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What causes the unfrozen water in polymers: hydrogen bonds between water and polymer chains?

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Abstract

Differential scanning calorimetry (DSC), positron annihilation lifetime spectroscopy (PALS), and NMR technique were used to explore the origin of unfrozen bound water in gelatin samples with various water contents. The results reveal that the content of unfrozen bound water (W_b) increases upon increasing water content up to 3.75 g water/g gelatin, above which, W_b declines. Moreover at 50 g water/g gelatin, no unfrozen bound water was detected. It is proposed that the 'nanocavity' in polymers is an important reason for the formation of unfrozen water; hydrogen-bonding is not the 'exclusive' factor influencing water crystallization, and hydrogen-bond bound water is only one of the different physical states of water in polymers. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Hydrogen bond; Unfrozen water; Nanocavity

The different physical states of water in polymers have provoked keen interest in many researchers over the past decades [1]. In general, water in a polymer is considered to be categorized into three types, "free water", which freezes at the usual freezing point; "intermediate water", which freezes at temperature lower than the usual freezing point; and "unfrozen bound water", which cannot freeze at the usual freezing point [2,3]. It has been experimentally verified that unfrozen bound water plays an important role in biological systems. For example, unfrozen bound water can contribute favorably to the conformational stability of protein [4]; the enzyme activity and drug delivery behavior are dependent upon the population of unfrozen bound water [5]. However, there still exist conflicting views regarding the formation mechanism of unfrozen bound water in a wealth of literature [6–9]. The frequently applied concept is that unfrozen bound water is formed by the hydrogen bonds between water molecules and polar groups in the polymer.

Recently, we examined the variation of unfrozen bound water in gelatin containing different amounts of water by differential scanning calorimeter (DSC). The experimental data made us think that it is necessary to re-explore the hydrogen-bond formation mechanism of unfrozen bound water in a polymer.

Gelatin (type B, extracted from bovine skin) was added to doubly de-ionized and distilled water and dissolved at 40°C to produce a series of solutions with different water contents. The gelatin–water solutions were cooled at 25°C until they formed gels. The maximum water content (W_m) guaranteed that gelatin was in sol state at 25°C. In this experiment, W_m was 50 g water/g gelatin. The gelatin samples were hermetically sealed in aluminum pans and the thermal transition curves were recorded on a Perkin–Elmer DSC-7 differential scanning calorimeter. The temperature ranged from 220 to 310 K and the heating rate was 5 K/min. The detailed experimental process has been reported in our previous paper [10]. Fig. 1 shows the DSC thermograms of gelatin samples with various water contents. It can be seen that the endothermic peaks have different shapes at different water contents. At 2.43 and 2.82 g/g water contents, there appear two endothermic peaks, one around 273 K and the other near 269 K. With water content ranging from 3.23 to 50.0, only one single endothermic peak is centered around 273 K. In order to check whether the thermal transition of gelatin itself imposes an effect on that of water, we recorded the DSC curve of dry gelatin (not shown) under the same conditions. In the given range of temperature, no thermal transitions of the gelatin itself were observed. Thus, it is reasonable to think that the endothermic peaks resulted from water itself. Based on the representative three-state-water model [11], the melting peak at lower temperature is due to the existence

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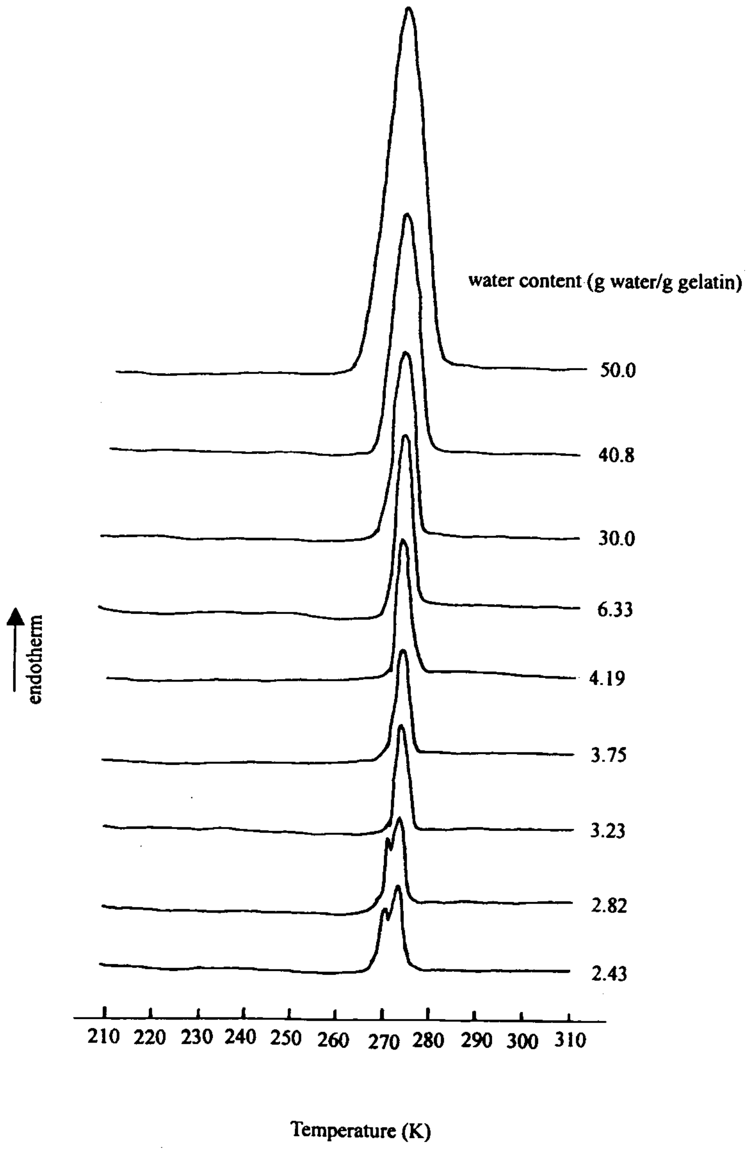

Fig. 1. DSC thermograms of gelatin samples with various water contents.

of intermediate water, whereas the peak at higher temperature is assigned to free water. In this study, to estimate the content of the different states of water, the fusion enthalpy of intermediate water is taken to be the same as that of free water (334 J/g) [12]. Since the endothermic peaks of intermediate and free water overlap, the heat of melting per unit sample weight calculated from the area of the melting peak is the total of two types of water. Herein, the calculated total content of free and intermediate water is defined as the content of frozen water (W_{tf}). The content of unfrozen bound water (W_b) is obtained by subtracting W_{tf} from total water content (W). Fig. 2 shows the variation in the contents of different states of water versus W. One can see that with the increase of W up to 3.75, W_b increases. Above this content, W_b decreases, and when W is 40.8, W_b diminishes drastically. It is noted that at 50, unfrozen bound water is equal to zero, and in this case, the gelatin is in a sol state. The result is somewhat surprising since on the basis of the hydrogen-bond formation mechanism of unfrozen bound water, W_b should increase with W, and should ultimately approach a maximum as the available specific groups such as C=O, –OH and –NH are all hydrogen-bonded by water molecules. Even in the sol state, there should exist unfrozen bound water because a large amount of water still interacts with the chains of dissolved gelatin. The above experimental facts allow one to question the concept that unfrozen bound water ‘merely’ originates from the hydrogen bonds between water and polar moieties in the polymer. It is well known that over a certain range of water content, the aqueous solution of gelatin can form physical gel by interaction of hydrogen bonds below 35°C [13]. In gel, there exist physical cross-linking points, which produce a variety of cavities. Some water molecules are buried in these cavities as “interstitial water”. Thus the volume of cavities

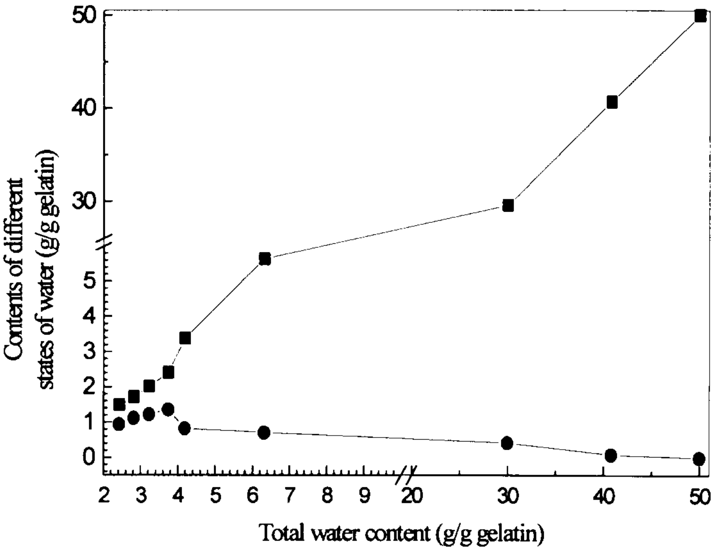

Fig. 2. Variation in the contents of different states of water versus W. (■) content of frozen water; (●)content of unfrozen bound water.

will exert an influence on the crystallization of buried water. Hori [14] found that when the distance between the glass walls was 1 μm, the freezing point of water between the glass walls was around −100°C. Raman spectroscopic study on water in gelatin gels demonstrated that the structure of water was perturbed by the entanglement of chain segments [15]. It has been corroborated experimentally that as the gelatin is in gelation, it partly renatures to form triple-stranded helical rods 15 Å in diameter; X-ray diagrams show that the rods are packed in parallel and form channel-like interstices, which cannot be more than several Angstroms long [16].

In this experiment, we measured the cavity size of gelatin samples with various water contents by positron annihilation lifetime spectroscopy (PALS). The PALS measurements were made using an EG&G Ortec fast–slow coincidence system with a ^{22}Na resolution of 250 ps. Data were collected at 22°C using a 20-μCi ^{22}Na kapton source sandwiched between two identical pieces of the sample. $1–2 \times 10^6$ counts were collected for each spectrum. The lifetime spectra were resolved into three components by the PATFIT-88 program. The o-Ps pickoff lifetime (τ_3) is related to the Ps cavity size and the intensity of Ps (I_3) is directly proportional to the content of free volume. τ_3 is estimated based on the following equation [17]

$$\tau_3^{-1} = 2\left[1 - \frac{R}{R_0} + \frac{1}{2\pi}\sin\left(\frac{2\pi R}{R_0}\right)\right] \tag{1}$$

The cavity volume is expressed as

$$V_c = \frac{4\pi R^3}{3} \tag{2}$$

The values of V_c and I_3 determined at various water contents are listed in Table 1. Their variation trend will be discussed later. First, it is noted that the values of V_c determined all fall within the range of nanometers. It is surmised that the mobility of water entrapped in this ‘nano-cavity’ will be vastly different from that of free water. In view of this, we used NMR to check the spin–lattice relaxation time of water in gelatin with various water contents. NMR experiments were carried out on a Varian Unity Plus-400 NMR spectrometer operating at a Lamor frequency of 400 MHz for protons. Gelatin was dissolved in different amounts of water at 40°C, and a certain amount of gelatin solution was injected into the NMR tubes. The solutions were cooled to the room temperature. Then the spin–lattice relaxation times (T_1) were determined at 298 K by means of the inversion recovery method [180°–t (variable)–90°–FID] using a solid-state probe with magic-angle spinning (MAS). The spinning rate was 3 kHz. A typical 90° pulse width for ^{1}H was 5 μs. In the experiment, only a single T_1 value was measured owing to the existence of a rapid exchange between different spin nuclei. Based on the Zimmermann–Brittin formula [18], the average spin–lattice relaxation time for different states of water can be

Table 1
The cavity volume and intensity of o-Ps determined at various water contents

Total water content (g/g gelatin)	V_c (nm^3)	I_3 (%)
2.43	0.072	14.2
2.82	0.081	16.6
3.23	0.146	18.5
3.75	0.154	19.6
4.19	0.155	20.2
6.33	0.156	22.3
30.0	0.155	25.2
40.8	0.157	27.4
50.0	0.157	29.1

written as

$$\frac{1}{T_1} = \frac{F}{T_{1F}} + \frac{I}{T_{1I}} + \frac{B}{T_{1B}} \quad (3)$$

where F, I, and B are the fraction of free, intermediate and bound water in gel, and T_{1F}, T_{1I}, T_{1B} are the spin–lattice relaxation times for free, intermediate and bound water, respectively.

In order to estimate T_1, four total water contents were selected, i.e. 2.43, 2.82, 3.23, and 3.75 g water/g gelatin, and the obtained values of T_1 are 0.62, 0.66, 0.70, and 0.72 s, respectively. T_{1F} is taken as that of pure water, 4.50 s [19]. In our previous work [20], T_{1I} was estimated as 1.06 s. Obviously, the average value of spin–lattice relaxation times for free and intermediate water is 2.78 s. Combining DSC results, the average value of T_{1B} was deduced as 0.3 s, 15 times less than that of free water. Note that the value of spin–lattice relaxation time for different states of water is only a rough estimation. By contrast, a conclusion can be drawn that the mobility of ‘bound water’ is markedly reduced compared to that of free water. Kinetically, the ‘nanocavity’ will hinder the crystallization of buried water. Therefore, with the decrease in temperature, the rearrangement of ‘bound water’ is fettered, and consequently, inhibits the crystallization of this fraction of water.

From the above discussion, it is natural to question whether the so-called ‘unfrozen water’ ‘merely’ originates from hydrogen bonds. In using extended lattice-liquid-hydrogen bond theory to predict the variation in bound water during volume phase transition of poly(*N*-isopropyl acrylamide) hydrogel, Lele et al. [21] held that only a small amount of hydrogen-bond bound water exists in network, and a vast majority of bound water exists by physically binding to polymeric chains. However, the physical binding does not necessarily produce bound water because the water molecules on the hydrophobic surface tend to form clusters [22]. On the other hand, they did not investigate the thermal dynamic behaviour of physically binding water. On the basis of the three-state-water model, the hypothesis for the formation of bound water is that water molecules are hydrogen-bonded to the polar moieties of the polymer. The determination of bound water is based on the thermal transition of water in the DSC curve, i.e. when no thermal transition peak of water appears, the bound water is considered to be generated, and becomes unfrozen presumably due to the formation of hydrogen bonds between water and polar groups of the polymer. And hence, the bound water is equivalent to ‘unfrozen water’. However, there are a variety of factors influencing the unfreezing of water. Reutner et al. [23] calculated the bound water contents of 15.1, 26.2 and 34.7% under the condition that every amino acid residue in gelatin binds one, two and three water molecules, respectively. By contrast, the content of unfrozen bound water determined by us is as high as 57.4%. Surprisingly, at 50 g/g gelatin water content, the unfrozen bound water is zero. It is proposed that there are two possible factors causing this peculiarity. On one hand, at this water content, the chain segments of gelatin in sol state behave like a liquid with high mobility, which renders the water molecules hydrogen-bonded to polymer chains mobile, behaving just like free water. On the other hand, the trace of unfrozen water originated from hydrogen-bonding might be beyond the reach of DCS detection. From the above analyses, we argue that the concept that the unfrozen water stems ‘exclusively’ from the hydrogen bonds between water and polar moieties is open to question, and the ‘nanocavity’ in the polymer is one cause that cannot be neglected for the generation of ‘unfrozen water’. Of course the effect of hydrogen bonds on the crystallization of water cannot be ruled out either. The finding of this work allows one to realize that the hydrogen-bond bound water should not be entirely equal to the unfrozen water; it is only one of the different physical states of water in the polymer.

A careful observation of Table 1 reveals that V_c increases upon increasing water content, and levels off at 3.75 g/g; I_3 increases monotonically with the increase of water content until it reaches the value of pure water, 28 ± 2%. Hodge et al. [24] proposed that the annihilation of *o*-Ps is the sum of the contribution from free water and polymeric cavity volume. From the above result, it is speculated that the cavity volume and content goes up with an increase in water content, which results in an increase in the population of unfrozen water entrapped there. Hence W_b increases. At 3.75 g/g water content, polymer chains strengthen out to the fullest, and the cavity size of the polymer becomes constant, and at this content, all the cavities of gelatin network are occupied by water molecules; thus the cavity volume remains nearly constant, and W_b attains a maximum value. The variation trend of I_3 suggests that an increase in water content leads to an increase in the content of cavity volume; at a critical water content, most likely 3.75, some neighboring cavities tend to link together, reducing the content of cavity of polymer. Accordingly, the amount of water buried in the cavities is reduced. Therefore exceeding 3.75, W_b starts to decline, and more free water comes to play a part in the annihilation of *o*-Ps.

In conclusion, differential scanning calorimetry (DSC), positron annihilation lifetime spectroscopy (PALS) and NMR technique were employed to explore the origin of unfrozen bound water in gelatin samples with various water contents. The results reveal that the content of unfrozen bound water (W_b) increases upon increasing water content up to 3.75 g water/g gelatin, above which W_b declines. Moreover at 50 g water/g gelatin, no unfrozen bound water was detected. It is proposed that the ‘nanocavity’ in polymer is an important reason for the formation of unfrozen water; hydrogen-bonding is not the ‘exclusive’ factor influencing water crystallization, and it is more acceptable that hydrogen-bond bound water is only one of the different physical states of water in a polymer.

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