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Partial heat capacity change – Fundamental characteristic of the process of thermal denaturation of biological macromolecules (proteins and nucleic acids)[1]

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Abstract

We prove experimentally that for any biopolymers (proteins and nucleic acids), the process of thermal denaturation in aqueous medium is characterized by change of the heat capacity regardless a unique spatial structures of different conformations. Absolute values and sign of $\Delta C_p = C_{p,\text{denatured}} - C_{p,\text{native}}$, depends primarily on conformational peculiarities of macromolecules and concrete mechanisms of interaction of water molecules with biopolymers chains – interaction is very clearly found to be of critical importance for the stability of the spatial structure of any biopolymers in aqueous medium.

Keywords: Collagen; DNA; Heat capacity increment; Molten globule; Thermal denaturation

1. Introduction

It is well established that for a single biopolymer the process of destruction of genetically determined unique spatial structure of a macromolecule under thermal action (i.e. thermal denaturation) goes within a certain interval of temperature and takes a portion of energy.

The average enthalpy of a transition (ΔH) from a regular state of the polymer into the irregular can be measured, at present, with rather high accuracy. Besides ΔH we can establish transition entropy $\Delta S = \Delta H/T_m$ (T_m is the transition temperature) and ΔG is the difference of Gibbs energy for these states and, so in fact, the stability of structure [1,2]:

$$\Delta G(T) = (T_m - T)\Delta H/T_m - \int_T^{T_m} \Delta C_p dT + T\int_T^{T_m} \frac{\Delta C_p}{T} dT,$$

where $\Delta C_p = C_p^D - C_p^N$ is the heat capacity difference between a regular (native) and irregular (denatured) states.

In the description of heat effects of chemical reactions, it is common to use $d\Delta H/dT = \Delta C_p$ (where, ΔH is the reaction enthalpy, T the temperature and ΔC_p the difference of heat capacities of initial and final states). If the ΔC_p change is positive, the heat effect becomes more positive with the increase of temperature [3]:

$$\text{at } \Delta C_p > 0, \quad \frac{d\Delta H}{dT} > 0$$

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and vice versa

$$\Delta C_p < 0, \quad \frac{d\Delta H}{dT} < 0.$$

If the heat capacity does not change, i.e. the sum of heat capacities of the reaction product equals to the sum of heat capacities of initial matters, the heat effect of the process does not depend on temperature:

$$\Delta C_p = 0, \quad d\Delta H/dT = 0 \quad \text{and} \quad \Delta H = \text{const.}$$

Thus the problem is not only ΔC_p measurement, but also precise establishment of the temperature dependence of reaction enthalpy to compare experimental and calculated values of ΔC_p. Consequently, it is important to obtain accurate values of ΔC_p and use them to extrapolate thermodynamic functions (ΔH, ΔS, ΔG), a standard reference temperature (e.g. 298 K). We are offering the solution of the problem of denaturation increment of specific heat capacity (ΔC_p) peculiar in general to thermal denaturation process of biopolymers. The solution is supposed to be found through direct microcalorimetric (DSC) measurements of aqueous solutions and absolute heat capacity values of biopolymers of three quite different conformational classes with different primary chemical structure: globular proteins, fibrous proteins, nucleic acid (*ds* – DNA). While for globular proteins it is established for sure that the increase of the heat capacity of a molecule follows the decay of the regular structure, the value and sign of denaturation increment of heat capacity for other class of polymers still remains unknown, such as fibrous proteins. As to the value and sign of ΔC_p for double helix of polydeoxynucleotides the question, however paradoxical it seems, is still open both for natural DNA and synthetic homopolynucleotides.

The positive heat capacity change observed for the thermal denaturation of globular proteins indicates that hydrophobic interaction is the dominant driving force stabilizing the unique native structure of proteins [1–4].

By using a precise scanning microcalorimetry, it was also found that melting of molten globule i.e. unfolding of this intermediate state is associated with the significant heat capacity change. Some author classified this transition as a second-order phase transition because we see the second derivative of thermodynamic potential but not the enthalpy and entropy [5].

The advances in studying the thermodynamic parameters of helix-coil transition (including ΔC_p) in diluted water solutions of collagen and DNA are connected with the appearance of the precise scanning microcalorimetric technique. However, as it is correctly noticed in Ref. [2], some complications in these studies are: the first is that denaturation of collagen is much slower process than the denaturation of any other proteins. Therefore, its calorimetric study can be carried out only at low-heating rates at which the apparent melting curve is more or less close to the 'equilibrium' melting curve (this condition can be seen in [6–8]). The other difficulty is connected with a very high viscosity of collagen solution and with a drastic drop of it's viscosity at denaturation. This might induce an artifact in measuring the denaturational heat capacity change by scanning calorimetric technique. Consequently, the calorimetric study of collagen intramolecular melting can be done only by using a very sensitive instrument which allow measurements in highly dilute solutions and at very low-heating rates [2]. The capillary scanning microcalorimetric devises – the new generation of DSC – satisfy this claims. However, whenever one applies DSC, one must perform the heat capacity measurements at three or more different heating rates in order to extrapolate the heating rate to zero. Only in such a case, the obtained results characterized the 'equilibrium properties' [6–9] of the specimen. Precisely such experiments made us to study the denaturational increment of heat capacity in diluted water solutions of collagen.

The investigation of conformational change of nucleic acids has flourished due to two new developments: first, improvement of the biochemical preparation techniques of native DNAs and DNA fragments and our ability to manipulate native conformations or engineer new sequences, and, secondly, improvement of experimental techniques for determining the energy parameters of conformational transitions using highly sensitive instruments – new generation scanning microcalorimeters (DASM-4, 4A; (Russia), 'Microcal' (USA) and other modern 'nanocalorimeters' [10]).

The aim of the presented research is to prove experimentally that for any biopolymers the process

of thermal denaturation in aqueous medium is characterized by change of the heat capacity regardless a unique spatial structures of different conformations both for proteins and nucleic acids (DNA, RNA). This accurate values of ΔC_p and correct thermodynamic parameters (ΔH, ΔS, ΔG) determined as a function of base composition (for nucleic acids), aminoacids sequences local modifications (for proteins) and solution variables, will provide information about the contributions of various noncovalent interactions to biologically important macromolecules structures stability.

2. Experimental

2.1. DNA and proteins samples

Super pure samples of cow spleen DNA with protein concentration in samples <0.1%; RNA<0.5%; MW>10^7D were used. DNA solutions were prepared from the lyophilized materials by first dissolving the polymers in double-distilled water for 48 h and then dialyzing the solution against several changes of sodium phosphate buffer at different pH and ionic strength. The concentration of DNA was determined via UV spectrophotometric analysis.

The fiber protein – acid soluble rat skin collagen in 0.1 M citrat buffer at pH 3.7 in absence of salts were used. The concentration of collagen was determined via polarimetric (406 nm) analysis.

The globular protein – Horse cytochrome C was purchased from Sigma. 'Molten globule' state was obtained at pH 2.0 (glycine buffer solution, 0.5 M NaCl).

2.2. Calorimetry

(a) Differential adiabatic scanning microcalorimeter DASM-4 (made in Puschino, Russia). The well-known device (capillary microcalorimeter) has extra sensitivity and stability of base line.
(b) Differential adiabatic scanning microcalorimeter DASM-4A (made in Puschino, Russia). Besides the potentialities of DASM-4, DASM-4A allows dealing with preparations which have not constant viscosity and bad-heat conductivity. The other advantages are: high sensitivity; high reproducibility of base line; the control of the device is possible by the computer; the possibility of automatization of data processing.
(c) Adiabatic low-temperature calorimeter (Institute of Physics of the Georgian Academy Sci.) [6–9]. It represents a classical Nernst's calorimeter for the study of absolute heat capacity of a matter in thermal equilibrium conditions in a wide range of temperature (2–400 K).

3. Results and discussion

3.1. Globular proteins

The temperature dependence of the partial heat capacity of cytochrome C in solution at different pH values in the presence of salt (0.5 M NaCl) is given in Fig. 1. It is well known that at pH 1.8–2.0 and in the presence of salt, cytochrome C is in the state of molten globule. As we see that the transition:native state (pH 3.7)–molten globule (pH 2.0) accompanied by significant change in the heat capacity at 25°C. Note that the partial heat capacity value for molten globule is intermediate between native (folded) and denatured (unfolded) states (see Figs. 2 and 3). We measure the temperature dependence of the heat capacity at different scanning rate (0.125; 0.25; 0.50; 1.0; 2.0 K min^{-1}); (the reversibility of the process molten globule–unfolded state was checked by reheating; this process was found to be reversible and could be adequately represented by a two-state process (see also [11,12])).

So we suggest that thermal unfolding of the molten globule state of horse cytochrome C is a cooperative

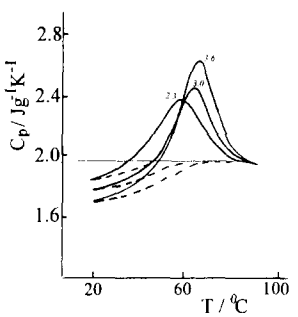

Fig. 1. Temperature dependence of partial specific heat capacity of cytochrome C in solution at different pH values.

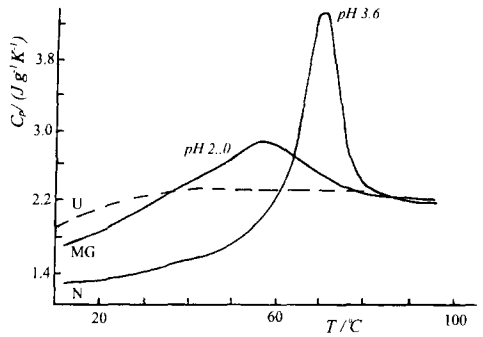

Fig. 2. Temperature dependence of partial specific heat capacity of cytochrome C in the various conformational states of protein: (1) Unfolded state, (2) Native state at pH 3.7, (3) Molten globule state at pH 2.0 (0.5 M NaCl).

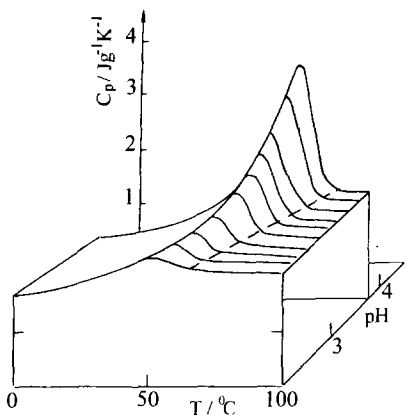

Fig. 3. Heat capacity surface for Cytochrome C at different temperatures and pH.

process with a small but distinct ΔC_p and the enthalpy change of unfolding ΔH=173±15 kJ mol^{-1}. The enthalpy of unfolding transition for molten globule is equal to only 1/2 of total enthalpy differences between folded and unfolded state (381± 30 J mol^{-1} K^{-1}); heat capacity change is small, but distinct and positive (Table 1); all this results suggests that transition 'molten globule–unfolded state' for cytochrome is like that of first-order phase transition, or may be same mixed transition. The last possibility is demonstrated in precise statistical theory of globule-coil transition [13]. Thus, for the rigid chains globule-coil transition is represented by abrupt first-order phase transition with definite change of density. However by certain attribute

Table 1
Heat capacity change of thermal denaturation for biopolymers in aqueous solutions

Biopolymer	ΔC_p (J K^{-1} g^{-1})	ΔC_p (J K^{-1} mol^{-1})
Globular proteins		
Citochrome C	+0.6	+7400/$M_{Cyt.}$
Citochrome C (pH 2.0, 0.5 M NaCl)	+0.15	+1900/$M_{Cyt.}$
Fibrous proteins		
Collagen pH 3.9	+0.22	+60/$M_{threepept.}$
Nucleic acides		
RNA [a]	+0.24	+110/M_{BP}
rRNA [b]	+0.25	+1500/M_{tRNA}
DNA		
pH 7.0	+0.15	+105/M_{BP}
pH 2–6	+0.2–0.3	+245/M_{BP}
pH 10–12	+0.10	+70/M_{BP}
pH 8–10	−0.10	−70/M_{BP}

[a] V.V. Filimonov, in: H. Hintz (Ed.), Thermod. Data for Bioch. & Biotechn., Springer, 1986.
[b] J.M. Sturtevant, PNAS USA, 74 (1977) 2236.

this transition is closer to second-order phase transition, for example, the heat of transition is small [13].

Our experimental results is very important for detailed computer simulations and calculations of realistic protein folding energy landscapes which resembles a funnel [14] in which the protein is guided through a myriad of pathways to its natural shape, referred as its 'native structure.' Although the folding protein eventually finds its way to a minimum-energy state, the energy landscape has rugged features. These features correspond to 'local' minimums in which the still-folding protein may be temporarily trapped because different chain segments are 'frustrated,' i.e., they have conflicting interactions when brought together at random. The 'molten globule' is placed in the middle of funnel (see [14]), where the polymer chains are half-way towards the folded structure. Our thermodynamic measurements give the funnel's slope (the effective search size is reduced, making the computational task easier) while separate measurements on the motions of molten globules by NMR gives us the possibility to estimate the motions of the ruggedness.

3.2. Fibrous proteins

The remarkable feature of water soluble fibrillar proteins is that they represent a coiled-coil structure. Collagen – the fibrillar protein of connective tissues represent triple-stranded coiled coils of the poly-prolyne type. At an increase of temperature above some critical level, the regular structure of collagen molecule breaks and the chains separate and fold into random coils [2]:

$$\alpha 3_{\text{helix}} \rightarrow 3\alpha_{\text{random coils}}$$

Melting of collagen in salt-free water solution on heating proceeds with an extremely intensive heat adsorbtion [2,15]; this transition is much sharper than that of any other proteins studied so far [2].

For the first time the question about denaturational heat capacity increment in collagen solution was discussed by Andronikashvili et al. [7,8]; authors had measured the temperature dependence of collagen in the helical and coil states over the temperature range 4–350 K; the heat capacity for dehydrated collagen in the helical and coiled states $\Delta C_p = C_{p,\,\text{coil}} - C_{p,\,\text{helix}} = 15\ \text{J mol}^{-1}\ \text{K}^{-1}$ (Fig. 4). It is significant that these results are obtained by means of adiabatic absolute calorimetry at equilibrium conditions.

Fig. 5 presents the partial heat capacity functions of the collagen obtained at various heating rates. Fig. 6 presents a plot of the transition enthalpies and temperatures versus the scanning rate. (the width of transition in this condition is constant $\Delta T = 2.2$ K). The 'equilibrium' values of temperature and enthalpies of transition is: $T_m = 36.8 \pm 0.5$°C and $\Delta H_m = 60 \pm 3\ \text{J g}^{-1}$, that is in good agreement with values obtained in Ref. [2]. This results indicate that

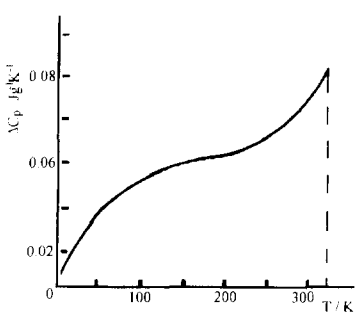

Fig. 4. Temperature dependence of heat capacity difference (ΔC_p) of dehydrated collagen ($\Delta C_p = C_{p,\,\text{coil}} - C_{p,\,\text{helix}}$).

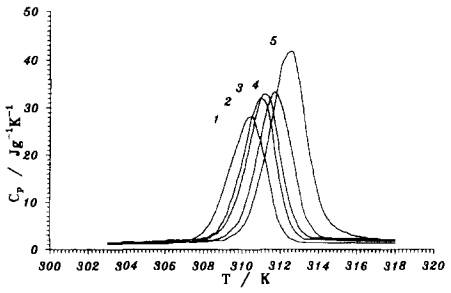

Fig. 5. Temperature dependence of partial specific heat capacity of collagen in solution at different scanning rates: ((1) 0.125; (2) 0.25; (3) 0.5; (4) 1.0; (5) 2.0 K min^{-1})).

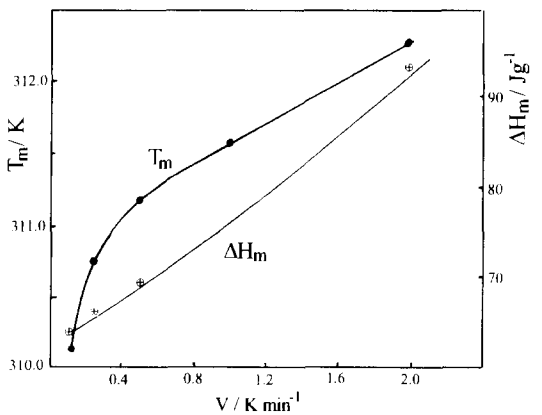

Fig. 6. Temperature and enthalpy of helix-coil transition in collagen solution versus scanning rate.

for description of the helix-coil transition in collagen solution, it is necessary to undertake detailed kinetic analysis of this process (see [16,17]).

The observed decrease of enthalpy with variation of scanning rate might be partly the result of a decrease of transition temperature. The direct measurements of the heat capacity of collagen (Fig. 7) indeed revealed a change of the heat capacity at melting and in compliance with obvious thermodynamic relation $\Delta C_p = \text{d}\Delta H/\text{d}T$, the experimental value of denaturational change of heat capacity also depend on scanning rate; extrapolated value of $\Delta C_p = 0.18 \pm 0.05\ \text{J g}^{-1}\ \text{K}^{-1}$. This value which coincides with ΔC_p is also measured directly in salt-free water solution of collagen [2].

Murphy et al. [18] suggest that protein unfolding and the dissolution of hydrophobic compounds (including solids, liquids and gases) in water are characterized by a linear relation between entropy

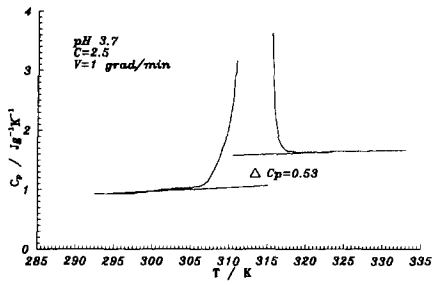

Fig. 7. A fragment in magnified scale of temperature dependence of partial specific heat capacity of collagen (at scanning rate 1 K min^{-1}).

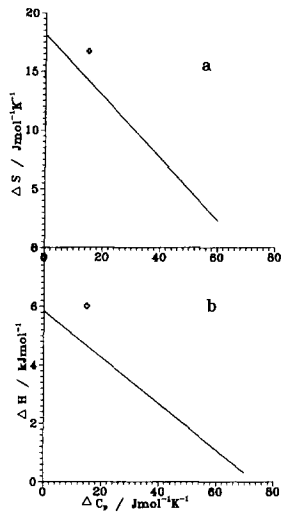

Fig. 8. Entropy (ΔS) and enthalpy (ΔH) of denaturation versus heat capacity change (ΔC_p) from collagen at 25°C. Linear correlation of thermodynamic properties for denaturation at 25°C for globular proteins also indicated on figures (line).

change and heat capacity change. The same slope is found for various classes of compounds, whereas the intercept depends on the particular class. The close similarity of properties of solid model compounds to those found in proteins suggests that the solid nature of protein core plays a significant role in the formation of the folded state.

Fig. 8 presents the dependences of $\Delta H=f(\Delta C_p)$ and $\Delta S=f(\Delta C_p)$ at 25°C for collagen. In this figure, we also indicate the linear correlation of thermodynamic properties for denaturation according to Ref. [18]. We see that data for collagen completely distorted this correlation tabulated from globular proteins, which indicates that the mechanism of stabilization of their structure is fundamentally different.

The thermodynamic specificity of collagen was considered in our previous investigations (including low-temperature heat capacity data [6–8] and NMR-measurements) of collagen melting process and it was shown that it cannot be understood without considering the specific role of water, which seems to be incorporated in triple-helical coiled-coil structure of collagen (see water–carbonyl helix model of collagen [19,20]). Later Privalov [2] also arrive to similar conclusion on the basis of thermodynamic studies of collagen and suggest that 'water,' is not only a solvent for it (collagen-G.M.).

3.3. Nucleic acids

In addition to the characteristic heat effects, the 'helix-coil' transition in DNA are also accompanied by changes in the volume [21,22] and in the heat capacity $\Delta C_p=C_{p,\,coil}-C_{p,\,helix}$ [23]. The volume and absolute heat capacity properties reflect more directly the degree of hydration [21–23], an interaction found very clearly to be of critical importance for the stability of the double-helical structures in aqueous solutions. However the quantitative role of water in maintaining native DNA conformation as well as its participation in helix-coil transitions accompanying varying solution conditions remains unknown. Our general effort, therefore, has been directed toward determining variations in heat capacity (ΔC_p) as well as those of the standard thermodynamic functions (ΔH, ΔS, ΔG) upon helix-coil transition of DNA (at different pH and ionic strength).

Fig. 9 shows the temperature dependence of absolute heat capacity of DNA in 'solid state' (hydrated DNA, when water content ~0.61 g_{H_2O}/g_{DNA}) in the helix-coil transition temperature region. So we demonstrate the average value of $\Delta C_p=$ 0.36 J g^{-1} K^{-1}.

The temperature dependence of the partial heat capacity of DNA in solution at different ionic strength is given in Figs. 10. Fig. 11 presents a fragment in magnified scale of the partial specific heat capacity of DNA as a function of temperature, at pH 4.

The remarkable feature of the functions $\Delta H=f$(pH) and $\Delta H=f(T)$ is that they are not linear [23]. With an increase of temperature the enthalpy of the conforma-

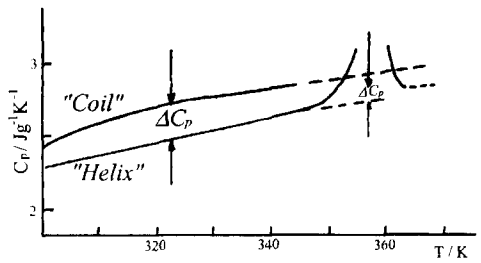

Fig. 9. The absolute heat capacity of hydrated DNA (0.61 g_{H_2O}/g_{DNA}) in temperature region of helix-coil transition.

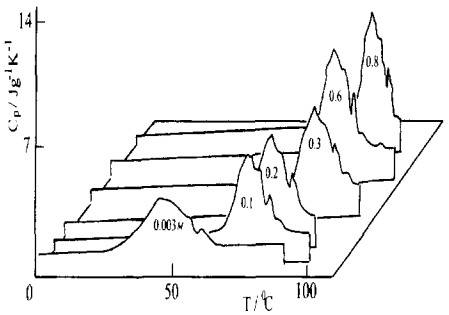

Fig. 10. Temperature dependence of partial specific heat capacity of DNA at different ionic strength (pH 7.0).

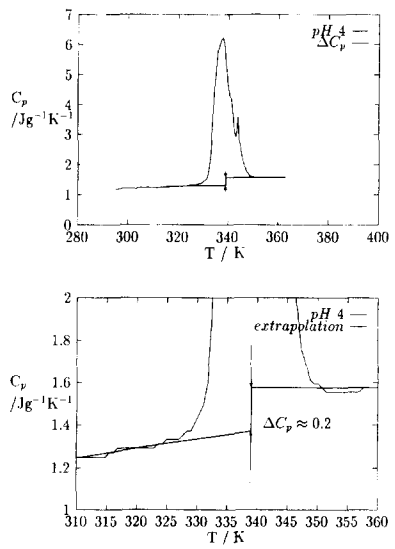

Fig. 11. A fragment in magnified scale of temperature dependence of heat capacity of DNA at pH 4.

tional transition increases over the pH interval 2.5–6.0, slightly changes at pH 6.0–7.0 and decreases in the interval pH>7.0.

In accordance with the thermodynamic equation $\Delta C_p = d(\Delta H_m)/dT_m$, dependence of the transition enthalpy on temperature shows up the difference of the heat capacity of native and denatured states of DNA. In this case, the absolute value and sign of ΔC_p will be determined by the properties of the solvent (ionic strength, pH, etc.). At neutral pH ΔH_m changes slightly [23]. So we can consider this specific feature of $\Delta H_m = f(\text{pH})$ and $T_m = f(\text{pH})$ dependencies in neutral pH area to be the reflection of the enthalpy–entropy compensation effect [23], which also brings to zero ΔC_p in winding–unwinding reactions. Therefore, even slight changes in the chemical composition of the solvent can strongly influence the balance of forces responsible for double-helix structure (coulomb-electrostatic, stacking, and hydrophobic interaction as well as hydrogen bonds). In this case we, must bear in mind the temperature dependence of transition enthalpy to derive a proper equation for free energy and that compels us to take into account ΔC_p. According to Ref. [23] calculated values of $d(\Delta H_m)/dT_m$, for ΔC_p are: $\Delta C_p = 0.6 \pm 0.06$ J g^{-1} K^{-1} (pH 2.0–7.0) and $\Delta C_p = 0.07 \pm 0.01$ J g^{-1} K^{-1} (pH 7.0–12).

To find the accurate values of ΔH and ΔC_p we have to take into consideration (as was recommended by Privalov) buffer ionization effects. The absolute value of ionization effects in case of ΔH is up to 2.25×10^{-6} J that is nearly 0.07% of measured value; in the case of ΔC_p the absolute value of the buffer ionization effect is 8×10^{-7} J K^{-1} that is up to 0.45% and is also negligibly small.

There is no analysis of data on the nature of the denaturation increment of DNA heat capacity in the literature simply because it was generally considered for many years that in dilute aqueous solutions of DNA $\Delta C_p = C_{p,\ coils} - C_{p,\ helix} = 0$. As shown in Ref. [23], it is not excluded that denaturation change of heat capacity is caused by 'melting' of the ordered water clusters incorporated in double-helix structure together with an increase of the number of the degrees of freedom in atomic groups of polynucleotide chains in the state of statistical coils as well as hydrophobic effects.

4. Conclusions

We prove experimentally that for any biopolymers the process of thermal denaturation in aqueous

medium is characterized by change of the heat capacity regardless either a unique spatial structures of different conformations both for proteins or nucleic acids (see table). Absolute values and sign of ΔC_p depends primarily on conformational peculiarities of macromolecules and concrete mechanism of interaction of water molecules with biopolymers chains – interaction very clearly found to be of critical importance for the stability of the spatial structure of any biopolymers in aqueous medium.

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