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Modification of collagen with a natural cross-linker, procyanidin

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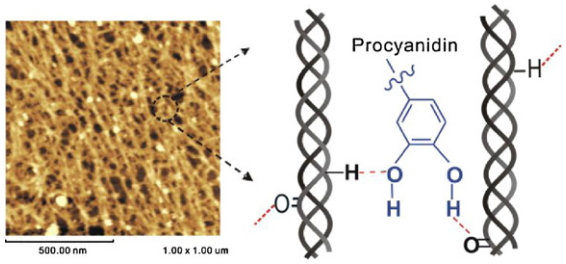
ABSTRACT

We have investigated the modification of collagen with a natural plant polyphenol, procyanidin under acidic conditions. Fourier transform infrared spectroscopy (FTIR) and Atomic force microscopy (AFM) studies demonstrate that the hydrogen bond interactions between collagen and procyanidin does not destroy the triple helix conformation of collagen, and the fibril aggregation occurs because of the cross-linking with procyanidin. The water contact angle (WCA) tests indicate that the hydrophobicity of the procyanidin modified collagen films can be improved. Whereas, the water vapor permeability (WVP) of the films decrease with the increasing procyanidin content due to the formation of denser structure. Moreover, differential scanning calorimetry (DSC) and thermogravimetric (TG) measurements reveal that the collagen/procyanidin films have improved thermal stability in comparison with pure collagen. The present study reveals that procyanidin stabilizes collagen as a cross-linker and preserves its triple helical structure.



1. Introduction

Collagen is nowadays one of the most widely investigated proteins, because it not only represents the main structural protein accounting for approximately one-third of all vertebrate body proteins [1], but also has commercial and industrial significance, as exemplified by traditional leather industry and current biomedical applications [2–4]. Generally, the isolated collagen from skin or tendon exhibits poor thermal stability, mechanical strength and water resistance, due to the destruction of natural cross-linking and assembly structure by neutral salt, acid, alkali, or proteases during the extraction process [5]. Therefore, they are often modified for practical uses by various methods, such as UV-light irradiation, multivalent metal ions, and synthetic aldehyde-based cross-linkers [6,7]. However, the treatment by UV only modifies the surface instead of the bulk of the collagen [8], and the toxic nature of aldehyde compounds reflected in the process of manufacturing and application prohibits their utilization in food and biomaterials [9–11]. Thus, some natural polymers with favorable biocompatibility have been exploited as protein cross-linkers, such as genipin, oxidized alginate and dialdehyde starch [7,12,13].

Procyanidin is a kind of condensed plant polyphenol, ubiquitously found in vegetables and fruits [14]. Due to its free-radical scavenging capacity and high affinity to protein, procyanidin has gained recent interest in dietary supplement and pharmacological applications. And the benefit of antioxidant activity, pharmacological activity and therapeutic potential from procyanidin has been extensively manifested [15,16]. In fact, natural plant polyphenols including procyanidin have long been used to treat animal hide collagen under acidic conditions and confer enhanced stability

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against heat and putrefaction to the resultant leather [17–19]. In practical leather-making, the required amounts of the vegetable materials can be 10–40% (w/w) depend on its affinity for hide substance [20]. The interactions between protein and polyphenol can involve hydrogen bond, covalent linkage, ionic and hydrophobic bonding [21–26]. Nevertheless, the effect of plant polyphenol on the microstructure of collagen, i.e. from triple helixes to fibrils, remains largely unknown. It has been well documented that the most abundant Type I collagen comprises two identical α1(I) chains and a different α2(I) chain. The three α-chains twist together into a unique triple helical molecule of ~300 nm in length and ~1.5 nm in diameter. The quarter staggered arrangement of collagen molecules leads to collagen microfibrils (~40 nm in diameter) and fibrils (100–200 nm in diameter), and the fibrils further assemble into collagen fibres [27]. As increasing use of collagen in biomedical applications, understanding the role of procanidin in the modification of collagen structure and property is helpful to development of new biological cross-linkers and fabrication of novel functionalized biomaterials [2].

In the present work, we have investigated the structure of Type I collagen molecules and microfibrils in the presence of procyanidin by Fourier transform infrared spectroscopy (FTIR) and atomic force microscopy (AFM). The surface hydrophilicity/hydrophobicity, water vapor permeability and thermal stability of the collagen/procyanidin films have also been examined. Our aim is to further explore collagen-polyphenol interactions and understand the structure-property relations of procyanidin modified collagen films.

2. Experimental

2.1. Materials

The acid soluble collagen used in this work was extracted from the fresh adult bovine Achilles tendon. The details can be found in our previous report [28]. The analysis of the extracted collagen by SDS-PAGE (Bio-Rad Powerpac 300, USA) has been made in our laboratory to verify its purity and structural integrity (Fig. 1) [29]. The isoelectric point for collagen is at pH 7.4 as measured by potentiometric titration method using Nano ZS instrument (Malvern Co., UK). The procyanidin, grape seed extract, was obtained from Tianjing Jian Feng Co. Ltd., China. Widely present procyanidin monomer (catechin or epicatechin) and dimmer are illustratively shown in Fig. 2. The content of oligomer procyanidin was more than 85.0% [30].

2.2. Preparation of film

After a 5 mg mL^{-1} aqueous collagen solution was prepared, procyanidin solution with the concentration 0.1–0.4 mg mL^{-1} was incorporated under magnetic stirring. Due to the weak acidic nature of procyanidin and the reasonable solubility of collagen in weak acids, the reaction pH of the mixture was adjusted to 3.0 with acetic acid solution. The final contents for procyanidin were 0%, 2%, 4%, 6% and 8% (w/w on dry collagen mass), respectively. And the collagen concentration was kept at 2.5 mg mL^{-1} for all the samples. Subsequently, the mixture was placed under mild ultrasonic (40 kHz, 120 W) for about 1 min to remove air bubbles and then several drops of ethanol was added to further eliminate air bubbles [31]. The collagen/procyanidin films with thickness around 0.03 mm were formed by casting the solution on a polytetrafluoroethylene (PTFE) plate with a diameter of 15 cm then dried at room temperature for about 1 week.

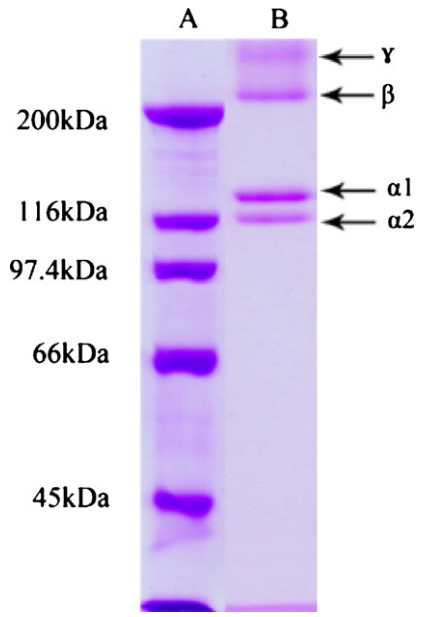

Fig. 1. SDS-PAGE analysis of the extracted collagen (B) in comparison with protein markers (A). The protein bands were visualized by staining with 0.1% Coomassie Brilliant Blue R-250.

2.3. FTIR spectra measurements

Fourier transform infrared spectroscopy (FTIR) spectra were obtained from the films equilibrated in a desiccator containing silica gel for 24 h at room temperature by a FTIR spectrometer (Nicolet Magna-IR560, USA). All spectra were obtained with a resolution of 4 cm^{-1} in the range of 400–4000 cm^{-1}. The spectra plots represent the average of 10 scans.

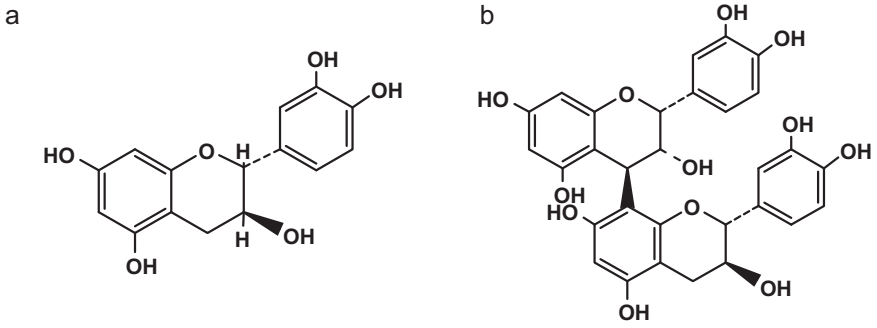

Fig. 2. Widely present procyanidin monomer (a) and dimmer (b).