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CALORIMETRIC STUDY OF THE GLASS TRANSITION PROCESS IN HUMID PROTEINS AND DNA

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Abstract

By method of differential scanning calorimetry the absolute values of heat capacity for the system water–biopolymer (globular and fibrillar proteins and DNA) were measured in a wide range of temperatures (from –30 up to 130°C) and concentrations of proteins both in native and denatured states. Thermal properties of humid denatured biopolymers demonstrate a characteristic anomaly in the form of the heat capacity jump at temperature depending on the bound water content. It has been shown that in the systems studied a glass transition, where water serves as a native plasticizer, is observed. It has been established that the S-shaped character of all heat capacity curves obtained on dehydration for native and denatured biopolymers is due to the gradual transition to the glassy state of both native and denatured samples. It was found that thermally denatured humid small globular proteins at subsequent dissolving in water at room temperature are able to restore their native structure.

Keywords: DNA, glass transition, humid proteins

Introduction

In many papers published during the last decade it has been shown, that native biopolymers, containing water in the amount close to that of the bound water under certain conditions demonstrate some properties characteristic of the glassy state. One of the first indications has been obtained by Goldanskii *et al*. through the analysis of the values of heat capacity of several proteins and DNA as measured by equilibrium adiabatic calorimetry at very low-temperatures [1]. Similarity of mechanical, optical and thermal properties of native biopolymers to that of systems with glass transitions has been discussed also in references [2–4]. It should be noted that all these papers were considering the temperature region well below 0°C, and the temperature limits of glass-like state were never discussed. Neither have the transformation of the glass-like properties of the biopolymer systems at the increase of temperature and the nature of the high-temperature state after glass transition.

These very important problems are considered in our present paper which summarizes our studies during the recent years of calorimetric manifestation of glass transitions in different biopolymers and its effect on the thermal properties [5–13].

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We would like to demonstrate that in the discussion of interaction of water solvent with the dehydrated solid biopolymer at the formation of water solution and in separation of contributions of definite components of biopolymer–water system into the total heat capacity the glassification process should be taken into account.

We are presenting the results of DSC measurements of the absolute values of specific heat capacity of the biopolymer–water system for several globular proteins [6, 9, 12, 13], for fibrillar protein collagen [5, 7, 8] and DNA [5, 10, 11]. A very wide range of compositions has been studied starting from practically dehydrated samples up to concentrated solutions. The measurements were performed both for native and denatured samples of the above mentioned proteins in wide temperature region from –40 up to 130°C. In the discussion of our results we shall confine ourselves to the established general features of calorimetric manifestation of the glass transitions in denatured and native biopolymers, which are essentially common for the proteins with quite different chemical and steric structure.

Experimental

For the study of heat capacity we used a Setaram DSC-111 differential scanning calorimeter with a sensitivity of $3 \cdot 10^{-5}$ J s^{-1}. The sample mass was 50–100 mg. Temperature was controlled with a precision of 0.1 deg. The error in the absolute values of denaturation heat and heat capacity of systems biopolymer–water was less than 3 and 1% respectively. The values of denaturation heat are related to the dry weight of sample. All the biopolymer studied were commercial products of Sigma, except the collagen, which was extracted in our laboratory from the rat tail tendon in accordance with the procedure described in [5].

Results and discussion

Figure 1 shows the temperature dependences of the absolute values of heat capacity of all the studied biopolymer–water systems at some specified water concentrations. It should be noted, that for all cases presented in Fig.1 the water in the system is bound and the mechanical properties of proteins are characteristic of the solid state. For every substance the first heating cycle is performed for the sample in original native state and the second – for denatured sample. The endothermic maximum at first heating corresponds to the denaturation process. The complete absence of such maximum at the second-heating curves indicates the irreversible character of denaturation under the experimental conditions used.

First, we would like to discuss the relation between the values of heat capacities of native (C_{pn}) and denatured (C_{pd}) states in the temperature region below the denaturation temperatures. As it can be seen from Fig.1, the C_{pn} and C_{pd} values differ for all proteins, and the magnitude of the difference depends on the temperature. It should be noted that such difference for protein solutions has been discussed for a long time by several biophysical groups [14]. However, for solid state samples containing only the bound water

such difference had been first established for collagen in [5]. The proposed interpretation is based on the analogy between the denaturation processes of humid proteins and the melting of partially crystalline synthetic polymers. In both cases, as a result of heating a partly ordered solid structure converts to disorder with the absorption of the transition heat. In case of synthetic polymers, the heating causes melting of the crystallites and the resulting high-temperature phase is amorphous. Depending on the temperature, the amorphous polymer can exist either in highly elastic (rubber-like) high-temperature state or in glass-like low-temperature state [15, 16].

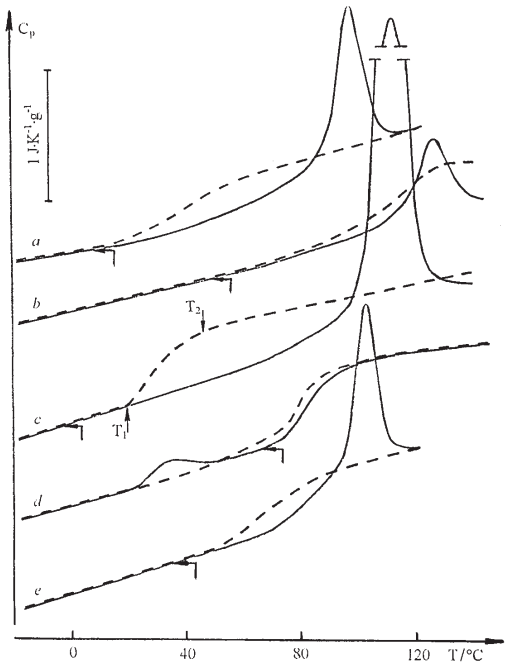

Fig. 1 Temperature dependence of the absolute values of the heat capacity of biopolymer–water systems in the presence of the bound water: a – ribonuclease (14% H_2O), b – myoglobin (5% H_2O), c – collagen (18% H_2O), d – DNA (20% H_2O), e – lysozyme (11% H_2O); 1 – heat capacity of native samples (first heating), 2 – heat capacity of denatured samples (second heating); the arrows on the curves point to the C_p=1.6 J g^{-1} K^{-1}

As we can conclude from our results, and also considering the paper [17], a similar classification and terminology can be applied to the studied protein–water systems. Prior to the discussion of the glass-like properties of denatured state we shall present the results of the study of denaturation transition from the native to the denatured state.

From our measurements we have deduced the values of the basic parameters of the denaturation process for three globular proteins studied for a wide range of com-

ponent composition (Fig. 2). First, we shall discuss the composition range, in which all the water in the system is bound.

The observed decrease of the denaturation heat with the decrease of the water content is quite expectable, since the stabilization effect of the water built-in into the protein structure is well known. However, on the same grounds, for all proteins the observed increase of denaturation thermostability amounting up to several tens of degrees on passing from solutions to the dehydrated protein, seems to be rather surprising. Stabilization of protein on dehydration was observed for collagen by one of the present authors a while ago [5]. As a possible explanation, it has been assumed, that the increase of thermostability of collagen can be attributed to the substantial decrease at dehydration of the entropy increment ΔS during the denaturation transition. We believe, that the same argument can also explain the observed anomalous increase of the temperature of denaturation T_d in dehydrated globular proteins. It can also be assumed that after denaturation of humid protein some position memory is retained; and the state of the globule of humid protein in solid is similar to that of the melted globule in solution [18]. Our further studies of renaturation of thermally denatured humid proteins, which will be reported at the end of the paper, provide a solid experimental evidence of the existence of such intermediates for low hydration levels.

Now we shall discuss the established by us properties of denatured proteins, which are similar to that of classical polymer systems with glass transitions. It has been found, that the curves of temperature dependence of heat capacity for denatured state containing only the bound water demonstrate a characteristic irregularity in the

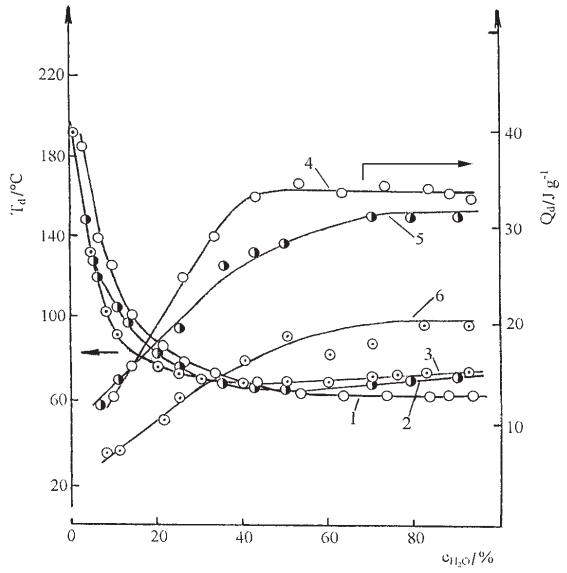

Fig. 2 Dependence of denaturation temperature (1, 2, 3) and denaturation heat (4, 5, 6) on water content for globular protein: 1,4 – ribonuclease, 2,5 – lysozyme, 3,6 – myoglobin

form of a 'jump' of heat capacity, the temperature position of the jump being dependent on the water content (Fig. 1, curves 2). This jump can be characterized by the transition temperature T_g, which corresponds to the inflection point of the heat capacity curve, and by the magnitude of ΔC_p, which represents the difference in heat capacity values extrapolated to T_g along the linear sections of the temperature dependence from higher and lower temperatures.

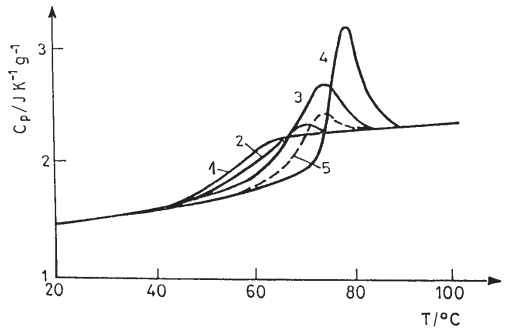

Fig. 3 Temperature dependence of heat capacity for the denatured collagen annealed at T=22°C during the different times: 1 – without annealing, 2–18 h, 3–3 days, 4 and 5–10 days. Heating rate is 5°C min^{-1} for curves 1–4 and 1°C min^{-1} for curve 5. Concentration of water is 14%

It has been shown, that the anomaly in the temperature dependence of the heat capacity of denatured biopolymers under the study can be assigned to the calorimetric manifestation of the glass transition. We have studied the influence of the heating rate and the thermal history of the denatured biopolymer samples on the behavior of the heat capacity in the jump region. We shall discuss this behavior in more details for the case of collagen. With the increase of the heating rate V_h from 1 to 5°C min^{-1} the transition temperature increases by 3–5 deg. The annealing of denatured samples at temperatures slightly below T_1 produces an additional maximum on the curve of temperature dependence in the region of the jump (Fig. 3). The temperature position of this maximum and its intensity increase with the increase of annealing time. This maximum is the most intensive in collagen and DNA for comparable values of annealing time and temperature. In synthetic polymers this additional maximum is known to have not a structural, but a relaxation origin. In reality, we have observed such a decrease when using the lowest heating rate, as it can be seen in Fig. 3 for the case of collagen. On the basis of our kinetic studies, we can conclude that the anomaly in the temperature dependence of the heat capacity of denatured biopolymers reveals all the features characteristic of the glass transitions in synthetic amorphous polymers [15, 16].

It has been found that the temperature dependence of heat capacity exhibits behavior similar to the above-mentioned for all studied biopolymers with concentrations of bound water varying from 5 to 25% in proteins and from 10 to 40% in DNA. As it is known, the amount of bound water in DNA is substantially higher than in pro-

teins [19]. The magnitude of the heat capacity jump, related to the dry mass of protein, is close for all biopolymers and is 0.50±0.10 J g^{-1} K^{-1}.

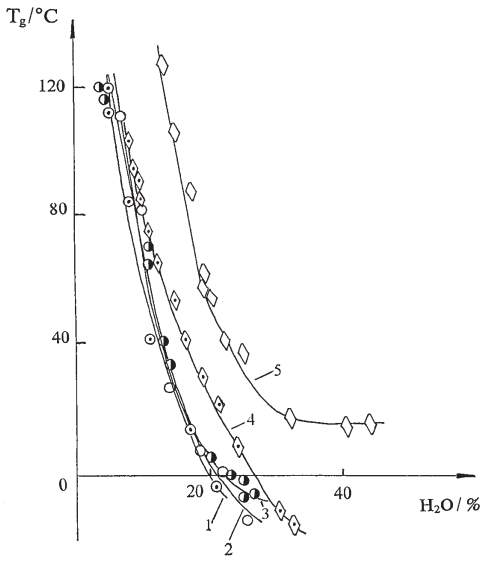

Fig. 4 Dependence of glass transition temperature of denatured biopolymers on water content: 1 – myoglobin, 2 – ribonuclease, 3 – lysozyme, 4 – collagen, 5 – DNA

It has been shown that the glass transition temperature strongly depends on the water concentration, especially for the samples with a small water content (Fig. 4). It can be concluded that the bound water, which constitutes an intrinsic element of the ordered structure of native biopolymers, at the same time acts as a natural plasticizer, lowering the glass transition temperature of denatured biopolymers. Our experimental data on the dependence of the values of glass transition temperatures on the water content for a wide class of denatured proteins and nucleic acids are in good consistence. Obviously, the denaturation process destroys the unique native structure of biopolymers so drastically that denatured polymers can be considered just as statistical copolymers composed of the chains with different combinations of side groups. The essential difference of the biopolymer–water system from the partially crystalline synthetic polymer–plasticizer systems can be formulated as follows. In the later case the inclusion of plasticizer influences only the melting parameters of the polymer crystals, whereas in the case of biopolymers the removal of bound water as plasticizer destroys the ordered native structure.

We would like to stress that the calorimetric study of humid native DNA not subjected to any preliminary treatment has shown that in this case the temperature dependence of heat capacity in the region of the glass transition is practically identical to that of denaturated DNA with the same humidity (Fig. 1, curve d). That reflects the well known fact, that the native structure of DNA is completely destroyed at the removal of bound water [19].

Our experimental results reveal how the glassification process influences the changes of thermal properties of biopolymers at hydration. Consequently, we shall demonstrate it for the case of collagen. From the obtained results we have plotted the curves (Fig.5), each of which demonstrates, for a fixed temperature, how the values of the total specific heat capacity of the biopolymer–water system change on passing from the completely dehydrated samples to those containing 90% of water (Fig. 5). Since all the experimental data points lie above the straight line, joining the heat capacity values of dehydrated protein at room temperature and that of volumetric water, it can be concluded that the heat capacity of the protein–water system over a wide range of concentrations cannot be calculated by using a simple additive model [20].

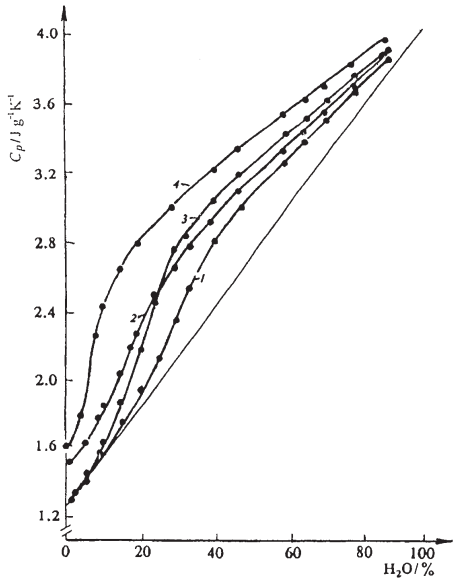

Fig. 5 Dependences of the heat capacity of a collagen–water system for native (1, 2) and denatured (3, 4) samples on the water content at different temperatures: 1, 3 – 20°C; 2 – 60°C; 4 – 90°C

Also, from the data on the total heat capacity of the collagen–water system, taking the heat capacity of water to be equal to 4.18 J g^{-1} K^{-1}, we have calculated the heat capacities of protein itself at different temperatures as a function of water content (Fig. 6). On passing from dehydrated protein to protein, completely surrounded by water, both in native and denatured states, an S-shape increase in heat capacity both in protein–water systems and in protein itself occurs. It was found, that starting from 50% water concentration the heat capacity of protein itself does not depend on the water content and equals to the heat capacity of protein in solution.

We shall analyze now the curves in Fig. 5 and Fig. 6 in the region of bound water content. We shall first consider the behavior of the heat capacity of denatured colla-

gen, as illustrated by the curves of C_{pd} at temperature 20°C for the system as a whole (Fig. 5, curve 1) and collagen itself (Fig. 6, curve 1). For the water content below 10% at T=20°C, the denatured protein is in the vitreous state (Fig. 1). Whereas for dry protein the vitrification range lies in the high-temperature region, for proteins with a 25% water content it is below the room temperature. It can be concluded that the S-shaped form of the obtained curves is due to a gradual transition of denatured collagen from vitreous to the rubber-like state.

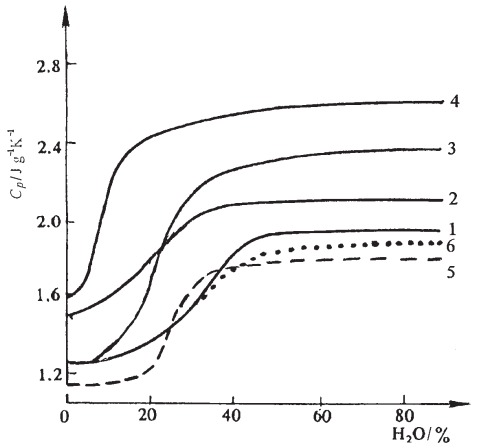

Fig. 6 Dependences of the heat capacity of collagen (1–4), ribonuclease (5), and DNA (6) on the water content at different temperatures in the native (1, 2, 5, 6) and denatured (3, 4) states: 1, 5, 6 – 20°C, 2 – 60°C, 4 – 90°C

As it also follows from our results, all the studied denatured biopolymers with water content about 15 % at room temperature are in a glassy state. This fact is crucial for the discussion of the results on protein renaturation, which will be discussed in the last part of the present paper.

In other temperature ranges, as in the case of T=20°C, the largest changes of the heat capacity values correspond to a narrower range of water concentrations so the values of heat capacity of the rubber-like state reached lower humidity levels.

Our results show that although the vitrification in the case of native samples does not show up directly as a heat capacity jump on its temperature dependence (Fig. 1, curve c), this process is reflected in the form of the S-shaped curve of the dependence of the heat capacity on the water content (Fig. 5, curve 1; Fig. 6, curve 1).

It has been found that, in case of other proteins studied and DNA, the dependence of heat capacity on temperature and/or hydration degree is essentially similar to that described above for collagen (Fig. 6). Consider the results obtained in terms of the concepts of the conformation of protein macromolecules and their conformational mobility. It is well known that changing from the glass to the rubber-like state or from a molecular crystal to the melt, a new type of thermal motion appears due to the appearance of the translation motion of individual segments of the macromolecules [15, 16]. These ideas, in our view, may be successfully used to examine the

phase and relaxational transitions in biopolymers. On denaturing of native proteins in solution and devitrification of wet denatured proteins, thermal motion destroys those bonds and structures, which freeze translation segmental mobility.

As to the native and denatured protein molecule deprived of its aqueous environs, we suppose that it exists in the vitreous state. It is considered that the dry native protein has the same or close conformation as the truly native protein in solution [21], but, as we have shown, it does not have the conformational mobility inherent to the native form. The molecular mobility of dehydrated native protein, which determines its heat capacity, is identical to that of the dehydrated denatured form in the glassy state. At the same time, in the course of gradual moistening the conformation of native protein remains unchanged, and its molecular mobility increases due to the interaction with the attached water. The translation motion of the segments of the macromolecules in such a system is impossible and only transition to the denatured state leads to the appearance of translation motion.

In conclusion, we would like to note that the difference of the values of heat capacities of native protein in solutions and in dry state at room temperature was established long ago [21]. At the same time, as it has been shown above, the application of such an approach requires a considerable caution since it does not allow for the interaction between the components of the protein–water systems to be considered. We would like to emphasize once more that the increase of the heat capacity of native protein–water and denatured protein–water systems, when they are moistened, is due to the sharp S-shape change in the heat capacity of protein itself, owing to its strong interaction with water, which also leads to a shift of vitrification temperature from the high-temperature region for dry proteins to the temperature region below 0°C for solutions. An increase in the heat capacity both of native and denatured proteins is a calorimetric manifestation of the passing of protein through the vitrification region.

Finally, we shall discuss the results of calorimetric study of renaturation processes for globular proteins. A systematic study of all thermally induced conformational transitions and relaxation processes in globular proteins has revealed a previously unknown ability of thermally denaturated humid proteins to restore the native structure at subsequent dissolving in water [22]. Such revitalization of denatured protein with the help of water has been observed for all three globular proteins under the study leading us to conclusion that the behavior established is very general and inherent property of proteins.

We consider that the discovered effect of the ‘reviving water’ can be understood within the framework of the model of melted globule, assuming that the denaturation of globular proteins in solutions takes place through the formation of stable intermediates with a structure intermediate between the totally ordered native state and the statistically disordered state, which was earlier proposed as a model structure for denaturated state [18]. We suppose that similar intermediates can exist not only in protein solutions, but also in solid phases such as humid protein. In other words, we assume that at the denaturation the protein with a low content of water transforms to some intermediate state, for which a steric memory partly persists like in the melted globule. Storing it at high-temperature it leads to the gradual deconvolution of the in-

termediate and the life-time of the intermediate at high-temperature is short. Cooling it down to the room temperature results in bringing the intermediate into the glass state, in which it is kinetically stable and can be practically kept for unlimited time without structural changes. At subsequent dissolving water the intermediate easily converts to the native structure. The effect established by our studies demonstrates the interrelation between the purely biological ability of protein to form the native structure through the self-organization and the characteristic polymer properties of protein as a system with glass transition.

Conclusions

Our research has shown that the biopolymers of different types (globular, fibrillar and DNA) at specified levels of humudity and temperature can exist in glassy state. The denatured proteins, unlike native, with the increase of the temperature exhibit the transition from glassy to rubber-like state in the form of characteristic heat capacity jump. The transition temperature changes from below 0°C in the samples with maximum content of bound water to above 100°C in dehydrated samples. The transition from glassy to rubber-like state and, consequently, to water solution in both native and denatured bioploymers is accompanied by S-shaped increase in the heat capacity caused by the increase of molecular mobility in biopolymers. The interaction with water leads to a shift in the vitrification temperature from the high-temperature region for dry biopolymers to the temperature region below 0°C for solutions. The value of the heat capacity of denatured protein in solutions must be greater than that in the dehydrated state, at least, by the magnitude of the vitrification jump. Earlier unknown renaturation ability of thermal denatured humid small globular proteins to restore its native structure is closely related to the formation of the glass state at low water contents. This very important property is based on the formation during thermal denaturation of rather stable intermediates with the structure, intermediate between that of native and denatured protein. These intermediates have short life-time at high-temperatures, however can last for unlimited time at room temperature. Freezing of intermediate structures during the glass transition provides the ability of biopolymers to restore its native structure at the addition of water.

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