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DSC STUDY OF THE POSTDENATURATED STRUCTURES IN BIOPOLYMER–WATER SYSTEMS

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Abstract

The temperature dependences of heat capacity for water–denaturated biopolymer (globular proteins, collagen and DNA) were measured in a wide range of temperatures (0–140°C) and water content of the systems.. It has been shown that thermally denaturated globular proteins (lysozyme, myoglobin and catalase) are able to form the thermoreversible heat-set structures under the certain conditions studied. The additional endothermal maximum observed is the calorimetric manifestation of the phase transition related to the melting of these thermotropic non-native structures. The melting gels are completely formed just after denaturation during relatively short time and only their prolonged state at $T>T_d$ leads to their transformation to thermoirreversible non-melting ones. The postdenaturated structures from water–denaturated protein (Mb, Lys and RN-ase) systems with a different amount of free water were also studied. The thermoreversible cold-set gels are formed from both water–denaturated DNA and water–denaturated collagen systems. These thermotropic structures are metastable. A spatial gel network of both collagen and DNA is formed from the native-like renaturated structures.

Keywords: differential scanning calorimetry, biopolymer–water systems, thermoreversible postdenaturated structures

Introduction

The interest in the fundamental studies of the gels which are formed from biopolymers has been growing in the recent years due to their importance in the first turn in food science and technology. This paper presents the calorimetric study of aggregation structures, which originate in biopolymer–water systems after irreversible denaturation. As it is known, with the change of temperature at some definite conditions the denaturation of native biopolymer molecules can be accompanied by formation of thermotropic gel network. Both denaturation and gelation correspond to sharp structural changes. Whereas the denaturation is connected with the change of the structure of individual biopolymer molecule, the gel formation represents the change of biopolymer–water system structure as a whole. It is also well known that the formation of two types of thermotropic gels, the so-called heat-set and cold-set gels can take place after denaturation [1–3]. All physical and chemical properties of the post-

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denaturated thermotropic structures depend on both the structure and properties of the denaturated biopolymer itself and the conditions of denaturation. The gels which cannot be melted are most often formed after the denaturation. However, the melting molecular gels can also be formed at certain conditions. All these gel types will be discussed in the present paper. By now, the structure and properties of different types of gels have been studied by different physico-chemical techniques such as electron microscopy, X-ray analysis, infrared spectroscopy and the method of circular dichroism, measurement of elasticity modulus and dissolubility in aqueous urea solutions [4–8]. The most general conclusion of these studies is that the forces binding the biopolymers into the thermotropic gel network are of the same origin as the forces stabilizing their native structure [9]. The difference in thermostability is evidently connected with the structure peculiarities of gel networks for heat-set and cold-set gels. It has been shown that in the case of heat-set gels (globular proteins) the structure is based on bound globules, which are not completely unfolded during denaturation [4, 5]. Whereas in the case of cold-set gels (fibrillar protein collagen) the gel network consists of short sections of ternary helix which is similar to the one of the native collagen molecule [10].

However, the thermal properties of the different gels have not been completely investigated up to now. Differential scanning calorimetry (DSC) is the best modern method for these purposes. The aim of the present work is to summarize our calorimetric investigations over several years in the field of thermotropic gelation from biopolymers and, in the first turn, to demonstrate the variety of melting gel structures which can be formed from the different biopolymers (globular or fibrillar proteins or DNA) after denaturation. The present paper reviews our published studies in this field and includes some new results as well. Most of the postdenaturated melting structures were found and systematically studied by our group with a help of DSC for the first time. The combined consideration of denaturation, gelation and gel melting as interconnected processes in our opinion has proved to be fruitful.

Materials and methods

The study was performed using the Setaram DSC-111 differential scanning microcalorimeter with a sensitivity of $3 \cdot 10^{-5}$ J s^{-1}. Temperature was controlled with a precision of ±0.1°C over the whole temperature interval 0–140°C. The errors in the absolute values of melting heat and heat capacity were less than 5% and 3%, correspondingly. Myoglobin (Mb), lysozyme (Lys), ribonuclease (RN-ase) and catalase, supplied by (Sigma), were used as the samples of widespread globular proteins. Besides, the fibrillar protein collagen, prepared in our laboratory from rat tail tendon [11], as well as calf thymus DNA, (Sigma) production, were measured.

The role of each of the main parameters of biopolymer environment affecting the gelation process was studied, including pH, ionic strength and buffer molarity of the biopolymer solutions, as well as the concentration at their variation in the wide ranges (for more details [12, 13]). The solutions of either myoglobin in the pH range 4.5–11.0 or lysozyme in the pH range 1.7–9.0 or ribonuclease in the pH range 4.0–8.5

or catalase in the pH range 4.0–10.0 were measured. The effect of concentration on the structure formation processes in denaturated biopolymers was investigated not only for their concentrated solutions (1.5–20%) but for a considerably wider range of the water content change in a biopolymer–water system – up to biopolymer with a small amount of free water.

Calorimetric studies were complemented by visual control of some rheological properties of the studied systems in special transparent glass ampoules under identical heating conditions. The tests of gels dissolubility in aqueous urea solutions were also carried out.

Results and discussion

Heat-set gels from globular protein concentrated solutions

This part of the work is devoted to our last years DSC studies of gel formation from globular protein–water systems in a wide range of all the protein environment parameters. Not discussing these studies in detail, we would like to present here only the most general features which are characteristic of the gelation process in such a system. And in the first turn, we consider the results obtained for the concentrated solutions of all the proteins studied ($C_{prot.}$=1.5–20%), but in so doing only one parameter

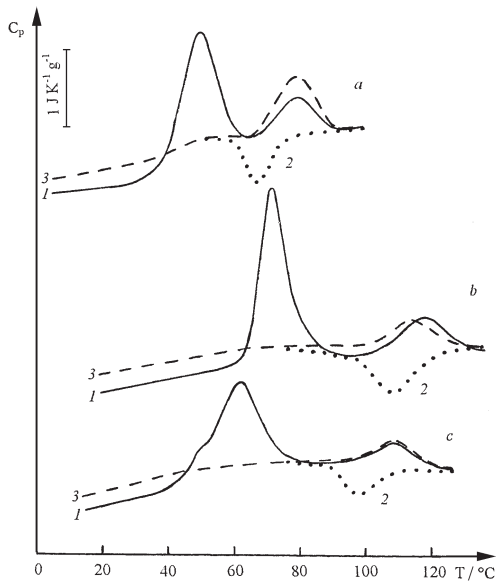

Fig. 1 Temperature dependence of heat capacity of concentrated globular protein solutions. a – lysozyme solution in 1.0 M glycine buffer at pH 2.0; b – *myoglobin* solution in 0.5 M Gl-NaOH at pH 9.1 and c – catalase solution in 0.5 M tris-buffer at pH 9.2. 1 – the first heating, 2 – cooling and 3 – reheating. Concentration of protein is about 15% for all samples; $V_{heat}=V_{cool}$=5°C min^{-1}

(pH) is being varied (Figs 1 and 2). We shall use both published results [12, 13] and the new ones. All the DSC curves present the temperature dependences of protein heat capacity, normalized to the protein mass in the solution, given for each typical experiment at the conditions considered. It should be mentioned, that all the proteins chosen belong to the globular ones with single-stage denaturation transition and demonstrate only one peak of denaturation heat absorption on heating curves of diluted solutions [14].

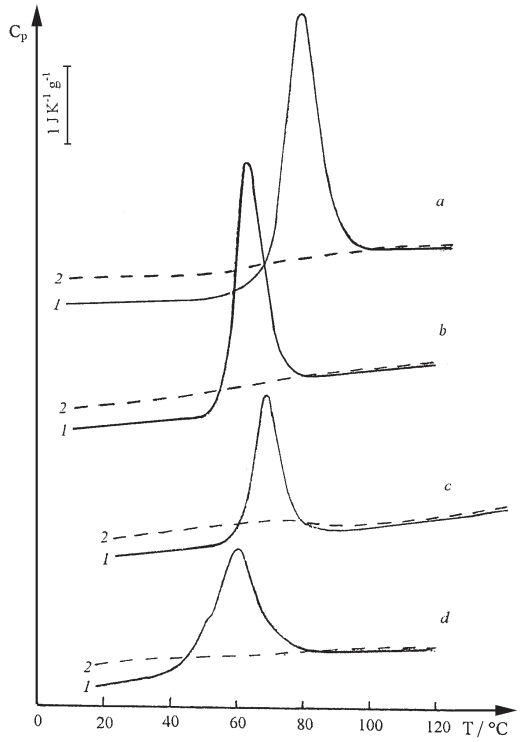

Fig. 2 Temperature dependence of heat capacity of concentrated globular protein solutions. a – lysozyme solution in 1.0 M glycine buffer at pH 5.3; b – ribonuclease solution in 0.5 M Gl-NaOH at pH 4.1; c – myoglobin solution in 0.5 M glycine buffer at pH 4.5 and d – catalase solution in 0.5 M tris-buffer at pH 6.9. 1 – the first heating, 2 – the second heating. Concentration of protein is about 15% for all samples; V_{heat}=5°C min^{-1}

One can see (Figs 1 and 2) that two different situations take place depending on pH of the studied protein solution. In the first case (Fig. 1) the curves obtained consist of two endothermic peaks, whereas in the second case (Fig. 2) there is only one peak of heat absorption corresponding to the denaturation transition. The globular protein denaturation has proved to be irreversible over the whole pH range for all the solutions studied. This means that the maximum corresponding to the protein denaturation disappears at the repeated heating of the sample. One should consider as the

most important result the appearance of an additional endothermic high-temperature maximum (HTM) in the temperature dependence of heat capacity of the protein solutions (Fig. 1) at the certain pH range for each protein studied (either Lys pH 1.7–2.7 or Mb pH 8.5–10.2 or catalase pH 9.2). As an exception, the DSC curves of ribonuclease solutions studied under these conditions have only one denaturation maximum over the whole pH range. The additional endotherm was shown to be connected with the melting of the heat-set gel of denaturated globular proteins, suggesting an additional phase transition which was not previously observed for these systems by DSC. These ordered heat-set structures are thermally reversible, HTM on the DSC curves persists for the multiply repeated heating cycles (Fig. 1, curve 2).

The visual observations of the solutions during their heating, performed at temperatures up to 140°C over the whole pH range, have shown that as a result of aggregation after protein denaturation, three different states arise, depending on the pH value. Firstly, just after denaturation a clear elastic weak gel is formed, which does not repel water under mechanical action (hereafter type 1). In this case the DSC curves of Lys, Mb, as well as catalase, have HTM which corresponds to gel melting because the sample becomes liquid on further temperature increase. As it has been shown the cooling of the melted protein gel under these conditions leads to gel restoration at the temperatures close to T_{melt}. The corresponding exothermic effect, which closely matches the value of heat gel melting during the subsequent heating, is presented on Fig. 1. Secondly, the gel also arises, though it is quite different from type 1 gel in its rheological and mechanical properties, being non-transparent and more elastic gel (hereafter type 2). Under mechanical action and heating this gel repels water quite easily. In this case HTM in the DSC curves of Lys as well as catalase is absent

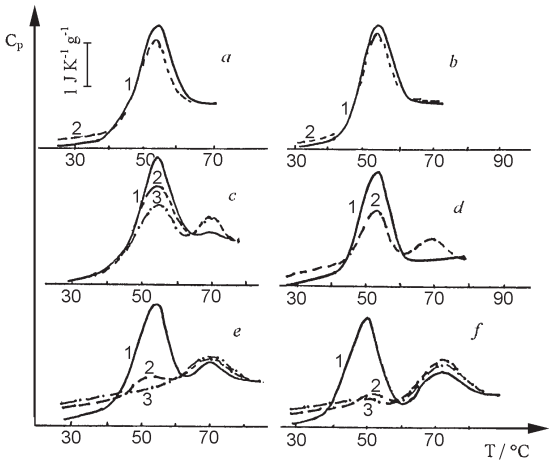

Fig. 3 Temperature dependence of heat capacity of lysozyme solutions. Reversible denaturation a – at 0.5 M, C_{prot}=7%; b – at 1.0 M, C_{prot}=1.5%; e – irreversible denaturation – at 0.5 M, C_{prot}=10% and f – at 1.0 M, C_{prot}=3%; passing from reversible to irreversible denaturation; c – at 0.5 M, C_{prot}=15% and d – at 1.0 M, C_{prot}=13%. 1, 2, 3 – sequence of heating cycles pH 2.0, V_{heat}=5°C min^{-1}

(Fig. 2a, 2d). The decrease of the denaturation thermal effect due to post-denaturation aggregation of protein molecules is the only calorimetric manifestation of this type gel formation. And finally, one more situation, in which HTM is also absent, exists. In this case, after denaturation, a turbid viscous solution occurs, as it takes place in the RN-ase solutions over the whole pH range studied, and in the Mb solutions at pH<5.0 (Fig. 2b, 2c) under the conditions chosen.

In such a way it has been proved that the heat-set gel from globular protein concentrated solution is formed during the denaturation process as early as inside its temperature range. The HTM is the calorimetric manifestation of the new phase transition which happens in type 1 gel upon melting. It is very important that both formation and melting of these new non-native melting structures are thermoreversible processes (Fig. 4b).

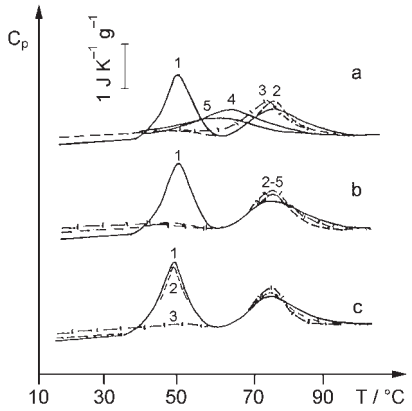

Fig. 4 Melting temperature and thermal effect of lysozyme gel as a function of the melt prehistory. a – The dependence on maximum heating temperature. 1 – the first heating up to 100°C; 2, 3, 4, and 5 – after heatings up to 100, 110, 125 and 135°C, respectively. b – The repeated heatings of identical heating-cooling regimes. 1-5 – the succession of heating. c – The effect of cooling rate on ability to renaturate. 1, 2 and 3 denote the succession of sample heating. 2 – after quenching; 3 – after cooling in the device. pH 2.0; C_{prot}=10%; V_{heat}=5°C min^{-1}

The present results of DSC study are in a good agreement with the structural investigations [4, 5], in which two types of heat-set gels of globular proteins were also found. It has been shown there for the most part the non-transparent gels of the type 2 are formed, which have a strong cross-linked structure based on bonded, partially unfolded protein molecules. For such a gel the dense regions are randomly distributed in the network. The homogeneous clear gel (in our classification gel of type 1) was found in the only case, namely, in the concentrated lysozyme solution at pH 2.0. This gel has regular linear structure formed by successive attachment of disc-shaped globules, as a whole, which is due to generation of β-sheets between aggregating globules [4, 6]. In this case a uniform protein density distribution is characteristic for the net-

work structure over large distances. It should be stressed that according to the work [6] the amid 1 band in the IR spectra of the lysozyme solution (at pH 2.0) connected with this β-structure appears, disappears and appears again at the same temperatures at which the formation, melting and restoration of the type 1 gel, correspondingly, take place as it follows from the DSC studies. The similar structural investigations for the other globular proteins either Mb or catalase, in which the type 1 gels can also be formed, are absent. But since the heat-set gels of these proteins have the thermal properties close to those of lysozyme we can suppose that their structures are also alike.

Thus, the results cited above have shown that the role of pH (other parameters being constant) in the globular protein gelation is very important. The effect of either protein concentration or molarity or ionic strength of the studied protein solutions (at the fixed pH) on the gelation was also investigated [12, 13]. As a consequence, we can demonstrate it for the case of the lysozyme solutions (Fig. 3). These results show that the critical value exists for each environment parameter for the incipient stage of the melting gel formation during the globular protein denaturation process. A definite set of the parameters, for which the heat of gel melting reaches its maximum and which indicates the optimum gelation conditions also exists. Note here, that a three-fold decrease of scanning rate in these experiments only slightly increases the heat of the gel melting. It means that the rate of gel formation is comparable with the heating rates used. It has been found that the process of gel formation is practically completed at V_{heat}=1.5°C min^{-1}.

It has been shown that further increase of the protein concentration, up to 20%, leads to the increase of both the temperature and the heat of the gel melting. Whereas it has been obtained that the NaCl addition which changes the ionic strength of the solution noticeably increases the temperature of gel melting but practically does not have any influence on the melting heat. It should be noticed that it is possible to broaden considerably the pH range in which the melting gel can be formed. (All the data in detail see in [12, 15].) It has been established that one can provide by varying all environment parameters such changes in the structure of the branched type 2 gel, which causes its melting. It results in the appearance of HTM in the DSC curves of such a gel (hereafter type 3). In this case HTM relates to the melting of the ordered part of the mixed-type gel formed. Such postdenaturated type 3 gels were found not only for the lysozyme concentrated solutions but for the myoglobin and catalase ones, as well, under the definite conditions.

It should be stressed that in the case of globular proteins the thermoreversible heat-set gels are formed at $T>T_d$. This refers to both processes of gelation at the first heating of the native solution and the gel restoration from the melt. Further cooling down to room temperature and keeping at T_{room} for a long time introduce no essential changes into the heat-set gel structure. (as well as according to [7] the elastic properties of the protein gel also change insignificantly under cooling down to T_{room}). It means that the postdenaturated structures which we study are completely formed during relatively short time by contrast to those which are arisen under long annealing at $T>T_d$ as it takes place in other approaches [8, 9].

It has been shown that thermostability and, hence, gel structure depend noticeably on its thermal prehistory, in particular on the temperature to which the sample is heated and on its exposure at this temperature. It has been found that the heat-set gel's thermostability decreases with the increase of both factors. The dependence of gel melting on maximum heating temperature for the lysozyme solutions, as an example, is demonstrated in Fig. 4a. The effect of different time of the sample's stay at high temperatures on the thermal properties of the lysozyme gels is the same [13, 15]. Similar results have also been obtained for the other protein solutions studied. Whereas visual observations have shown that the heat treatment has practically no influence on the elastic properties of gels formed. Consequently, it is possible to receive thermoreversible melting gels with different thermal stability and ordering degree by varying of both factors until the state of thermoirreversible non-melting ones.

It appeared rather unexpected that one could avoid gel restoration at cooling down to T_{room} and obtain again the renatured globular protein solution by quenching (sharp cooling) the sample immediately after the gel melting. Then the DSC curves of the subsequent heating of such a solution are close to the initial ones (Fig. 4c) and contain again the denaturation maximum. It has been concluded that in this case the protein, after irreversible denaturation (due to gelation), acquires the possibility to renaturate. The same experiments were also carried out for both Mb and catalase solutions. These results mean, in our opinion, that just after denaturation the melting gels are formed by interaction between denaturated practically folded globules. And we can suppose that such globule state is similar to the well known one of melted globule in dilute solution [23]. Keeping the melted gels at high temperatures leads to unfolding of the globule and to forming thermoirreversible non-melting gels after their cooling.

Thermotropic structures from globular protein–water systems in a wide concentration range of the components

All the results cited above are related to the gelation process in the concentrated buffer solutions of globular proteins. Now we consider the process of non-native structure formation in protein–water systems as a function of the water content change over a considerably wider range – from a concentrated solution to protein with a small amount of free water (from 80 up to 25% of water). These researches were carried out for all Mb–water, Lys–water, and RN-ase–water systems. Along with DSC measurements, before, after, and during heating visual observations of the state of these systems, where possible (80–45% of water), were made. The tests of dissolubility in urea solutions of some of the formed gels were also carried out.

As a result, it has been shown that all the systems studied are able to form gels or gel-like structures after thermal denaturation, but gel formation process takes place differently for each protein studied. It has been obtained that in the case of Lys–water or RN-ase–water systems the non-melting type 2 structures are formed. As this takes place, the lysozyme non-native structures arise just after denaturation, whereas the ribonuclease ones form at the temperatures strongly above T_d and by special thermal treatment (for example, 30 min at T=120°C). Visual control of these systems con-

firmed the formation of thermotropic structure which cannot be melted. The corresponding DSC curves have only one endothermal peak connected with irreversible denaturation in all the cases studied. The test of dissolubility in urea solutions (from 1.0 to 11.0 M) of the postdenatured structures of both Lys and RN-ase with different amount of free water has shown that all these structures are not dissolved. This indicates that these thermotropic structures exist through the presence of strong chemical or covalent bonds, in particular –S–S– bonds of the proteins studied [7].

On the contrary, in Mb–water systems, under the same measurement conditions the melting non-native structures are always generated [16]. This permitted us to use Mb–water system as a model for the study of the melting postdenaturated structures formation, while varying the water concentration in a very wide range.

The temperature dependences of the heat capacity of the Mb–water systems have been obtained as the water concentration varied from 80 to 25% (Fig. 5). As it follows from Fig.5, in all the cases studied at the first heating of the samples the curves have both denaturation heat absorption and HTM. Visual observation of the state of the samples has confirmed the formation of the thermotropic gel or like-gel structures in all the cases. As this takes place, it has been shown that in the case of the aqueous Mb concentrated solutions (pH 7.0) the melting mixed-type gels are generated and HTM is related to the melting of the ordered part of such a gel. Since the thermal properties of the Mb postdenaturated structures from the systems with a different water content are very close to the ones of the protein solutions, we can con-

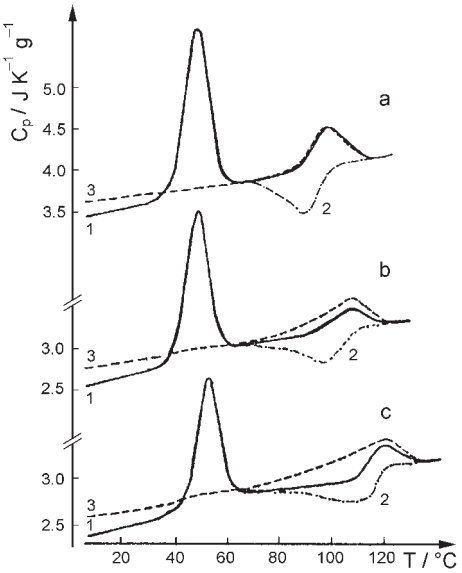

Fig. 5 Temperature dependence of heat capacity of myoglobin–water systems for different water contents: a, b and c – 70, 40 and 30% of H_2O, respectively. 1 – the first heating, 2 – cooling and 3 – reheating. $V_{heat}=V_{cool}=5$°C min^{-1}

sider that HTM in their DSC curves at the first and subsequent heatings (Fig. 5) is also attributed to the melting of the mixed-type non-native thermotropic structures, which are thermally reversible. It has also been established that their restoration under cooling just after melting occurs in all the cases at temperatures far exceeding that of denaturation (Fig. 5). It is important that the Mb postdenatured structures can be practically dissolved, for example in 7.0 M urea solution during several days. This demonstrates that these structures are supported by weaker non-covalent bonds, mainly, by hydrogen bonds [7]. It should be stressed, that the temperature range where the melting mixed-type structures can be formed from denatured globular proteins with a different water content is quite wide: $T_d<T_{gel}<T_{melt}$.

It has been shown that the nature of the formation and melting of non-native structures in denaturated Mb changes smoothly under decreasing free-water content. Whereas a decrease in the amount of water, which gradually changes the conditions for the formation of thermally reversible structures in denaturated protein, leads to appreciable increase in their dispersion in respect of the melting and formation temperatures (Fig. 5) and thereby, being compared with partially crystalline polymers, in the degree of their perfection [17]. Really, one can see that the wide asymmetrical exothermal effect with T_{max} close to T_{melt} is observed at cooling of the sample with the small quantity of free water just after its melting. Then the wide asymmetrical endothermal effect with $T_{max}=T_{melt}$ is obtained after the repeated heatings of such a system. These results taken together mean that the main part of the melting non-native structure forms and melts in the temperature range close to T_{melt}. The other part of this structure arises under cooling as far as the denaturation temperature range and starts to melt under heating just above T_d.

Cold-set gels of collagen and DNA

Now let's consider the thermotropic gels formed from biopolymers after their denaturation under cooling down to room temperature, i.e. cold-set gels. It is well known that denaturated collagen can form thermotropic cold-set gels [2, 18, 19] and their structural, mechanical and thermal properties have been widely discussed. Recently new data on the formation of the similar DNA gels have been obtained in our laboratory as a result of the investigations of the renaturation process in DNA [20]. It has been found that the curves of the temperature dependence of heat capacity of the denaturated DNA samples display an endothermic maximum at more lower temperatures than those of denaturation over the whole range of different free water content. This maximum may be attributed to the melting of ordered thermotropic structures. Visual observations of the state of such samples have shown that during the cooling process to room temperature the denaturated DNA becomes an elastic gel, which can hold a form during long time.

As it can be concluded from our results of the DSC studies, the gels of DNA and denaturated collagen have the following important peculiarities. First, these gels are thermally reversible. It means that thermodynamic parameters of destruction of such gels are reproduced completely with the reproducing of the gel formation conditions.

Second, it has been established that these postdenaturated structures are metastable since their thermal stability changes over time (Figs 6, 7).

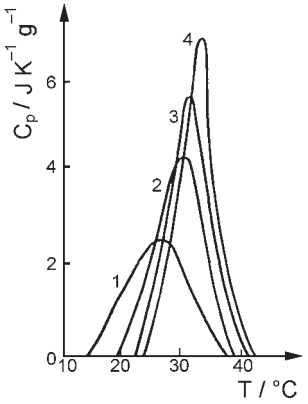

Fig. 6 Evolution of melting curves for denaturated collagen in dependence on the time of gel formation at T=22°C. 1, 2, 3 and 4 – formation times of 30 min, 3 h, 18 h and 7 days, respectively. C_{prot} =20%, V_{heat}=1°C min^{-1}

Figure 6 presents the temperature dependence of the apparent heat capacity for the samples of denaturated collagen (C_{prot}=20%) which after denaturation were kept at constant temperature T=22°C for different periods of time [18]. It has been shown that the maxima in the curves of the postdenaturated gel structures observed correspond to the phase transition of gel melting. The results in Fig. 6 demonstrate the kinetics of gel formation. One can see, that with the increase of the gel formation time, the transformation of the melting curves is typical of that of metastable structures [17]. The melting heat Q_m and the melting temperature T_m increase, whereas the halfwidth of the melting curve ΔT decreases.

The similar transformations of the melting curves as a function of the gel formation time have been observed for DNA samples (Fig. 7). However, as it follows from the results presented in Fig. 7 for DNA samples, the process of gel formation in high

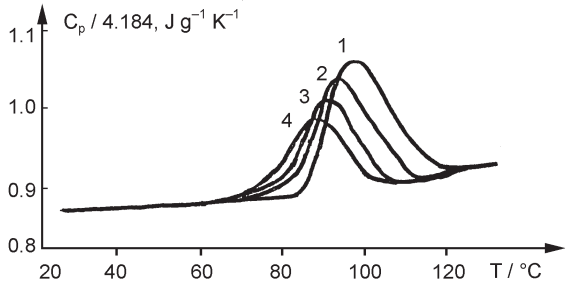

Fig. 7 Evolution of melting curves for DNA in dependence on the time of gel formation at T=70°C. 1 – native DNA; 2, 3, 4 – gel formation times of 3 h, 30 min and 15 min, respectively. Concentration of DNA is 20%. V_{heat}=5°C min^{-1}

concentrated solutions of DNA takes place at temperatures which are about 20–25 degrees lower than the denaturation temperature [20].

It has also been shown that the limit (although practically not achievable) values of the thermodynamic parameters of the gel melting both of collagen and DNA should be given by those of denaturation of these biopolymers with the same concentrations. Thus, it can be concluded that a spatial gel network of both collagen and DNA is formed from the native-like renatured structures, which in their initial states belong to different native biomacromolecules. As it is known, in strongly diluted solutions the isolated molecules of the collagen and DNA can restore their native structures after denaturation [2, 21, 22]. Consequently, it can be presumed that a gel formation process is a process of the partial renaturation.

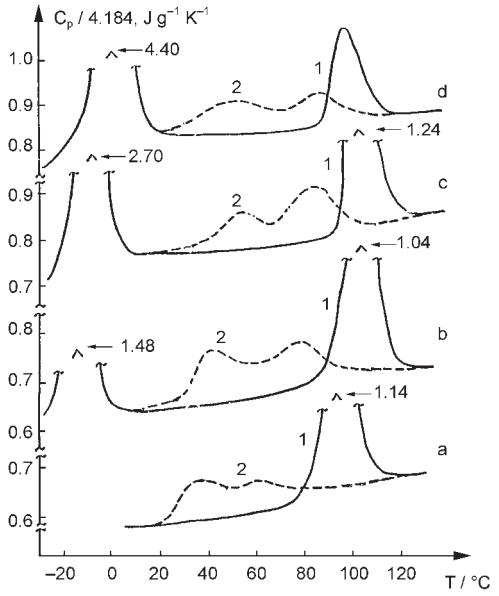

Fig. 8 The heat capacity of DNA samples with different water contents as a function of temperature. a – 41.6%; b – 51.1%; c – 62.5%; d – 80.0% of H_2O. 1 – native DNA; 2 – DNA denaturated after the first heating. $V_{heat}=V_{cool}=5°C\ min^{-1}$

It has been obtained that the further increase (above 20%) of the amount of the biopolymer in either DNA–water or collagen–water systems leads to an appreciable decrease of thermostability of the regular structures formed from these biopolymers. As it has been shown for both denaturated collagen [11, 18] and denaturated DNA [20], the melting heat Q_m and melting temperature T_m decrease when the water content of these systems decreases. In what follows we shall demonstrate it for the case of DNA (Fig. 8). The experimental data presented in Fig. 8 shows the temperature dependence of heat capacity for the samples of DNA both in native and denaturated states with a water concentration varying from 80 to 40%. One should note that the decrease of water content leads to appearance of the second maximum on the DSC

curves of the denaturated DNA, but at lower temperatures. Besides, one can see that with the increase of DNA concentration the low temperature maximum increases, whereas the high temperature one decreases. At the same time, when the DNA humidity decreases the total melting heat of both maxima decreases, as well. Summarizing all these results, we can see that quality and quantity of the DNA melting structures decreases gradually. It can be supposed that native-like structures formed become more imperfect in time. Note, the destruction parameters of a three-dimentional network of the gels never exceed the denaturation heat and temperature values of a native one-dimentional structures, which can serve as an indication that the native-like structures formed are not completely perfect.

Conclusions

The analysis of the whole range of the obtained results shows that the thermally denaturated globular proteins are able to form the thermoreversible heat-set structures under certain conditions studied. It is an important fact that the meltable structures are formed promptly (V_{scan}=1–5°C min^{-1}) after irreversible denaturation. At the same time, the prolonged stay (for several tens of minutes) of the systems under study at $T>T_d$ leads to gradual loss of the gel's ability to melt. Apparently, the protein melting gel is formed by aggregation of practically folded globules. And only its gradual unfolding at high temperatures leads to the formation of non-melting gel.

Unlike the heat-set gels of globular proteins, the cold-set gels of denaturated collagen–water and denaturated DNA–water systems are formed due to the native-like metastable structures. Nevertheless, the thermodynamic parameters of such gels are strongly dependent on the biopolymer concentration and the temperature of the gel formation, as well as in globular proteins. But, in contrast to the gels of globular proteins, the thermodynamic parameters of the denaturation of the native structures serve as limit values to the thermodynamic parameters of melting of the DNA and collagen gels.

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