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Interaction study of collagen and sericin in blending solution

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ABSTRACT

The interactions of collagen and sericin were studied by fluorescence spectra, ultraviolet spectra, FTIR spectra and dynamic light scattering. The fluorescence quenching in emission spectra and red-shift (283–330 nm) in synchronous fluorescence spectra suggested the Tyr of collagen and sericin overlapped with a distance of 3 Å, generating excimer. The overlapped Tyr of collagen and sericin decreased the hydrophobicity of collagen, which resulted in the red-shifts (233–240 nm) in ultraviolet spectra. Moreover, the red-shifts of amide bands of collagen in FTIR spectra indicated the hydrogen bonds of collagen were weaken and it could also be explained by the overlapped Tyr. The results of 2D-FTIR spectra demonstrated the backbone of collagen molecule was varied and the most susceptible structure of collagen was the triple helix with the presence of sericin. Based on dynamic light scattering, we conjectured large pure collagen aggregates were replaced by hybrid aggregates of collagen and sericin particles after the addition of sericin. With ascending sericin ratio, the diameters of the hybrid aggregates increased and attained maximum with 60% ratio of sericin, which were on account of the increasing excimer number. The results of DSC demonstrated the presence of sericin enhanced the thermal stability of collagen.

1. Introduction

As an extracellular matrix protein, collagen have been found in many tissues, such as skin, bone, tendon and blood vessels [1]. Collagen is widely used in medicines, foods and tissue engineering due to the good biological activity and low immunogenicity [2–5]. Collagen is usually mixed with other materials to enhance its advantages or overcome instinctive defects such as low thermal stability and mechanical properties for application. Especially, biopolymers, such as fibroin, hyaluronic acid and chondroitin sulfate, are often adopted to blend with collagen. The addition of fibroin increased the storage modulus (G′) of collagen gels from about 1kPa to 10 kPa [6] while it improved the biocompatible and water content of collagen membrane [7]. The thermal stability and swelling capacity of collagen scaffold were improved by the presence of hyaluronic acid [8]. The report of Tian et al. [9] showed that the thermal stability of collagen fibrils could be increased with the ascending dose of chondroitin sulfate. Mixed scaffold consisted of collagen and chondroitin sulfate had a better performance in promoting proliferation of chondrocytes than pure collagen scaffold in vitro [10].

Sericin, a water-soluble protein produced by silkworms, is being the glue that wraps the fibroin fibers and forms the silk [11,12]. The previous reports about sericin illustrated that sericin was a hydrophilic, biodegradable, antibacterial, biocompatible, antioxidative and negatively-charged material [13,14]. Sericin promoted the proliferation of human epithelial Hela cells or fibroblasts and prevented the apoptosis, which was ultraviolet induced, in human skin keratinocytes [15–17]. Because of the high solubility and weak structural properties, Sericin was often blended with natural polymer, such as collagen, to prepare membranes, scaffolds and hydrogels for cosmetics, tissue engineering and wound dressing application. The research of Mitran et al. showed collagen hydrogel with 40% sericin had the best results of preadipocyte proliferation status and survival while both the hydrogels with 40% and 20% sericin had a better results than hydrogels of pure collagen [18]. Bi et al. [19] prepared a scaffold consisted of collagen and sericin for the reconstruction of anterior cruciate ligament (ACL), the results indicated that the scaffold was appropriate for the reconstruction of ACL in a rabbit model and had potential for clinical application. Akturk et al. [20] investigated the wound dressing membranes of collagen and sericin, found the hybrid membranes, which had a less or equal sericin dose to collagen component, would be com-

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mendable as wound dressing materials. Vulpe et al. [21] produced a hydrogels consisted of collagen, sericin and hyaluronic acid, the results indicated the addition of sericin improved the biocompatibility of gels.

The hybrid membranes, scaffolds or hydrogels mentioned above were usually produced from the blending solutions of collagen and sericin [19–21]. Their properties were closely related to the blending solutions. For examples, the structure of collagen sponges were determined by the concentration of collagen solutions: collagen sponges obtained from 1.0% and 0.5% collagen solutions had a porous structure while those obtained from 1.5% collagen solution were nonporous [22]. The collagen sponges prepared from the solution with 50% hydroxypropyl methylcellulose had a more compact structure and a less homogenous morphology than pure collagen sponges [23]. However, the blending solution of collagen and sericin had not attracted much attention: the interactions between collagen and sericin in solution had not been identified; the properties of blending solutions with different ratio lacked sufficient characterization yet. In the present work, collagen solution and sericin solution mixed with different ratios. Fluorescence spectroscopy, ultraviolet spectroscopy, Fourier transform infrared spectroscopy and dynamic lighting scattering were adopted to investigate the interactions between collagen and sericin. Moreover, the material properties of the mixtures would be systematically measured and analyzed in future study. We expected that the experimental data may provided theoretical basis for the optimizing of manufacturing and acquirement of stable hybrid product.

2. Experiments and methods

2.1. Materials

Calf skin and silkworm cocoons were purchased from a market in Beibei district, Chongqing municipalities. Lyophilized type I collagen was prepared from calf skin according to the method of Zhang et al. [24] while lyophilized sericin was extracted from cocoons according to the method of Aramwit [25]. The results of SDS-PAGE displayed the collagen consisted of two α bands (about 100 kDa for α1 and α2) and one β band (about 200 kDa) while sericin was a mixture of different polypeptide chains, ranging in size from about 40–150 kDa, similar with the results reported by Zhang [24] and Aramwit [25].

2.2. Preparation of blending solution

Lyophilized collagen and sericin were dissolved in acetic acid (0.5 mol/L) respectively. Collagen solution and sericin solution with the concentration of 1 mg/mL were obtained and then mixed with the volume ratio of 10: 0, 8: 2, 6: 4, 4: 6, 2: 8 and 0: 10. The solutions were mixed uniformly by stirring. Then, the samples were stored at 4 °C for 48 h before measurement.

2.3. Fluorescence spectra

The fluorescence spectra were measured by a fluorescence spectrophotometer (F-4600, Hitachi, Japan), with a scanning rate of 120 nm/min. Each solution was equilibrated for 20 min before the scanning at room temperature (20 °C). The emission and the excitation slit openings were both set as 5 nm. The solutions were excited at 275 nm and the emission spectra of 290–350 nm were collected for fluorescence emission spectra. The initial wavelength was set at 250 nm while Δl was 12 nm for the synchronous fluorescence spectra.

2.4. Ultraviolet spectra

The ultraviolet spectra of the blending solutions were recorded in the region of 220–400 nm by a spectrophotometer (Lambda 25, PerkinElmer, USA) at room temperature.

2.5. FTIR spectra

The blending solutions were transferred onto the polythene disc and then air dried into membranes at room temperature to perform the Fourier transform infrared spectroscopy (FTIR) measurement. For removing the residual acetic acid, the membranes were soaked in distilled water for 3 days. Then, the membranes were dried and stored in a silica gel desiccator before test. The FTIR spectra with a resolution of 4 cm^{-1} were recorder in the range of 400–4000 cm^{-1} by Nicolet iS10 spectrometer (Thermo Fisher Scientific, USA).

Two-dimensional (2D) correlation spectra were derived from the data of FTIR spectra with a software named 2D shige (developed by Shigeaki Morita and Yukihiro Ozaki, Kwansei Gakuin University, Japan). The dashed and solid lines in 2D spectra represented negative and positive correlation intensities, respectively.

2.6. Dynamic light scattering (DLS)

DLS measurements were performed with BI-200SM goniometer and a BI-9000AT digital correlator (Brookhaven, USA) equipped with an Argon laser. DLS were taken at room temperature and the scattering angle was set at 90°. The data of DLS were analyzed with the Brookhaven software.

2.7. Diifferential scanning calorimetry (DSC)

The thermal stabilities of collagen solutions with different dose of sericin were measured by DSC (Netzsch DSC 214, Germany). The concentration of the previous samples (1 mg/mL) were too low to observe the transition temperature (T_m) of collagen. In this case, we improved the concentration to 5 mg/mL in DSC measurement. Approximately 5 mg samples was hermetically encapsulated in aluminum pans and an equal weight of solvent in anther pan was used as the reference. The DSC curves of the samples were recorded from 30 to 55 °C at a scanning rate of 5 °C/min in a nitrogen atmosphere.

3. Results and discussion

3.1. Fluorescence spectra analysis

In proteins, phenylalnine (Phe), tyrosine (Tyr) and tryptophan (Try) were fluorescent because of the aromatic ring in their structure [26]. The works of Li. et al. [27] and Wu. et. al [28] suggested that the intrinsic fluorescence of collagen, which provided considerable information about folding and association reactions, was derived from Phe and Tyr. Fig. 1a showed the emission fluorescence spectra of blending solutions with an excitation wavelength of 275 nm. For pure collagen solution, the maxima peak at 306 nm was attributed to the Tyr while the excitation wavelength was 275 nm [29]. With the increasing ratio of sericin, the peak derived from Tyr of collagen shifted towards the longer wavelength side obviously (306–318 nm). According to the reports of Royer, the shift of fluorescence spectra was referred to the variation of hydrophobicity and protein folding [26]. The red-shift of emission peak indicated sericin decreased the hydrophobic property of microenviroment of collagen while the peptides of collagen became more stretch. Besides, Fig. 1a showed that the fluorescence quenching occurred with the ascending sericin dose. Nevertheless, there were several possible reasons for the fluorescence quenching, such as the wrapping of Tyr by the addition of sericin, the decreased concentration