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Structural properties of pepsin-solubilized collagen acylated by lauroyl chloride along with succinic anhydride

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ABSTRACT

The structural properties of pepsin-solubilized calf skin collagen acylated by lauroyl chloride along with succinic anhydride were investigated in this paper. Compared with native collagen, acylated collagen retained the unique triple helix conformation, as determined by amino acid analysis, circular dichroism and X-ray diffraction. Meanwhile, the thermostability of acylated collagen using thermogravimetric measurements was enhanced as the residual weight increased by 5%. With the temperature increased from 25 to 115 °C, the secondary structure of native and acylated collagens using Fourier transform infrared spectroscopy measurements was destroyed since the intensity of the major amide bands decreased and the positions of the major amide bands shifted to lower wavenumber, respectively. Meanwhile, two-dimensional correlation spectroscopy revealed that the most sensitive bands for acylated and native collagens were amide I and II bands, respectively. Additionally, the corresponding order of the groups between native and acylated collagens was different and the correlation degree for acylated collagen was weaker than that of native collagen, suggesting that temperature played a small influence on the conformation of acylated collagen, which might be concluded that the hydrophobic interaction improved the thermostability of collagen.

1. Introduction

Type I collagen, with an approximate molecular weight of 300 kDa [1], is composed of two α1 chains and one α2 chain [2]. Each polypeptide chain is formed by around 1000 amino acids, which contains the repeated typical tripeptide units Gly-X-Y, where Gly is glycine, and X and Y are often proline and hydroxyproline, respectively [3]. The three polypeptide chains folding a left-handed helical configuration intertwine with a right-hand triple helix structure which is stabilized by inter-/intra-chain hydrogen bonds [4]. Type I collagen has been widely used as biomaterials for drug delivery [5] and wound healing [1] due to its advantages such as biocompatibility, weak antigenicity and biodegradability [6].

Usually, collagen with integrated triple helix structure is extracted from skin, tendon and bone by the treatment of acetic acid (pH 2.5–3.0) or pepsine [7], and could not dissolve in water since its isoelectric point (pI) is close to neutral pH value. To improve the water-solubility of collagen, Zhang et al. [8] prepared water-soluble collagen via introducing a large amount of carboxyl groups to decrease the pI of native collagen up to 3.5 based on the method of acylation of collagen with succinic anhydride, however, the thermostability of the water-soluble collagen decreased, which limited the potential of the water-soluble collagen used as biomaterial. Therefore, the authors [9,10] introduced hydrophobic lauroyl and hydrophilic succinic residues on the free amino groups of native collagen molecules, and the results showed that acylated collagen not only exhibited water solubility and better surface activity, but also had stronger thermostability, which might be suitable for promoting the dispersion and the sustained release for hydrophobic drugs [11].

All the practical applications of collagen are often based on its triple helix conformation that determined its specific functions. When the triple helix structure of collagen was destroyed, its biological activity might be lost. For instance, gelatin, the denatured collagen, lost the fibril formation ability and was easily attacked by proteinase, and had weak proliferation ability for epidermal keratinocyte, suggesting that collagen with a triple helix structure had greater potential biomaterial utilization than gelatin [12,13]. However, collagen was subjected to high temperatures during the production, storage and use, resulting in the helix–coil transition [14]. Therefore, understanding the change of the triple helix conformation for collagen or its derivate during heating process was of great importance to the practical application of this bio-macromolecule. Liu et al. [15,16] investigated the thermal denaturation of collagen using differential scanning calorimetry, and found that the three-state model could describe the denaturation process for collagen. By the method of Fourier transform infrared spectroscopy (FTIR), Chen et al. [17] found that the intensity and the positions of the major amide bands for collagen decreased and shifted to lower wavenumber with the temperature increased from 25 to 115 °C, suggesting that the secondary structure of collagen was destroyed during heating process.

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To study the interactions at the molecular level in various kinds of biopolymers, 2D correlation spectroscopy was successful in systematically extracting the specific nature of spectral intensity variations induced by an external perturbation since 2D correlation spectroscopy could spread, identify and enhance many overlapped bands [18–20]. Tian et al. [21] applied composition-dependent 2D Fourier transform-infrared (2D-FTIR) to investigate the conformational change of collagen and the specific interactions between collagen and chondroitin sulfate, and found that the hydrogen bond and the electrostatic interactions played an important role in the blends when the chondroitin sulfate content was more than 50 wt.%. However, there was little information concerning about the micro-information of the conformational change for collagen or its derivate induced by temperature using the method of 2D-FTIR.

In previous works [9,10], the authors prepared acylated collagen by introducing lauroyl and succinic residues on the native collagen molecules. The lauroyl and the succinic residues in the structure provided the hydrophobic and the hydrophilic moiety respectively, which endowed acylated collagen with water solubility and surface activity. Since the conformation was correlated with the performance of acylated collagen, it was much important to understand the structural properties of acylated collagen. In this paper, the structural properties for this kind of acylated collagen were investigated using amino acid analysis, circular dichroism spectrum (CD) and X-ray diffraction (XRD). Meanwhile, the thermal stability of acylated collagen was also detected by thermogravimetry (TG) and the structural transformation during heating process was analyzed by FTIR. Finally, temperature-dependent 2D-FTIR was selected to analyze the results of FTIR to further understand the conformational change of acylated collagen.

2. Experimental

2.1. Preparation of acylated collagen

The preparation of acylated collagen could be carried out by two steps, which had been described by the authors in previous report (Scheme 1) [9]. Briefly, 1.0 g native collagen sponge was added into 100 mL dimethyl sulfoxide (DMSO) containing two equivalent of triethyl amine, then 1.0 g lauroyl chloride (Sigma-Aldrich, St. Louis, MO) was added in the reaction system for stirring around 24 h at 20 °C and the intermediate product was dialyzed against 0.1 mol/L acetic acid for 3 days. Subsequently, DMSO solution (10 mL) containing 0.2 g succinic anhydride (Sigma-Aldrich, St. Louis, MO) was added into the intermediate product solution with a constant pH value of 9.0 for stirring about 4 h. Finally, acylated collagen was dialyzed against with deionized water for 3 days. The purified acylated collagen solution was lyophilized by a freeze dryer at −50 °C for 2 days and stored at 4 °C until used.

2.2. Amino acid analysis

Lyophilized acylated and native collagens (10–20 mg) were hydrolyzed in 6 mol/L HCl at 110 °C for 24 h. The hydrolysate was vaporized and the residues were dissolved in 25 mL citric acid buffer solution. After that, an aliquot of 50 μL was applied to an amino acid analyzer (Hitachi L-8900 Amino Acid Analyzer, Japan).

2.3. CD studies

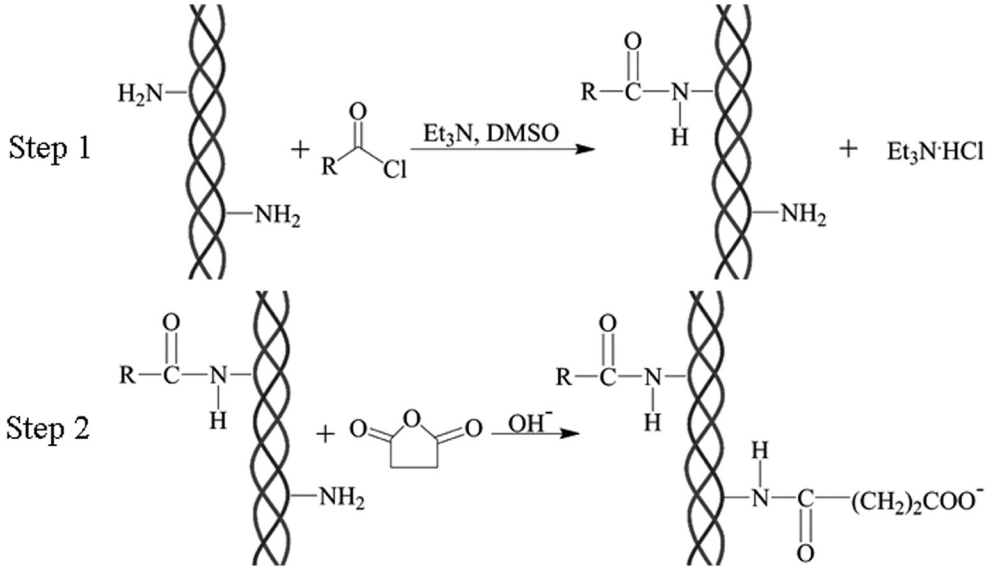
CD experiments of native and acylated collagens were carried out by the method of Fathima and Dhathathreyan with slight modification [22]. Lyophilized acylated collagen and native collagen were dissolved in 0.01 mol/L phosphate saline buffer (PBS, pH 7.4) and 0.01 mol/L acetic acid solution respectively, to obtain the concentrations of 0.1 mg/mL. Native and acylated collagen solutions were scanned in a wavelength range from 190 to 250 nm at 25 °C and their molar ellipticity [θ] was recorded using a circular dichroism apparatus (CD spectrometer model 400, AVIV, USA). The reference spectrum containing acetic acid and PBS with the concentrations of 0.01 mol/L was also recorded. The CD spectra of native and acylated collagen solutions were obtained after subtracting the reference spectrum of acetic acid and PBS, respectively.

2.4. XRD analysis

The XRD analysis patterns of lyophilized acylated and native collagens were recorded on an X-ray diffractometer (DX-100, Fangyuan Instrument Co., Ltd, Dandong, China) with Cu Kα radiation (λ = 0.1541 nm) at 40 kV. The 2θ range was from 4 to 40°, and the electricity was 25 mA.

2.5. TG measurements

Lyophilized native and acylated collagens with the quantity of approximate 4 mg were sealed in crucibles. Then, thermal weight loss of the specimen was determined by a thermogravimetric analyzer (Netzsch TG 209F1, Germany) from 40 to 600 °C at a heating rate of 5 °C/min under nitrogen atmosphere.



Scheme 1. The schematic illustration of acylation of native collagen with lauroyl chloride and succinic anhydride. R: $CH_3(CH_2)_9CH_2-$ [9].