Journal of Thermal Analysis and Calorimetry, Vol. 92 (2008) 3, 711–716

DEHYDRATED NATIVE BIOPOLYMERS – A UNIQUE REPRESENTATIVE OF GLASSY SYSTEMS

G. I. Tsereteli, T. V. Belopolskaya and N. A. Grunina*

Institute for Physics named by V. A. Fock, St.-Petersburg State University, Ulyanovskaya 1, Peterhoff 198504, Russia

DSC study of native and denatured biopolymers with different chemical and steric structure was carried out in a wide range of temperatures and water contents. It was shown that all the native and denatured humid biopolymers studied are glassy systems. The residues of native structures surviving after partial dehydration prevent the glass transition at the glass transition temperatures of the denatured biopolymers. In dehydrated native biopolymers the processes of melting and glass transition take place in the same temperature range that leads to a large change of the heat capacity across denaturation.

Keywords: *dehydration, denaturation, denatured biopolymer, DSC, glass transition, native biopolymer, thermal destruction*

Introduction

As known, the glass transition is the main relaxation process occurring in amorphous or partially crystalline synthetic polymers [1]. We have reported in previous papers on calorimetric studies of biopolymers (proteins and nucleic acids) that thermally denatured humid biopolymers show typical signals of glass transition. It was found that the temperature of the glass transition of denatured biopolymer depends on the content of bound water which plays a role of natural plasticizer [2]. In present work we show that the glass transition has an important effect also on the thermal properties of native dehydrated biopolymers despite the fact that the characteristic jump of heat capacity is not detectable in the curves across the temperature range of the glass transition of the same biopolymers in denatured state. It should be noted that at the time when our first papers on the glass transition in denatured biopolymers were published [3, 4] it was found that the polysaccharides too can undergo a glass transition [5]. Later on, a large number of papers were published which were treating the thermal properties of starches that are the most abundant biopolymers in living organisms [6, 7].

The present work summarizes the results of our studies of the heat induced conformational and relaxation transitions in all the three main classes of biopolymers at different hydration degree. Detection of the excess heat capacity drop and absolute values of the heat capacity in a wide range of temperatures and water content allowed us to get a deeper insight in the nature of the processes occurring in these biopolymers. As a result, we were able to distinguish the individual features of the thermal properties related to the peculiarities of the steric structure for each biopolymer studied and assess common features for every biopolymer system undergoing a glass transition.

Experimental

All the studies were performed using the Setaram DSC-111 differential scanning microcalorimeter with sensitivity of $3 \cdot 10^{-5}$ J s^{-1}. Temperature was controlled with a precision not less than ±0.2°C over the whole measured interval –30–180°C. The errors relevant to the melting heat for the broad melting curves and to the absolute values of heat capacity were not more than 5 and 1%, respectively, for a 5°C min^{-1} heating rate.

Biopolymer–water systems with different water content from 10 to 75% were studied. To determine the actual water content, control samples were dried in vacuum at T=105°C until no change could be observed. The sample masses were varied from 50 to 120 mg. To obtain the uniform water distribution, the hydrated sealed samples were kept for one day at room temperature before the measurement. We have studied:

- globular proteins, like lysozyme (Lys), myoglobin (Mb), ribonuclease (RNase),
- fibrillar proteins, like elastin and calf thymus DNA, which were all supplied by 'Sigma',
- collagen that was prepared from rat-tail tendon in our laboratory,
- potato starch from 'Aldrich' (USA) and rice starch 'Lazurnyi' received from the RAS Institute for Biochemical Physics.

* Author for correspondence: Tatiana.Belopolskaya@pobox.spbu.ru

1388–6150/$20.00

Akadémiai Kiadó, Budapest, Hungary
Springer, Dordrecht, The Netherlands

Results and discussion

The results of the studies on the glass transition of humid native biopolymers can be better understood taking into account changes of the thermal properties of biopolymers that occur during their dehydration. Figures 1 and 2a show, that the values of denaturation heat (Q_m) and temperature (T_m) related to the endothermic maximum observed in every curve of native biopolymers change on varying the water from practically zero to beyond the threshold of the 'bound water', here referred to as the unfreezable water. The mass% of the bound water decreases in the following order: DNA (42–40%), proteins (27–25%) and starches (24–22%) with minor variations related to the sample origin. As it is seen from Fig. 1, in the region of high water content Q_m attains a constant value. Some exception can be observed for globular proteins, which tend to aggregate just after the unfolding [8]. It was shown, that in some biopolymers after the thermal destruction of native structures, the non-native ordered structures can be formed [9]. In most biopolymers (collagen, DNA, starches) this process occurs on decreasing the temperature below the denaturation threshold so favoring the formation of the so-called cold-set gels. In globular proteins the gels are formed at temperatures above the denaturation temperature (heat-set gels) [10].

The most sensitive changes of Q_m take place for water contents close to that of the bound water. This increased sensitivity reflects the fact that on dehydration produces severe modifications of the steric structure of the biopolymer. The biopolymers can be considered as really native (i.e. possessing the biological activity) only when surrounded by the water which stabilizes their steric structure. Our experimental data are in a good agreement with the results of other researchers for concentration ranges where the published data are available [11].

The large experience in the calorimetric investigations enabled us to carry out for the first time the systematic comparative DSC study of the gradual destruction of native structures on dehydration for biopolymers of different classes. Presently, different multi-component systems involving in the 'classical' biopolymers attracts the large and permanently growing interest due to their wide practical application [12, 13]. Therefore the studies of the fundamental thermodynamic properties of biopolymers are still actual and important.

It should be noted that up to now the number of studies of gradual destruction of native structures on dehydration by other experimental methods, including the X-ray analysis, is not large. The exception is the DNA for which the scrutinized studies were performed to establish the steric structure of this 'hegemonic' molecule [14, 15]. It should be noted that the X-ray measurements necessary to obtain the information allowing to restore the conformational structure of native biopolymer represent a laborious task especially in cases when the crystals of the macroscopic size do not exist in nature (e.g. DNA or globular proteins). In the cases of collagens and starches the situation is facilitated by the existence in nature of macroscopic ordered structures for which the processes of destruction on dehydration are studied rather well [16, 17].

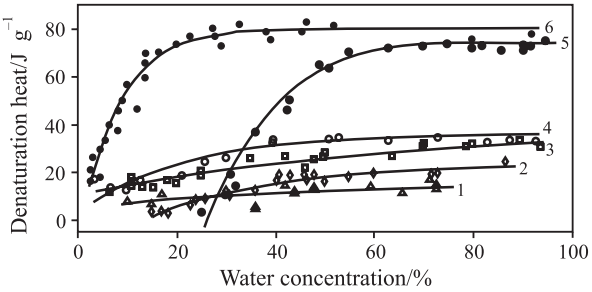

Fig. 1 Dependence of melting heat on water concentration for: 1 – rice starch, 2 – potato starch, 3 – lysozyme, 4 – ribonuclease, 5 – DNA, 6 – collagen

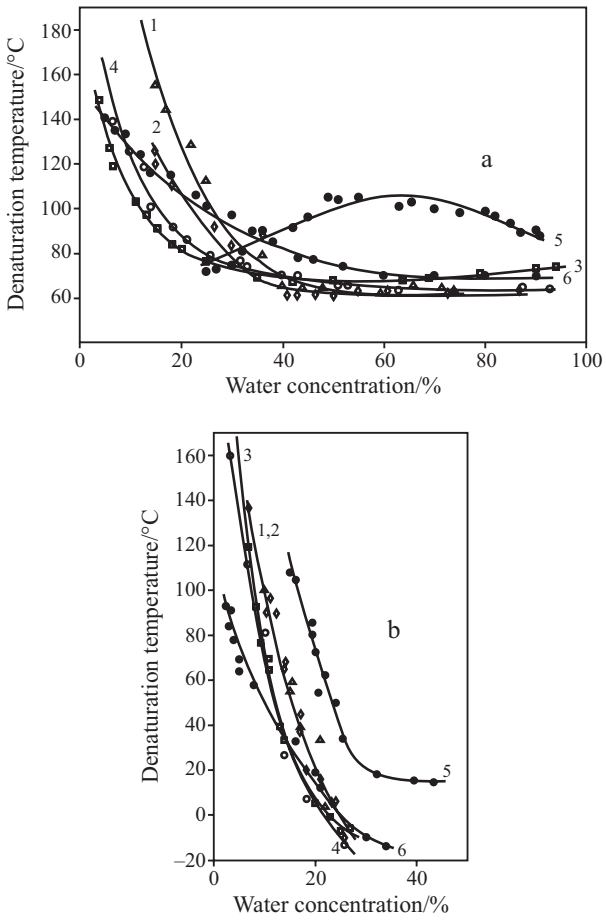

Fig. 2 Dependence of a – melting temperature and b – glass transition temperature on water concentration for: 1 – rice starch, 2 – potato starch, 3 – lysozyme, 4 – ribonuclease, 5 – DNA, 6 – collagen

As is clear from the DSC data presented in Fig. 1, in the region of low water concentration the value of Q_m strongly decreases, especially for DNA. One can see that in DNA with the water content ~25% the native structures are completely destroyed. This result is in a good agreement with the X-ray data which indicate that at such humidity only the so-called disordered P-form is observable [14, 15].

For the denaturation temperature T_m of the biopolymers studied the main changes also take place at low water content. As shown in Fig. 2, for all biopolymers, except the DNA, the effect of dehydration on the native structures produces a strong increase of T_m. As a tentative interpretation it was argued this increase could be of entropic nature being caused by a partial preservation of the structure during the denaturation process [18, 19]. This hypothesis was confirmed by the studies of the thermal properties of gels formed after denaturation. We showed that the longer the time of the high-temperature annealing, the lower the crystal quality of the secondary structures formed on cooling (collagen, DNA) and heating (lysozyme) [9].

In the case of DNA the role of water in maintaining the conformational structure of this macromolecule is also high, but counterbalanced by the degradation of the native structure produced by the dehydration: this process prevails and leads to a decrease of T_m. The heating run of the native DNA with 20% water content shows a curve that is very close to that obtained from denatured DNA with the same humidity, including a 'jump' of the heat capacity typical for the glass transition. As for humid starches, their thermal properties are strongly depen- dent on type of crystal lattice [20]. At the same humidity, thermal destruction of the rice starch ordered structures takes place at temperatures higher than those of potato starch (Fig. 2a, curves 1 and 2).

Figure 2b displays the temperatures of glass transition, T_g, of heat denatured biopolymers belonging to different basic classes. It can be seen that all proteins and starches have very close T_g values. Only for DNA the values of T_g as well as the range of humidities at which the glass transition is observed are slightly different. It should be noted that the region in which the glass transition is observed is limited from the high humidity limit where free water appears which forms an ice matrix that hinders the translational motion of the macromolecular segments [21]. The obtained dependences of T_g on water content indicate that the heat denatured biopolymers have thermal properties similar to those of amorphous synthetic polymers added with plasticizers. An essential difference, however, is that in biopolymers the water not only plays a role of natural plasticizer but also represents an essential element of the structure, the removal of which leads to the gradual destruction of the native structure.

As shown in Figs. 2a and b the lower the humidity, the smaller the difference ΔT between the denaturation temperature of biopolymers T_m (on first heating) and the temperature of glass transition in denatured biopolymers (on second heating).

Now we shall discuss the humidity range where ΔT practically vanishes. To elucidate the mechanism of the processes that take place, the example of lysozyme may be of help. Figure 3 shows the denaturation curves, normalized on the dry mass basis, obtained from samples with different water content. It should be noted that the curve of the humid native sample does not show any 'jumps' of the heat capacity before the unfolding peak. The value of Q_m (peak area) for the sample with higher water content is considerably larger than that for the poorly hydrated biopolymer. Nonetheless the relevant heat capacity drops are comparable. We suppose that such increase of the C_p in the melting endotherm range for this humid native biopolymer can not be only due to the destruction of its residual ordered structures, the amount of which approximately in four times is less than in a solution (Fig. 1). The similar behavior was observed for all the humid biopolymers studied. As is clear from the experiments the glass transition in the disordered phase forming on dehydration takes place just after the thermal destruction of the residual native structures. Hence, until then while the residues of native structures will not be thermally destroyed the glass transition 'jump' can not be observed before the

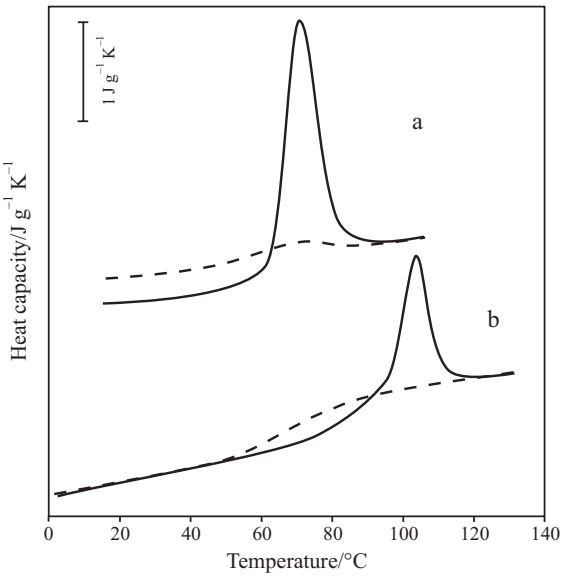

Fig. 3 Thermograms for lysozyme with different water content normalized on dry mass a – C_{H_2O}=80%; b – C_{H_2O}=11%. — – the first heating, - - - – the second heating. V_{heat}=5°C min^{-1}

unfolding peak. In the work [22] we have shown experimentally what is the minimum amount of ordered structures in the dehydrated potato and rice starches, as an example, is sufficient to prevent to appearance to the corresponding 'jump'. Thus, the large 'jump' of heat capacity displayed in Fig. 3b for the humid biopolymer reflects the increase of the molecular mobility due to the two processes occurring simultaneously in the same temperature region – the destruction of the residues of native structures and the glass transition of the amorphous phase.

It should be emphasized that there is a substantial distinction between the endothermic maximum observable in the discussed temperature region and the similar maximum which can appear in the glass transition range on annealing of denaturated biopolymers of the same humidity. For the sake of comparison we shall discuss our experimental data on the thermal properties of elastin which, as known, in natural state does not have any ordered structures and can be considered as a completely amorphous system [8, 23, 24]. It was shown that in the case of the quenched humid elastin with any degree of hydration any melting endotherm is not observed in DSC curves [25]. The curves of the dehydrated native elastin are typical for those of amorphous biopolymer systems with the 'jump' ΔC_p reflecting the transition on heating from the glassy state into the highly elastic one.

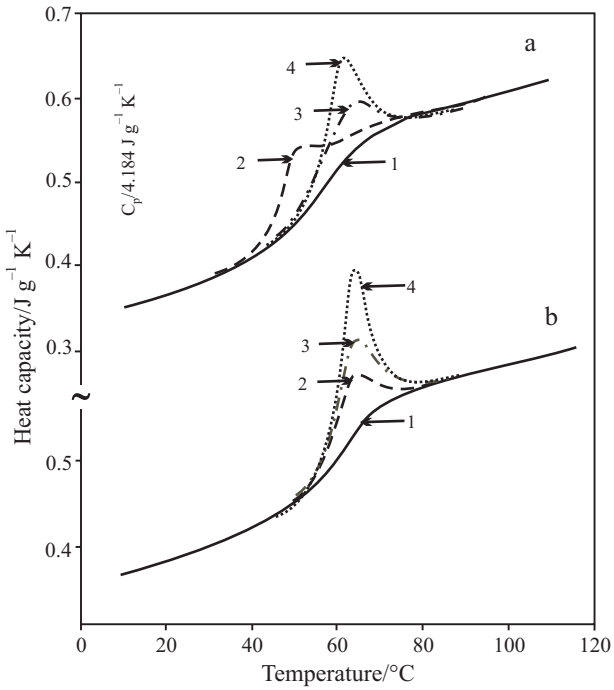

Fig. 4 Influence of the sample annealing on the behaviour of heat capacity in the jump region for elastin. 1 – unannealed sample. a – t_{anneal}=72 h, T_{anneal}=2–0°C, 3 – 20°C, 4 – 25°C; b – T_{anneal}=45°C, t_{anneal}=2–24 h, 3 – 96 h, 4 – 248 h. C_{H_2O}=17%. V_{heat}=5°C min^{-1}

The study of thermal behaviour of the annealed elastin in the glass transition region showed that the annealing at temperatures close to its lower border leads to an occurrence of the maximum with the T_{max} and the value depending on the conditions of the thermal treatment (Fig. 4). In particular, the annealing of the elastin with 17% water content at T_{room} for 3 days results into the appearance of the additional maximum in the region of the heat capacity 'jump' which disappears just after any elevation temperature inside the glass transition range.

Besides, it was found that the change of a hydration degree in such annealed elastin samples (e.g. from 17 to 12%) also leads to the disappearance of the relevant thermal effect. The both facts, as known, are typical for the relaxation transition and the relevant peak area in this case can be referred to as 'relaxation enthalpy' [1, 7]. Remember here that the destruction of biopolymer native structures on dehydration is a fully reversible process. It means that neither temperature position nor the value of the unfolding peak for dehydrated native biopolymers does not depend on a way of the task of water concentration in the samples. So, on the basis of the experimental results it can be decisive concluded that in the case of the studied dehydrated native biopolymers with the residual ordered structures the endothermical maximum in the temperature dependence of the heat capacity reflects exactly their thermal destruction superimposed on the glass transition.

Finally, we shall discuss the results on the determination of the absolute values of the heat capacity for every studied biopolymers. All the results presented above were obtained at the study of the excessive thermal capacity in the temperature range of conformational and relaxation transitions. The determination of the absolute values of C_p provided a

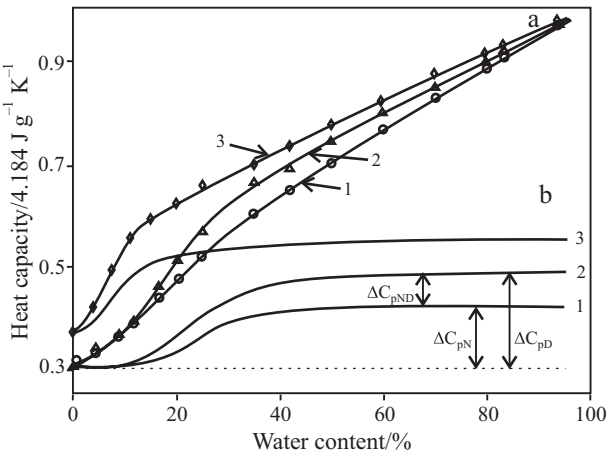

Fig. 5 Dependence of the heat capacity of a – RNase-water system and b – of the protein itself for 1 – native and 2, 3 – denatured states on the water content at different temperatures: 1, 2 – 20°C; 3 – 90°C

deeper understanding of the nature and mechanisms of the processes under study.

The absolute values of heat capacity extracted from curves for the biopolymer-water systems were plotted *vs*. water concentration for every system studied. We shall illustrate the obtained dependences (some kind of the diagrams of state) for the case of RNase as an example. Figure 5, part a demonstrates how at different fixed temperatures the total specific heat capacity both of native and denatured biopolymer–water systems varies on passing from the completely dehydrated samples to those containing 90% of water. Then the C_p of native and denatured biopolymers themselves were calculated from these data as a function of water content at different temperatures, assuming C_p=4.184 J g^{-1} K^{-1} as for free and bound water at all temperatures considered (Fig. 5, part b).

These calculations revealed the non-linear character of biopolymers' heat capacity change during forming of their hydration shells for both native and denatured states. It was found that at low water content the molecular mobility of native biopolymer, which determines its heat capacity, is identical to that of denatured form in a glassy state. Remember that the coincidence of C_{pN} and C_{pD} takes place in the DSC curves in the glassy state region (Fig. 3b). At the further gradual moistening biopolymer, its molecular mobility increases due to the interaction with water. For biopolymers with water excess, the heat capacity for both native and denatured states ceased to depend on water content (Fig. 5b).

It seems natural to conclude, that the S-shaped increase of heat capacity observed in diagrams of state for the denatured RNase is the manifestation of the transition of dehydrated denatured biopolymer from glassy state to the rubber-like one. Hence, the similar S-shape increase of the C_p observed in the diagrams of state in the case of the native protein may be considered as the transition of dehydrated native biopolymer from glassy state to the native one with more intensive molecular mobility. At the same time, the translation motion of macromolecular segments, which corresponds to rubber-like state, appears only after denaturation. In Fig. 5 the arrows indicate the magnitudes of the heat capacity changes of the biopolymer at transitions between the different states: ΔC_{pN} – the increment for transition from the native dehydrated state into the solution, ΔC_{pD} – the increment for transition from the dehydrated denaturated state into the solution, ΔC_{pND} – the increment for transition from native into the denaturated state in the solution. Thus, the S-shaped increase of heat capacity on hydration for native as well as for denatured biopolymers can be also considered as the manifestation of their passing through the glass transition region. As it follows from the obtained data, the native biopolymers studied, as well as denatured ones, with water content about 15% at room temperature are in a glassy-state. The general character of the heat capacity changes for all biopolymers themselves at hydration is the same.

Conclusions

As follows from the realized DSC research, both the native and denatured biopolymers with different chemical and steric structure (proteins, DNA, starches) can exist in a glassy state at the certain ratio of humidity and temperature. On heating the denatured biopolymers, in contrary to the native ones, exhibit the transition from glassy to rubber-like state that is evidenced by characteristic heat capacity 'jump'. The value of increment ΔC_p at thermal denaturation of humid biopolymers is so large that it can not be accounted completely by the melting of the residual native structures survived after the partial dehydration. The main contribution to the ΔC_p in this case is due to the glass transition in the amorphous phase of the dehydrated biopolymer. It was shown that the thermal destruction of the residues of the ordered structures and glass transition in humid biopolymers take place in the same temperature range that leads to the found large increment of heat capacity.

On hydration the transition of biopolymers from dehydrated state to the state with excess of water is accompanied by the S-shaped increase of the absolute value of heat capacity, caused by the growth of molecular mobility in the systems, not only for denatured biopolymers but for native ones as well.

The found distinctions in the thermal properties of the native and denatured biopolymers in the region of glass transition can be attributed to different nature of their amorphization. As known, the thermal destruction of the native structures of proteins, DNA, and starches, named denaturation, melting or gelatinization, respectively, is usually, except the rare exceptions, irreversible. In contrary, the destruction of the native structures of these biopolymers by dehydration is completely reversible. Whereas the conformational changes of biopolymers at the thermal destruction are studied rather extensively, the steric changes of biomacromolecules on dehydration are under discussion up to now. The fact that the dehydrated native biopolymers rapidly and completely restore their structure on moistening allows to assume that the dehydration causes only the local changes in the steric positions of the side groups and does not influence on the general conformation of the biomacromolecules.

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DOI: 10.1007/s10973-008-9023-1