The glass transition process in humid biopolymers. DSC study

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Abstract. Thermal properties of native and denatured biopolymers with quite different chemical and steric structure (globular and fibrillar proteins, DNA, starches) were studied by means of differential scanning calorimetry in a wide range of temperatures and concentrations of water. It was shown that both native and denatured humid biopolymers are glassy systems. The glass transition temperature of these systems strongly depends on percentage of water, with water being simultaneously an intrinsic element of systems’ ordered structure and a plasticizer of its amorphous state. On the base of the absolute values of heat capacities for biopolymer-water systems as a whole, heat capacities for biopolymers themselves were calculated as functions on water concentration at fixed temperatures. The $S$-shaped change of heat capacity observed on diagrams of state both for native and denatured biopolymers is the manifestation of biopolymers’ passing through the vitrification region, as it occurs for denatured samples at heating.

1. Introduction

Obviously, the glass transition is one of the main processes forming thermal properties of synthetic polymers [1-3]. The main goal of the present work is to demonstrate that it is also not possible to describe thermal properties of biopolymers without considering vitrification. As it is well known, biopolymers keep the nativity (i.e., the ability for biological activity) exclusively in water environment, which maintains their steric structure. During dehydration (more precisely at the loss of bound water), biopolymers’ steric structure is destroyed. Note that here and further we use the term “bound water” for water that does not freeze at cooling. The process of destruction/restoration of the native structure at dehydration/hydration is, as a rule, completely reversible, providing the grounds for storage of biopolymers in a dry state [4-6]. The steric structure of partly or completely dehydrated “native” biopolymers is poorly investigated and is still under discussion. However, it is clear that this structure is not identical to the “true” native one. In this case, macromolecules cannot be represented by purely statistical coil, which is the case for biopolymers that lost their native structure during thermal denaturation in water solutions.

During last 15 years our group studied in details thermally induced conformational and relaxation transitions in biopolymers with quite different chemical and steric structure [7-12]. We considered small globular proteins, structural fibrillar proteins, DNA, and starches of different botanical origin. We were interested in the consequences of hydration/dehydration processes on the thermal properties of all biopolymers studied. The dependences of thermodynamical properties of denaturation process (temperature, $T_d$, and heat, $Q_d$) on the loss of bound water were established. The studies of biopolymer-water systems were carried out in a wide range of water percentage – from dry state to solution. The present paper summarizes our studies of calorimetric manifestation of glass transition for all these
biopolymers, containing only bound water, both in their native and denatured states. For partly or completely dehydrated biopolymers, the term “native” means only their possibility to restore the primary native structure after hydration. The comparative study of the molecular mobility, based on the analysis of the absolute values of heat capacity for biopolymers, whose native structure has been destroyed either by thermal denaturation or by dehydration, is one of the aims of the present work. In this work, we review our published earlier results and present the results of our recent investigations in this field.

2. Materials and method
The studies of all biopolymer-water systems were performed using the Setaram DSC-111 differential scanning calorimetry with a sensitivity of 3·10^3 J/sec. The error in the absolute values of heat capacity was about 1% for the heating rate 5°C/min. The temperature was controlled with a precision not less than ±0,2°C over the whole measured temperature interval: -30÷160°C. The heating rates used varied from 0,2°C/min to 5°C/min according to the particular task. Globular proteins, such as myoglobin (Mb), lysozyme (Lys), ribonuclease (RNase), fibrillar protein elastin, and DNA were supplied by «Sigma». The collagen studied was prepared in our laboratory from rat-tail tendon. We have studied also the potato starch from Aldrich (USA) and rice starch “Lazurnyi” received from the RAS Institute for Biochemical Physics (Moscow). The necessary concentration of water in the samples was achieved either by wetting of the samples or by vacuum drying of the original biopolymer. To determine the resulting water content the control samples were dried in vacuum at T=105°C unless their weight stopped changing. The samples’ masses used varied from 50 to 120 mg. In order to obtain the uniform water distribution, the sealed hydrated samples were kept for a day at room temperature before measurements.

3. Results and Discussion
Fig. 1 presents the temperature dependences of the absolute values of heat capacity for biopolymer-water systems containing only bound water. The chosen set of thermograms on Fig. 1 demonstrates both typical and individual features of thermal behavior of the biopolymers studied. Note, that the solid and dash lines on this figure are thermograms of the studied biopolymers in their native and denatured states. For partly or completely dehydrated biopolymers, the term “native” means only their possibility to restore the primary native structure after hydration. The comparative study of the molecular mobility, based on the analysis of the absolute values of heat capacity for biopolymers, whose native structure has been destroyed either by thermal denaturation or by dehydration, is one of the aims of the present work. In this work, we review our published earlier results and present the results of our recent investigations in this field.

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![Figure 1. Temperature dependences of the absolute values of the heat capacity for:
(a) DNA (C_H2O=18%),
(b) elastin (C_H2O=17%),
(c) lysozyme (C_H2O=11%),
(d) rice starch (C_H2O=15%),
(e) potato starch (C_H2O=15%).
Solid lines correspond to the first heating of samples, dash lines are the second heating.
V_{heating}=5°C/min.](image1)

![Figure 2. Dependences of the glass transition temperature on water content for:
1 - DNA (x),
2 - potato starch (Δ),
3 - rice starch (•),
4 - elastin (•),
5 - lysozyme (•).](image2)
denatured states, correspondingly (except Fig. 1b). In Fig. 1c, d, e the denatured states were obtained by heating of partly dehydrated native biopolymers up to the temperatures at which the denaturation process took place. The denatured state of DNA (Fig. 1a) was reached by special high-temperature treatment of DNA solution and its further drying up to the 18% of water concentration.

The thermograms (solid lines) demonstrate the different stages of native structure destruction at the loss of bound water. The bound water content decreases in the biopolymers studied in succession DNA, protein, starch as 42-40, 27-25, 22-20 percents by weight, depending on the origin of the sample. One can see that there exists only one peak of heat absorption at the first heating for globular protein lysozyme, potato and rice starches. According to the investigations of these biopolymers in a wide range of their hydration degrees [10-12], this peak corresponds to the cooperative destruction of their native structure at given conditions. This endothermic maximum is not observed at the repeated heating of the sample because the denaturation is irreversible. Note, that the native structure of different biopolymers is destroyed completely at different humidities. Whereas in Lys with 11% of water the heat denaturation is still equal about 1/2 \( Q_d \) of the native sample [11], in DNA the native structure is completely destroyed already at 18% of water (Fig. 1a). The corresponding maximum is absent on DNA thermogram at the first heating. It means that DNA in its original dehydrated state was disordered. Indeed, it is known that the native structure of DNA with the content of water less than 25% is completely disordered [6]. In the case of elastin, the endothermic maximum was not observed on the thermograms at the first heating both in the presence of bound water only and in the water environment. According to the existing structural studies, elastin is always in amorphous state [4]. In other words, elastin does not have regular melting structures at any degree of hydration and, hence, thermograms of its first and second heatings coincide (Fig. 1b). As for humid starches, their thermal properties are strongly dependent on the various contents of structural water. It is well known that granules of the rice and potato starches contain crystallites with different water content. Namely, the water percentage in rice starch is approximately four times smaller than in potato one [14]. Hence, at the same humidity, thermal destruction of the rice starch ordered structures takes place at higher temperatures than of potato starch ones (see Fig. 1d, e).

The character of the thermograms obtained at the second heating in all cases (Fig. 1, dash curves) is practically identical. It was found out that for all studied denatured biopolymers (and also for elastin) with low humidity there exists an anomaly in the form of a “jump” of heat capacity. Its temperature position depends on the bound water content. Obviously, the unique space native structure is destroyed after denaturation. Thus, biopolymer, as we think, can be considered just as a statistical amorphous copolymer, consisting of either chains with different side groups’ combinations (proteins and DNA) or an amorphous homopolymer (starch). The absence of the ordered structures in these systems makes denatured biopolymers similar to the amorphous synthetic polymers. It is well known that with temperature increasing the amorphous synthetic polymer can proceed from glass-like state to rubber-like one [3]. This transition, named glass transition, is accompanied with a jump of heat capacity. One observes similar effect for all denatured humid biopolymers studied and amorphous elastin (Fig. 1, dash curves). It is also known that the glass transition temperature \( T_g \) for amorphous synthetic polymers depends on the concentration of solvent, which plays the role of plasticizer [1-3]. Fig. 2 shows that the temperature position of the heat capacity jump, which can be treated as \( T_g \), strongly depends on the bound water concentration for all biopolymer-water systems studied.

Comparing the obtained results with the analogous ones for amorphous synthetic polymers one can conclude that for biopolymers the bound water, being an intrinsic element of their native structure, also acts as plasticizer in their disordered state. The hydration of biopolymer shifts the glass transition temperature to lower temperatures. Thus, accordingly to these ideas, the observed heat capacity jump (Fig. 1, dash curves) is the calorimetric manifestation of the, so-called, -glass transition in denatured humid biopolymers and amorphous elastin. Therefore, being dehydrated, they all can exist in a glassy state near room temperature (Fig. 2).

It should be stressed that the glassy state is not an equilibrium one and, thus, the heat capacity of disordered state strongly depends on thermal history and specific treatment. We investigated the influence of such factors as heating rate, time and annealing temperature, as well as change of annealed sample humidity, on the behaviour of heat capacity in the jump region. The experiments
showed that the character of the results was practically the same for all denatured biopolymers chosen. It was found that annealing of the amorphous biopolymer led to appearance of a maximum in the temperature region of heat capacity jump, similarly to the case of amorphous synthetic polymers [1-3]. Fig. 3 demonstrates the dependence of the temperature position and intensity of this maximum on annealing conditions for globular protein lysozyme and fibrillar protein elastin. One can observe the temperature T and intensity of the maximum grow with the increase of annealing time. The temperature of the maximum decreases with decrease of the heating rate. As an example, the change of the heating rate from 5°C/min to 0.2°C/min results in decrease of T from 45°C to 36°C for the annealed sample of elastin with 17% of water content.

**Figure 3.** Influence of the sample annealing on the behaviour of heat capacity in the jump region. Left part is for denatured lysozyme, right part is for elastin. 1-non-annealed sample. V_{heat} = 5°C/min. For lysozyme: a - t_{anneal}=72h, T_{anneal}= 25°C, 35°C, 45°C (2, 3, 4); b - T_{anneal}=25°C, t_{anneal}=3h, 18h, 72h (2, 3, 4).

For elastin: a - t_{anneal}=72h, T_{anneal}= 0°C, 20°C, 25°C (2, 3, 4); b - T_{anneal}=45°C, t_{anneal}=24h, 96h, 248h (2, 3, 4).

Taken all together, these data for the humid denatured biopolymers confirm that the discussed maximum in the glass transition region of annealed samples has not a structural, but relaxation origin. The following experiment can also serve as the additional confirmation of relaxation origin of the considered maximum. It was found out that the dehydration of annealed elastin sample with 17% water content, e.g., up to 12% led to the complete disappearance of the maximum discussed. The corresponding thermogram was similar to that of the sample quenched from the rubber-like state. Thus, the obtained results convincingly demonstrate the similarity of thermal properties of the denatured biopolymers with low humidity and of amorphous synthetic polymers.

Now let us discuss the change of thermal properties of the native biopolymers at dehydration. Consider again the thermograms of the dehydrated native globular proteins and starches studied at the first heating (Fig. 1, solid lines). One can see that for these native biopolymers a jump of heat capacity corresponding to the glass transition is absent. However, it was found that the heat capacity of the humid native biopolymers was not a strict function of temperature in the range corresponding to the glass transition of the same biopolymers in denatured state. It turned out, that there exists a dependence on the thermal prehistory of the sample. In particular, a small maximum appears in that temperature range for the native air-dried samples after long annealing at room temperature (Fig. 1c, dotted curve). It was also shown that additional dehydration of such annealed samples led to the disappearance of the maximum. This maximum can be probably assigned to the small-scale motions.
of molecular chains analogously to synthetic polymers. So, it can be considered as the calorimetric manifestation of β-glass transition [2]. As it was noticed above, the same effect was observed for the annealed samples of elastin in the region of α-glass transition.

However, the clear manifestation of glass transition in humid native biopolymers was revealed at hydration. The absolute values of heat capacity extracted from thermograms for the biopolymer-water systems were plotted against water concentration for every system studied. We shall illustrate the obtained dependences (diagrams of state) for the case of potato starch. Fig. 4, part a demonstrates how the total specific heat capacity both of native and denatured biopolymer-water system changed on passing from the completely dehydrated samples to those containing 90% of water at different fixed temperatures. 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·K for both free and bound water at all temperatures considered (Fig. 4, 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 the glassy state. Remember that the coincidence of $C_{pN}$ and $C_{pD}$ takes place also on the DSC thermograms in the glassy state region (Fig. 1). At further gradual moistening of biopolymer, its molecular mobility increases due to the interaction with water. For the biopolymers with water excess, the heat capacity for both native and denatured states ceased to depend on water content (Fig. 4b).

It seems natural to conclude, that the $S$-shaped increase of heat capacity observed on diagrams of state for denatured starch, as well as on its DSC thermograms, 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 on the diagrams of state in the case of native starch corresponds to 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. So the hydration increment of heat capacity for the native biopolymer ($C_{p^{\text{GN}}}$) is always smaller than that for the denatured one ($C_{p^{\text{GD}}}$). Thus, the $S$-shaped increase of heat capacity at hydration both for native and denatured biopolymers can be also considered as the manifestation of their passing through the vitrification region, as it

![Figure 4. Dependences of the heat capacity of potato starch-water system (a) and of starch itself (b): for native (1) and denatured states (2, 3, 4) on the water content at different temperatures: 1, 2 – 20°C; 3 – 50°C; 4 – 70°C.](image-url)
occurs for denatured samples at heating. 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 glassy state. This fact gives the evidence that just the glassy state of dehydrated native biopolymers reserves the ability to restore their native properties after dissolving due to freezing of segmental mobility in macromolecule. The general character of the heat capacity changes for all biopolymers themselves at hydration is the same.

4. Conclusion
Our DSC research has shown that the biopolymers of different types (proteins, DNA, starches) can exist in glassy state at some levels of humidity and temperature. The denatured biopolymers, in contrary to the native ones, at scanning exhibit the transition from glassy to rubber-like state evidenced by characteristic heat capacity jump. At the same time at hydration the transition of biopolymers from dehydrated state to the state with excess of water is accompanied by the S-shaped increase of $C_p$, caused by the growth of molecular mobility in the systems, not only for denatured biopolymers but for native ones as well. This fact permits us to conclude that the native biopolymers with low water content are also in glassy state. The increment of $C_p$ at the biopolymers’ hydration is greater than that at their transition from native to denatured state in solution at heating. The interaction of biopolymer with water leads to the shift of the $T_g$ from the high-temperature region for dry biopolymers to the temperatures below 0°C for solutions. All studied biopolymers with about 15% water content are in glassy state at room temperature, providing the grounds for their safe storage in a solid state.

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