard tissues (bone, dentine) and soft tissues (tendon, skin), was presented in the position of β chain dimers using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis. The additional band observed both in the bone and dentine collagen was putatively identified as β_{22} dimer (made of by an intermolecular cross-linking between two α_2-chains). Further investigations carried out on bovine bone and skin collagen, corresponding to hard tissue and soft tissue collagen respectively, confirmed this hypothesis. Successful separation of individual β_{22} dimer from bone collagen was achieved. The procedure involves molecular-sieve chromatography on a Sephacryl S-400 column followed by differential acetone precipitation. Identification was done by the widely used methods, such as SDS-PAGE and cyanogen bromide (CNBr)-cleaved peptide analysis. It was proposed that the dimer and consequently α_2-chains may play important roles in the morphological and biological differences between hard and soft tissues.

Keywords: Bone collagen; Intermolecular cross-link; β_{22} dimer; α_2-Chain

1. Introduction

Collagens are predominant structural proteins which fulfill important functional roles in different connective tissues such as bone, skin, tendon, blood vessels, and other tissue types. Collagens have complex supramolecular structure and their molecules interact with each other at different levels in order to form higher-order structures with specific functions [1–3].

At least 27 types of collagen have been discovered so far. Type I collagen, which is the most abundant and extensively studied collagen, is constituted of three polypeptide chains called α_1 (two chains) and α_2 which are accessible to polymerization through intra- and intermolecular bonds [4–6]. Intramolecular cross-links form between two α-chains in the non-helical section of the same molecule by aldol condensation of two aldehydes, while intermolecular cross-links occur between the telopeptide region of one collagen molecule and the helical region of a quarterly staggered, adjacent molecule [7]. Dimers of α-chains are called β-components (composed, e.g. of $\alpha_1\alpha_2$ or $\alpha_1\alpha_1$ chains). Trimers of three α-chains are designated γ-components. Analysis of these chain polymers is, aside from collagen type identification, the main task in studying the biological functions of collagens [4].

In order to obtain a clear pattern of intramolecular and intermolecular cross-links in collagen, many studies had been carried out in past years. But many of these studies focused on investigating the sites of the cross-links and isolating the precursors as well as actual cross-link peptides. Although the detection of the intermolecular cross-linked dimer β_{22} ($\alpha_2\alpha_2$) was mentioned by several investigations [8,9], its separation had received little research attention.

In recent years, many advanced separation techniques such as gel permeation, reversed-phase separations and capillary electromigration etc. have been used for the separation of collagen parent α-chains and their polymers [5]. Successful separations of the major constituting peptide chains (α_1 and α_2) and chain polymers (β_{12}, β_{11} and γ) from collagen type I were reported by many studies [4,10]. However, as far as the separation of individual β_{22} dimer is concerned, the information is still limited to our knowledge.

Abbreviations: SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; CNBr, cyanogen bromide; $CHCl_3$, chloroform; CH_3OH, methanol; GuHCl, guanidine hydrochloride

* Corresponding author. Tel.: +86 22 60601437; fax: +86 22 60601375.
E-mail address: grzhang@tust.edu.cn (G.-R. Zhang).

1359-5113/$ – see front matter © 2007 Published by Elsevier Ltd.
doi:10.1016/j.procbio.2006.10.008

In this paper, a successful separation of β_{22} dimer from bovine bone collagen was achieved. This study may provide useful information for understanding the roles of α_2-chain in the chemical structure and biological functions of collagen.

2. Materials and methods

2.1. Preparation of type I collagen from bovine bone

The bone type I collagen was extracted from bovine bone as described below. In brief, powdered bovine bone was washed with 1.0 M NaCl, 50 mM Tris–HCl, pH 7.4 containing protease inhibitors, and defatted with chloroform/methanol ($CHCl_3/CH_3OH$, 1:1). The defatted bone powder was demineralized in dilute HCl, keeping the pH constant to 2.0 by addition of 12 M HCl. The demineralized bone matrix was washed with 1% Na_2CO_3 to remove the noncollagenous proteins. Then the bone type I collagen was extracted by 6 M guanidine hydrochloride (GuHCl), 77 °C. The type I collagen solution was dialyzed and freeze dried. Preparation of the skin collagen was performed under the same conditions.

2.2. SDS-polyacrylamide gel electrophoresis (SDS-PAGE)

Laemmli's [11] SDS-polyacrylamide gel electrophoresis system was adapted with some modifications using 7% (for collagen analysis), and linear 10–18% (for CNBr-peptides analysis) polyacrylamide gradient gels in 0.25 M Tris–HCl buffer, pH 8.8. The samples were dissolved in SDS sample buffer solution containing 1% SDS, 1% β-mercaptoethanol, 1 mM EDTA and 20% glycerin, and heated for 5 min at 100 °C. The samples (15 μL) were subjected to gel electrophoresis at a constant current of 20 mA per gel. The running buffer solution contained 0.1% SDS and 25 mM Tris–glycine buffer, pH 6.8. After electrophoresis, the gels were stained with 0.1% (w/v) Coomassie Brilliant Blue R-250. Gel images were captured on a Gel Doc XR Gel Documentation System (Bio-Rad, Hercules, CA, USA), and analyzed with Quantity One software.

2.3. Cyanogen bromide digestion

The collagen peptides (α_1-chain, α_2-chain and β_{22} chain) were digested by cyanogen bromide (CNBr) in 70% (v/v) formic acid at room temperature as described by Liu et al. [12]. CNBr peptides produced from each collagen chains were resolved in linear 10–18% SDS-polyacrylamide gradient gels and stained with Coomassie Brilliant Blue R-250 as before.

2.4. Purification of β_{22} chain from bovine bone collagen

The first step of purification of β_{22} chain from bovine bone type I collagen is the preparation of β chain fraction and α-chain fraction, which was carried out using a Sephacryl S-400 column (2.0 cm × 100 cm). Two grams of type I collagen was dissolved in 10 mL of chromatography buffer (50 mM Tris–HCl, pH 7.4 containing 4 M guanidine hydrochloride). Then the samples were applied to the column and eluted with the same buffer. Each fraction of the chromatography was examined by SDS-gel electrophoresis. The second step of purification of β_{22} chain was carried out using a "differential precipitation method" by adding acetone gradually (56–82%) at 4 °C to β chain fraction and α-chain fraction, respectively. The precipitation sequence of each chain was the β_{11} chain (56%), β_{12} chain (60%), β_{22} chain (66%), α_1-chain (73%) and α_2-chain (82%). Each precipitated chain was collected by centrifuge at 10,000 × *g* for 30 min and examined by SDS-gel electrophoresis.

3. Results and discussion

It is interesting to investigate new methods in making more effective use of under-utilized resources and industrial wastes for many years. Many of animal wastes are potentially valuable resources. Skin and bone, for example, contain large amounts of collagen [13].

Recently, we studied collagens obtained from bovine hard tissues (bone, dentine) and soft tissues (tendon, skin). Collagen subspecies bands (α_1, α_2, β_{11}, β_{12} and γ, etc.) in all these samples typically appeared in a characteristic ladder-like array [10] in their SDS-PAGE patterns. The most noticeable difference was in the position of β chain dimmers. One more band was observed both in the bone and dentine collagens. The additional band was putatively identified as β_{22} dimer according to its electrophoretic mobility.

Because bovine bone and skin are the primary sources of industrial collagens and type I collagen accounts for approximately 90% of the total bone protein [14], bovine bone and skin were applied to analysis again in order to obtain reliable information. Similar results were observed, as shown in Fig. 1.

The presence of the additional band was further confirmed by the results of scanning density analysis of the α- and β-bands in the SDS-PAGE patterns (Fig. 2). In Fig. 2(1), the peak corresponding to putative β_{22}-band appeared. A comparison of

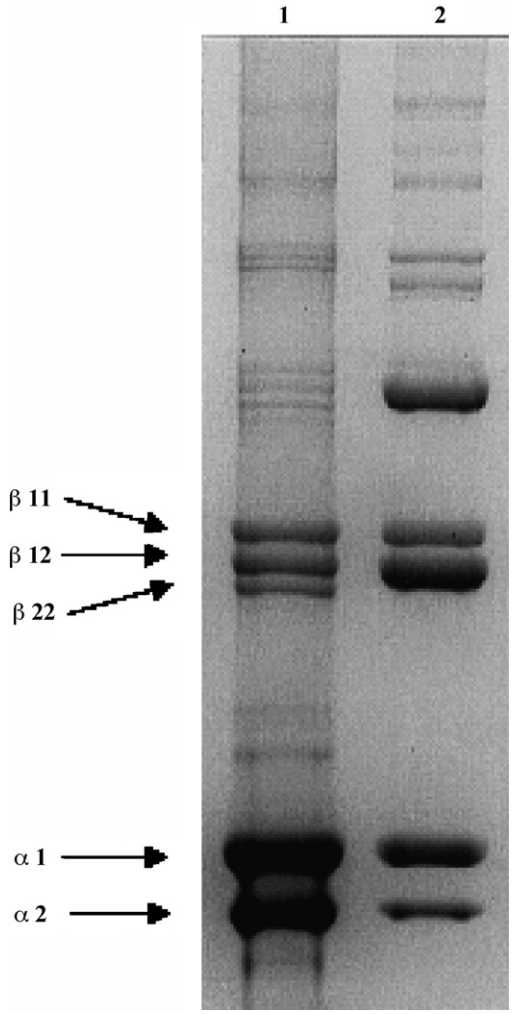

Fig. 1. SDS-PAGE pattern of bovine bone and skin collagen. The samples were dissolved in SDS sample buffer containing 1% SDS, 1% β-mercaptoethanol, 1 mM EDTA and 20% glycerin, and heated for 5 min at 100 °C. Lanes: 1 = bovine bone; 2 = bovine skin.