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DSC studies on bound water in silk fibroin/S-carboxymethyl kerateine blend films

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Abstract

The physical state of water, absorbed in silk fibroin (SF)/S-carboxymethyl kerateine (SCMK) blend films, was studied by differential scanning calorimetry (DSC) to investigate the change of internal structures of the blend films. The amount of non-freezing bound water in the blend film was decreased with the addition of SCMK because of the change in the secondary structure of SF from random coil form to β-structure form. This transition could be also monitored by thermal analysis using DSC. The maximum change of the secondary structure was observed at a blend ratio of 50/50 (wt/wt). © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Non-freezing bound water; S-carboxymethyl kerateine; Silk fibroin

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1. Introduction

Silk fibroin (SF) and wool keratin have drawn much attention as biomaterials for their natural origin and biocompatibility [1,2]. SF is largely composed of nonpolar amino acids and adopts a variety of secondary structures. The presence and amounts of the particular secondary structures of SF can be controlled by stretching, compression, annealing, and/or chemical treatments. This change of secondary structure has been applied to the formation of stable and thin SF films [3,4]. Wool keratin is one of the most representative fibrous proteins having many polar amino acids components. Since keratin is regarded as a three-dimensionally inter-crosslinked polymer by disulfide bonds, the cleavage of disulfide bonds improves the solubility of the wool keratin. There have been many reports on various kinds of keratin derivatives in terms of the reactivity of solubilized keratin [5–7].

The state of water absorbed in polymeric systems has been widely investigated using various techniques [8–12]. Water in polymeric systems is known to be affected by the specific interaction with polymer chains or the internal structure of the polymer matrix. Therefore, studies on the physical state of water absorbed in polymeric systems may provide useful suggestions on their internal structure. Specifically, water that does not freeze as a result of the strong interaction with polymer systems has been called ‘non-freezing bound water’ [13]. The amount of non-freezing bound water may be closely related to the internal structure of polymeric systems.

In this work, blend films of SF and S-carboxymethyl kerateine (SCMK) were prepared and the physical state of water absorbed in the blend films was investigated using differential scanning calorimetry (DSC). It was conjectured that the state and amounts of non-freezing bound water might be one of the potent indicators to confirm the internal structure of the blend films.

2. Experimental

SF was extracted from commercial raw silk using a solution of calcium chloride/ethanol/distilled water (1:2:8 mole ratio) at 70°C. After dialysis with cellulose tabular membranes (Sigma Co., 250-7 μ) in distilled water for 3 days, the fibroin solution was lyophilized [14]. SCMK was obtained by the reduction of Merino wool using 2-mercaptoethanol at pH 10.5 under an atmosphere of nitrogen [15]. Iodoacetic acid was added to prevent the reoxidation between thiol groups at pH 8.5. After dialysis in the same manner of the SF solution, the SCMK solution was poured into the chilled acetone (−10°C) and the precipitates were lyophilized. The SF/SCMK blend film was prepared by casting a 2% mixed solution of SF and SCMK in formic acid onto a Teflon plate. The blend ratio was expressed as a weight ratio (wt/wt).

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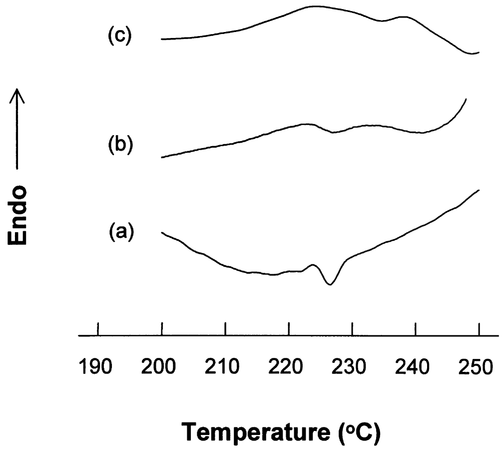

Fig. 1. DSC curves of (a) SF; (b) SF/SCMK = 50/50; and (c) SCMK film.

Thermal analysis of the dried films was performed on a Perkin-Elmer DSC7 at a heating rate of 10°C $\min^{-1}$. The transition of water absorbed in SF/SCMK blend films was also investigated by a Perkin-Elmer DSC7 equipped with a cooling apparatus [9,13]. The blend films were sufficiently immersed in deionized water and external surface water was removed by blotting with filter papers. Water content (W_c) was defined as following:

$$W_c = \text{weight of added water (g)/weight of dry sample (g)} \quad (1)$$

The hydrated film was carefully sealed in a hermetic aluminum pan that had been pretreated in boiling water for 1 h. The temperature range was −60°C ~ 50°C. The heating and cooling rate was 5°C $\min^{-1}$.

3. Results and discussion

DSC curves of the SF/SCMK blend films are given in Fig. 1. The DSC curve of SF exhibits an endothermic peak at 223°C and an exothermic peak at 227°C. The endothermic peak at 223°C corresponds to the weakening of interhelical interactions in the α-helix of SF [Fig. 1(a)]. The exothermic peak at 227°C can be attributed to the transition from random coil to β-structure of SF and can be diminished by treatments of polar solvents such as methanol [16]. In case of the SF/SCMK blend film [Fig. 1(b)], the exothermic peak of SF at 227°C is weakened and the transition peaks of α- and β-structure of keratin are still remained at 224°C and 238°C, respectively [17]. This means that the structural change of SF from random coil form to β-structure has occurred because of additional SCMK.

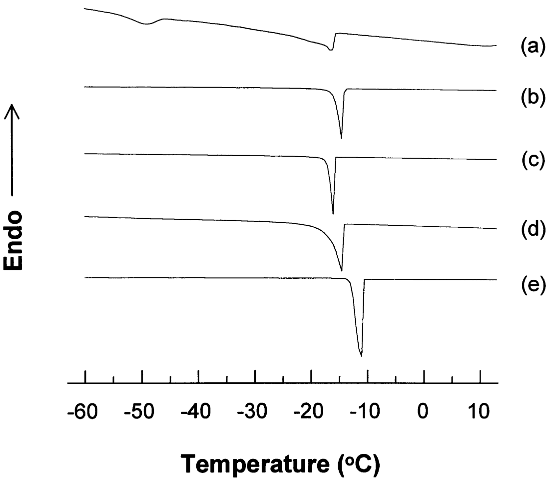

Fig. 2. DSC cooling curves of fully hydrated SF/SCMK blend films (cooling rate: 5°C $\min^{-1}$): (a) SF only; (b) SF/SCMK = 75/25; (c) 50/50; (d) 25/75; (e) SCMK only.

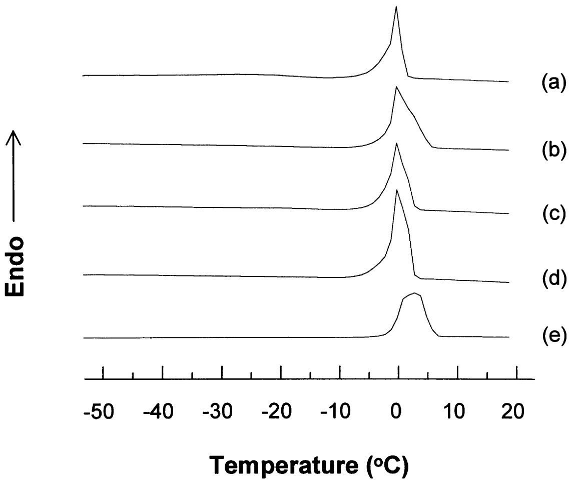

Fig. 3. DSC heating curves of fully hydrated SF/SCMK blend films (heating rate: 5°C $\min^{-1}$): (a) SF only; (b) SF/SCMK = 75/25; (c) 50/50; (d) 25/75; (e) SCMK only.

It is well known that the conformational transition in secondary structure of SF from random coil form to silk II crystalline form (antiparallel β-sheet) occurs by treatments such as stretching, heating, and/or immersion in polar solvents [18]. This transition can be monitored by various spectroscopic methods including FTIR spectroscopy [18–20]. The characteristic IR absorption bands of β-sheet form for the blend films were observed at 1623 (amide I), 1520 (amide II), and 1260 cm^{-1} (amide III). The absorption bands at 1650 (amide I), 1538 (amide II), and 1234 cm^{-1} (amide III) were assigned to random coil conformation. The characteristic absorption bands of β-sheet form of SF increased with addition of SCMK, resulting in the increase of crystallinity [14].

Water in polymeric systems can be classified into three species: freezing free water, freezing bound water, and non-freezing bound water. Non-freezing bound water is affected by the strong interaction with polymer systems and has no detectable phase transition. Freezing bound water, in other words, freezing intermediate water, has a phase transition at lower than 0°C. This can be attributed to the weak interaction between water and polymer. Freezing free water has a same transition temperature at 0°C as bulk water [21,22].

Table 1
Water content (W_c), the ratio of non-freezing bound water (W^c_{nf}/W^h_{nf}), and the difference of W_{nf} values (ΔW_{nf}) in SF/SCMK blend films

SCMK content (%)	W_c	W^c_{nf}/W^h_{nf}	ΔW^c_{nf}	ΔW^h_{nf}
0	0.78	1.06	–	–
25	0.54	1.00	0.46	0.34
50	1.44	1.13	0.72	0.60
75	1.98	1.12	0.19	0.07
100	14.33	1.22	–	–

DSC measurements of the blend films, fully hydrated in water, were performed and the results are shown in Figs. 2 and 3. During the cooling cycle (Fig. 2) one sharp exothermic peak is observed except the SF film. This peak can be attributed to the freezing free water. But water absorbed in the SF film shows two different exothermic peaks. One is for freezing of free water and the other is for freezing of freezable bound water. During the heating cycle (Fig. 3) one endothermic peak of a similar shape is observed.

The sum of freezing free water and freezing bound water was regarded as freezable water. The amount of freezable water (W_f) was calculated by using the transition enthalpy of water and corrected by weight of polymer matrix. Since the transition of bulk water under cooling and the freezing bound water occurs at lower than 0°C, the transition enthalpy should be corrected as following:

$$\Delta H(T) = \Delta H(0) - \int_T^0 \Delta C_p \mathrm{d}T \tag{2}$$

where ΔC_p is the difference of heat capacities between ice and supercooled water or freezing bound water in the polymer matrix [22,23]. The amount of non-freezing bound water (W_{nf}) can be obtained by subtraction of W_f from W_c. Water content (W_c) and the ratio of W_{nf} during cooling (W^c_{nf}) and heating (W^h_{nf}) cycles are listed in Table 1. Among three types of water in polymer systems, non-freezing bound water is considered to be closely related to the polymer chains and the change of their structures [24]. Therefore, we can calculate ΔW_{nf}, the difference of W_{nf} values between the calculated and the experimental ones.

$$\Delta W_{nf} = (W_{nf})_{calc} - (W_{nf})_{exp} \tag{3}$$

$$(W_{nf})_{calc} = c_1(W_{nf})_1 + c_2(W_{nf})_2 \tag{4}$$

where c_1 and c_2 are the weight fractions of polymers 1 and 2, respectively. If there is no structural changes in SF/SCMK blend films, W_{nf} may linearly increase with increasing blend ratios because of high polar properties of SCMK. But in SF/SCMK blends the structural change occurs and experimentally obtained W_{nf} values decrease. Therefore, increasing ΔW_{nf} values during cooling (ΔW^c_{nf}) or heating (ΔW^h_{nf}) cycles are observed (Table 1). As illustrated in Fig. 4, since the change from random coil form to the β-structure of SF induces an increase of silk II crystalline structure, $(W_{nf})_{exp}$ values are lower than the calculated ones.

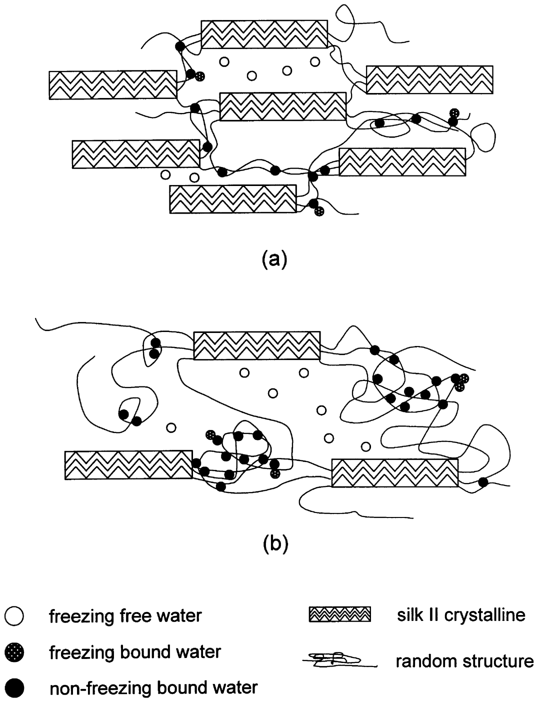

Fig. 4. Schematic description of water absorbed in (a) silk II crystalline-rich film and (b) silk II crystalline-poor film.

4. Conclusions

The change of secondary structure of the SF/SCMK blend films could be characterized by thermal analysis using DSC. The characterization of non-freezing bound water, absorbed in the blend films also indicated the change of the internal structure of the blend films indirectly. Since the transition from random coil form to β-sheet form of SF occurred by blending with SCMK, it was considered that SCMK plays a similar role of a polar solvent because of abundant polar groups in amino acid composition.

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