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Low-temperature DSC study of the hydration of ss-DNA and ds-DNA and the role of hydrogen-bonded network to the duplex transition thermodynamics

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Abstract

The study of thermodynamic parameters of ice–water phase transition in aqueous solutions of ss-DNA, at different concentration of polynucleotide single chains is reported and the critical concentration for which the enthalpy of transition is zero has been determined. The thermodynamic parameters of ice–water phase transitions, obtained for native ds-DNA and for ss-DNA in the whole concentration region (0.2–2.0 g H_2O/g DNA) and the hydration values (bound water quantity) for native (*helix*), denatured (*single strand coils*) and *unfolded* ss-DNA are compared. The critical values of the hydration at which all the water in the DNA–H_2O system exists in the bound (*unfrozen*) state are established, for these conformations, with great precision: $N^{\Sigma}_{\text{ds-DNA}} = (0.55 \pm 0.01)$ g H_2O/g DNA, $N^{\Sigma}_{\text{ss-DNA}} = (0.40 \pm 0.01)$ g H_2O/g DNA. According to this calorimetric data the transformation "*double stranded helix*" → "*single stranded chains*" is accompanied by dehydration of ds-DNA: $\Delta N = N^{\Sigma}_{\text{ds}} - N^{\Sigma}_{\text{ss}} = (0.15 \pm 0.01)$ g H_2O/g DNA. We suggest that the formation of B-DNA duplex from mixing of their complementary single strands is mostly accompanied by the uptake of structural water molecules and so, the overall hydration of a duplex is mainly determined by its conformation and stability.
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Keywords: ss-DNA; ds-DNA; Hydration; Low-temperature DSC; Stability of duplexes

1. Introduction

How "the aperiodic crystal of heredity" [1]—the three-dimensional structure of the DNA double helix—is stabilized is an attractive problem. Despite the increasing knowledge, and the considerable progress made in the X-ray analysis of short oligonucleotides, the very complex surface of double helix and its hydration and solvation are the most poorly defined parts of the structures obtained by crystallography methods, since they are topologically disordered or dynamically averaged and conformational fluctuated (special for high molecular DNA in aqueous solutions).

Using a low-temperature DSC we studied the hydration of high molecular ds-DNA [2]. We designed a new type of calorimetric vessel on the basis of the stainless steel crucible of the DSC-141 (SETARAM) [2]. The new ampoule gives us the ability of measuring the heat of melting of water at different concentrations

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of ds-DNA without changing the materials inside the calorimetric cells. This gives the possibility of obtaining the principal results for the correct dependence of the enthalpy of the ice–water phase transitions versus the concentration of biopolymers (starting at low water content and up to the regime of "semidilute" and "dilute" solutions (definition of [3])) and revealed the critical concentration at which the enthalpy of transitions becomes zero [2,4–6]. We measured the hydration of DNA for native (*helix*) and denaturated (*coils*) states and suggest that the main contribution in the enthalpy of the process of the heat denaturation of DNA duplex is the enthalpy of disruption of the ordered water structure in the hydration shell of the double helix [2]. It should be emphasized that other authors [7] obtained similar result on the basis of the analysis of data obtained by using different physical methods, measuring structural, mechanical, electrical and energetically properties of *DNA–ions–water* systems. Possibly this part of energy composes the non-specific general contribution (70%) in the enthalpy of transition of all type of duplexes [8]. It has been also established [2] that for DNA in the condensed state the ratio between the heat capacity increment (ΔC_P) and the entropy change (ΔS), $\alpha = \Delta C_P/\Delta S \approx 2$ is smaller than for DNA in dilute aqueous solutions ($\alpha \cong 2$–4). This means [2] that there are some other sources for the large heat capacity change in diluted solutions of DNA, for example, the hydrophobic effects [9] and the unfolding of single polynucleotide chains [10].

The main difficulty in identifying and characterizing the solvent-exposed groups of atoms of DNA in their "coil" state is related to the uncertainty associated with the degree of unfolding of the single stranded state [10–12] (Fig. 1). It should be noted that there are a number of experimental observations suggesting that the composition and sequence may retain considerable residual structure [11].

The total enthalpy of denaturation of ds-DNA, $\Delta H_{\text{melting}}$, includes the "specific" and "nonspecific" contributions $\{\Delta H^0_{\text{melting}}(T) = \Delta H_{\text{spec}}(T) + \Delta H_{\text{nonsp}}(T)\}$ [8]. The "specific" interactions includes the hydrogen bonds which play a central role in forming Watson–Crick (W–C) A·T and G·C base pairs. In addition to interstrand H-bonding, intrastrand base-stacking and interstrand cross-stacking interactions are important in maintaining the bases in a stacked structure along the length of the DNA backbone. In general, hydrogen bonds between W–C base pairs are viewed as "informational", whereas the base-stacking interactions are regarded as "noninformational" merely stabilizing the double helix [13]. It is important that the total enthalpy of denaturation of ds-DNA is equal for condensed DNA (concentrated solution, gels, crystals of DNA) and for the DNA in dilute solutions [2,7]. But the different topology of individual single polynucleotide strands in condensed states and in dilute solutions of DNA and the presence of the residual folded structures of ss-DNA can influence the values of hydration enthalpy ($\Delta H_{\text{hydration}}$) upon denaturation and denaturational heat capacity increment (ΔC_P) in these different regimes [2].

To reveal the main peculiarities in the thermodynamic characteristics of thermal denaturation of ds-DNA it is necessary to obtain independent data about thermal properties of high molecular single stranded DNA (ss-DNA) and hydration parameters for ss-DNA by using the same method: low-temperature DSC with the new calorimetric ampoules [2].

Accordingly, the aims of this investigation are the study of the thermodynamic parameters of ice–water phase transition in aqueous solutions of ss-DNA, at different concentration of polynucleotide single

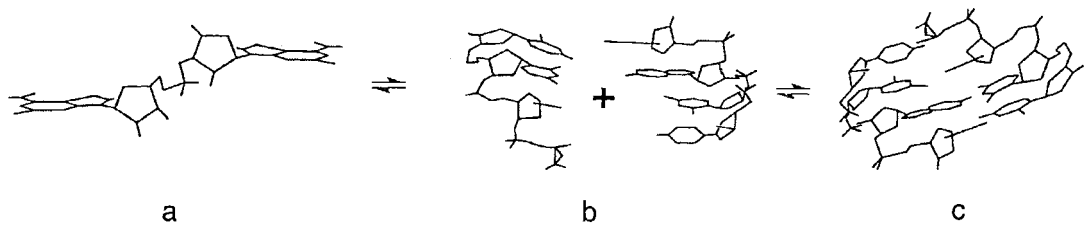

Fig. 1. Schematic representation of the processes of transition from unstacked single strands (a), to stacked conformations of two single strands (b), and transition from two single strands to a double helix (c).

chains, as well as to reveal the critical concentration for which the enthalpy of transition is zero and, to compare data of thermodynamic parameters of ice–water phase transitions obtained for native ds-DNA and for ss-DNA in the whole concentration region (0.2–2.0 g H_2O/g DNA) and the hydration value (bound water quantity) for native (*helix*), denatured (*single strand coils*) and *unfolded* ss-DNA.

2. Experimental

2.1. Materials

Lyophilized powder of calf thymus ss-DNA (Sigma), containing ∼65 wt.% DNA was used. Lyophilized samples of calf thymus Na–ds-DNA (Sigma), with protein concentration in samples <3% were also used. These lyophilized materials contained 14 wt.% of water; usually Na-salt DNA contained ∼7–10 wt.% salt.

2.2. Preparation of DNA samples

The ss- and ds-DNA were hydrated into high-pressure stainless steel calorimetric crucibles of volume 30 mm^3 (DSC 141, SETARAM), sealed and kept in this condition for several weeks. Water content was determined by weighing to ±0.01 mg, taking into account the water content of as-received Na–DNA. The accuracy of the given hydration values is estimated to be ±0.01 g of water/g of dry DNA. DNA hydration level was between (0.2 and 2.0) g H_2O/g DNA. The DSC is cooled by means of a liquid nitrogen stream. The DSC scans were recorded on heating from −100 to 120 °C at scanning rate 1 K/min. The weights of the samples used were between ≈10 and 20 mg.

2.3. Calorimeter

A DSC 141, SETARAM differential scanning calorimeter was used. The baseline was obtained with empty sample crucibles and after temperature correction and energy calibration, by making measurements in the working temperature region (−100 to +120 °C) using standard materials (benzoic acid, naphthalene and indium) for temperature and heat of fusion.

Our new designed type of calorimetric cell [2] on the basis of the stainless steel crucible of the DSC-141 was used. The new crucible has a hole sealed by a stainless steel thin screw and with teflon packing, which allows an easy modification of the composition of DNA solution, without changing the DNA quantity inside the cell. The new ampoule gives the ability to measure the heat of melting of water at different concentrations without change of materials inside the calorimetric cells. This gives the possibility of obtaining the principal results for the correct dependence of the enthalpy of the ice–water phase transitions versus the concentration of DNA (and other biopolymers) and revealed the critical concentration at which the enthalpy of transitions becomes zero (Figs. 2 and 3).

The bound water was calculated on the basis of the difference between the measured ice–water phase transition enthalpy in the gels of DNA and the enthalpy of melting of pure water (333 J/g water). The absence of heat absorption in the ice–water phase transition temperature interval indicates that all the water added to the dehydrated DNA (Figs. 2 and 3), became bound to the DNA macromolecules and so became a constituent part of the double helix or single stranded of DNA [4–6]. Since bound water does not participate in the freezing/melting process, the heat effect of the freezing/melting of such aqueous solutions (or gels) of DNA is smaller than expected for the total amount of water which is present in the solution. The total amount of water in the solution can be determined separately by subsequent drying the solution in desiccators under vacuum. Since the enthalpy of freezing of water is very large (−333 J/g), this method permits to determine, with very high accuracy, the amount of bound water [4–6]. We measured the heat effect of freezing/melting of the gels of ss- and ds-DNA when DNA is denaturated [2] or ss-DNA is *unfolded*, by heating the same solution to high temperatures. The difference between these two heat effects of freezing/melting will correspond to the process of hydration/dehydration of DNA upon denaturation or, ss-DNA upon *unfolding*. Since throughout this experiment the ss- and ds-DNA solutions are never removed from the hermetically sealed calorimetric cell, this heat effect difference can be measured with high accuracy. The accuracy in the determination of the bound water is ±0.01 g H_2O/g DNA [4–6].

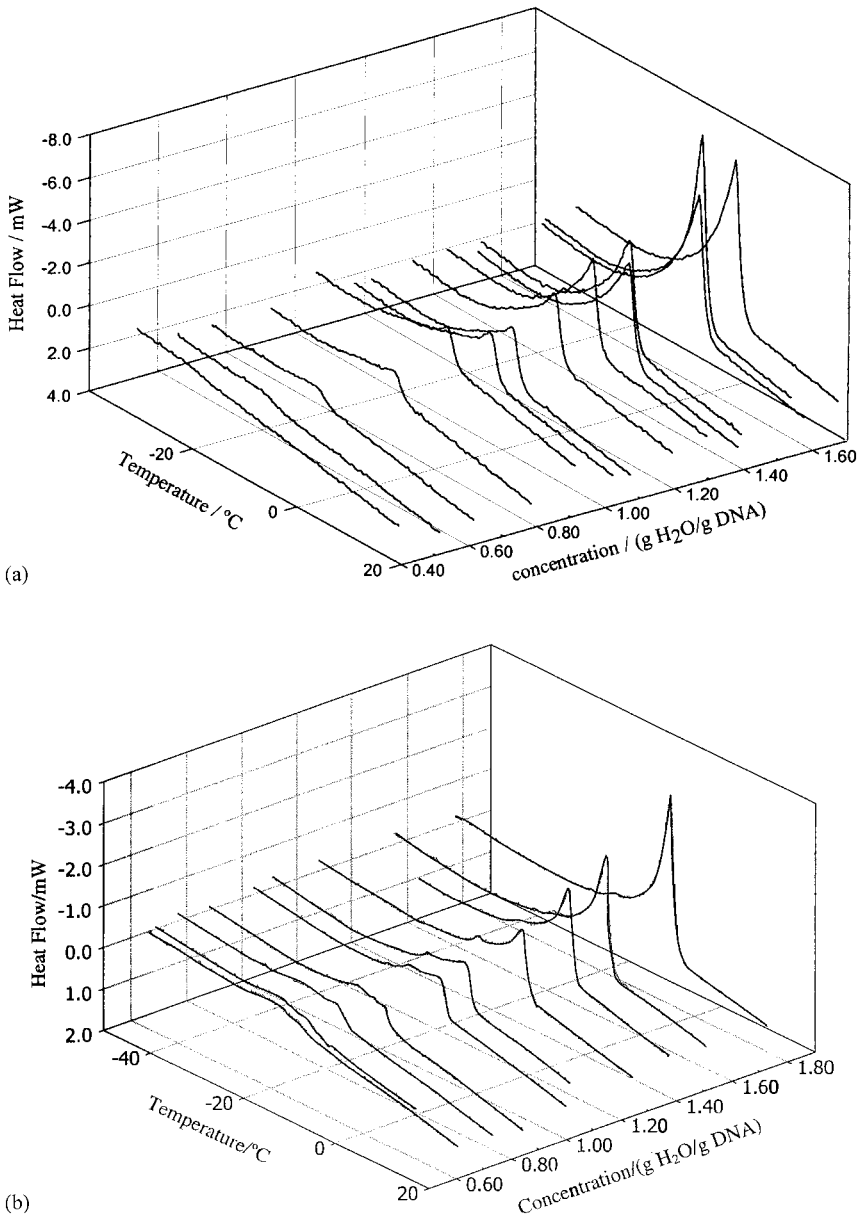

Fig. 2. Ice–water phase transitions in ss-DNA (Na–DNA of calf thymus) (a), and in ds-DNA (Na–DNA of calf thymus) (b), in coordinates: heat flow (mW)—temperatures (°C)—concentration (g H_2O/g DNA). Scanning rate 1 K/min.

3. Results and discussion

The detailed study of ice–water phase transition in the solution of ss-DNA at wide range of concentration of ss-DNA shows (Fig. 2a) that the melting of water in the single stranded DNA–H_2O system, loses the character of sharp phase transition and like in ds-DNA solutions (Fig. 2b), it degenerates into a transition of order–disorder type which is extended in temperature as explained in reference [4,5]. Both

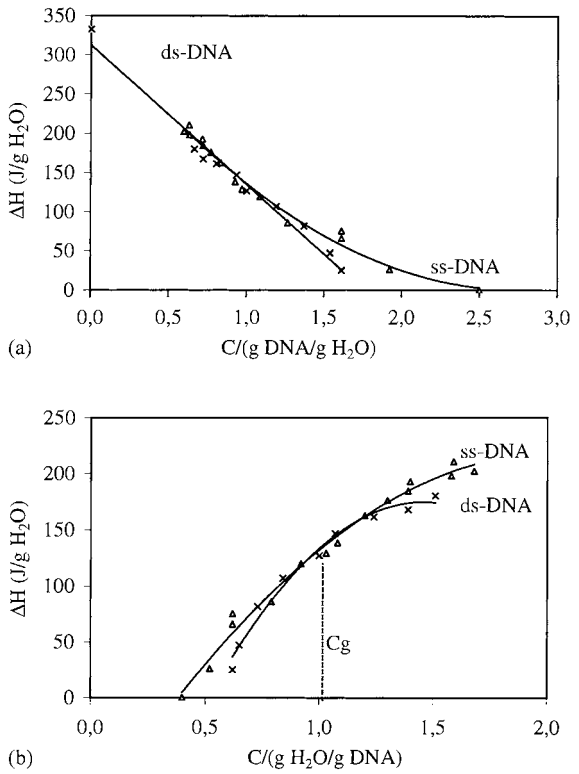

Fig. 3. (a) Enthalpy of fusion of water as a function of concentration of DNA (g DNA/g H_2O) for ss- and ds-DNA. (b) Enthalpy of fusion of water as a function of water content (g H_2O/g DNA) for ss- and ds-DNA.

dependencies of the enthalpy of fusion of bulk water versus concentration of DNA (g DNA/g H_2O) (Fig. 3a) and the enthalpy of transition versus concentration of water (g H_2O/g DNA) (Fig. 3b) reveal the dramatic difference in the character of interaction of water with the polynucleotide chains in the single stranded and double stranded conformations. The least squares line used to obtain a value for the critical hydration gives the two different quantities for this most important parameter for ss- and ds-DNA. The critical values of the hydration at which all the water in the DNA–H_2O system exists in the bound (*unfreeze*) state are established for this conformations with great precision: $N^{\Sigma}_{\text{ds-DNA}} = (0.55 \pm 0.01)$ g H_2O/g DNA, or (20 ± 1) mol H_2O/mol bp and $N^{\Sigma}_{\text{ss-DNA}} = (0.40 \pm 0.01)$ g H_2O/g DNA. According to this calorimetric data, for the transformation "*double stranded helix*" → "*single stranded chains*" accompanied by dehydration of ds-DNA: $\Delta N = N^{\Sigma}_{\text{ds}} - N^{\Sigma}_{\text{ss}} = (0.15 \pm 0.01)$ g H_2O/g DNA, or (5 ± 1) mol H_2O/mol bp). This quantity is equal to the value obtained in our previous investigation of the hydration change upon thermal denaturation of ds-DNA [2]; this definitely suggests that the "*double helix*" → "*single strands*" thermal transition is accompanied by disruption of the ordered water fraction in the hydration shell of the double helix. It is also important, that the large change in slope of the DSC plot at about 1 g H_2O/g DNA ($C = C_g$, Fig. 3b) characterizes only ds-DNA–H_2O system; usually this large change in slope on the dependence of enthalpy of fusion (ΔH_{H_2O}) versus concentration of biopolymers or water is interpreted as the glass–solution transition [14]. This is a very important point on the water/biopolymers concentration scale. At concentrations $C \gg C_g$ (with increasing of concentration of water) we have a dilute polymer solution where macromolecules are separated by large distance and hardly interact with each other at all. The properties of such solutions are governed merely by the properties of the individual macromolecules [3]. With increasing the concentration of the polymers, the polymer coils, or later, starts to overlap and then one has the regime of semidilute solution; obviously, the intermediate regime between "dilute" and "semidilute" solutions will be when the coils do not overlap, but just touch each other [3]. At concentrations $C < C_g$ this regime is changed to the regime of densely entangled coils. Hence, the transformations between the different regimes take place for ss-DNA without large change in the slope of the DSC plot in the measured concentration region (Fig. 3a). It is important, to point out that the difference between these two DSC plots can be interpreted as the enthalpy of dehydration upon transition "*ds-DNA → ss-DNA*".

After the critical hydration points ($C > N_{\text{ds}}$ and $C > N_{\text{ss}}$) for both conformations the hydration values are changed; for semidilute and intermediate regimes $N^{\Sigma}_{\text{ds}} = (0.60 \pm 0.01)$ g H_2O/g DNA, or (21 ± 1) mol H_2O/mol bp), and $N^{\Sigma}_{\text{ss}} = (0.62–0.67)$ g H_2O/g DNA. This means, that in these regimes the accessible total surface area for both conformations increases, but ss-DNA binds more additional water since the accessible total surface area for water is greater for this conformation when in double helix, with ordered hydrated waters in the grooves of the helix [2].

According to X-ray crystallographic analysis of the hydration of A- and B-DNA forms, at atomic resolution, Egli et al. [15] demonstrated that the overall hydration of B-DNA consists of three hydration shells: first shell contain (12,5), second shell (6) and third shell (2) mol H_2O/mol bp. Hence, the total hydration $N_{X\text{-ray}}$ = (20.5) mol H_2O/mol bp, which is in excellent agreement with our calorimetric value. It is important to remember that according to the X-ray data, the largest number of first shell water molecules

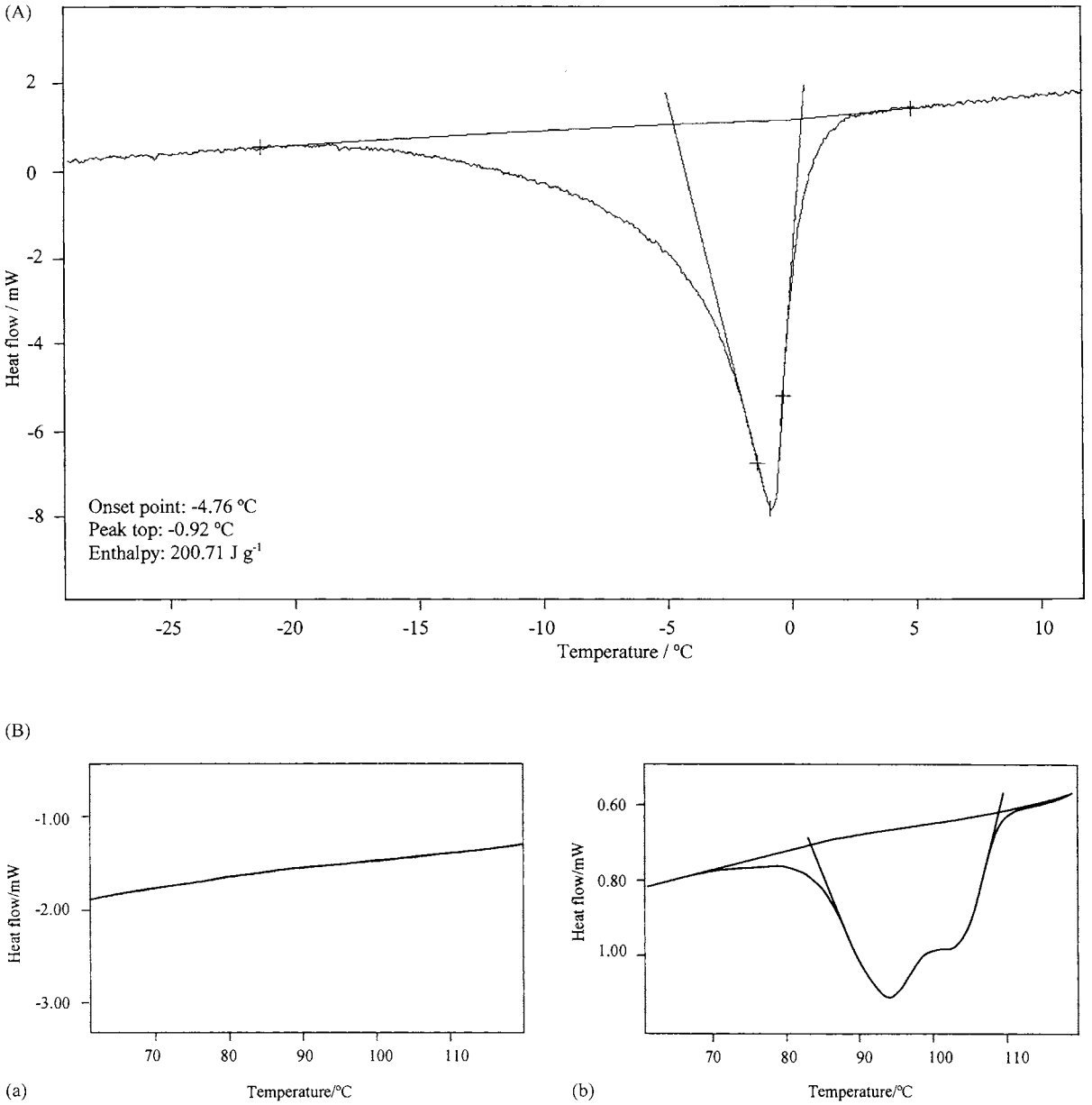

Fig. 4. (A) Ice–water phase transition in ss-DNA aqueous solution (water content 1.59 ± 0.01 g H_2O/g DNA). (B) Heat capacity change in the same ss-DNA solution at high temperature (a); heat absorption peaks in ds-DNA–water system (water content about 1.0 g H_2O/g DNA) (b). (C) Ice–water phase transition in the same ss-DNA aqueous solution after heating and cooling. Scanning rate 1 K/min.

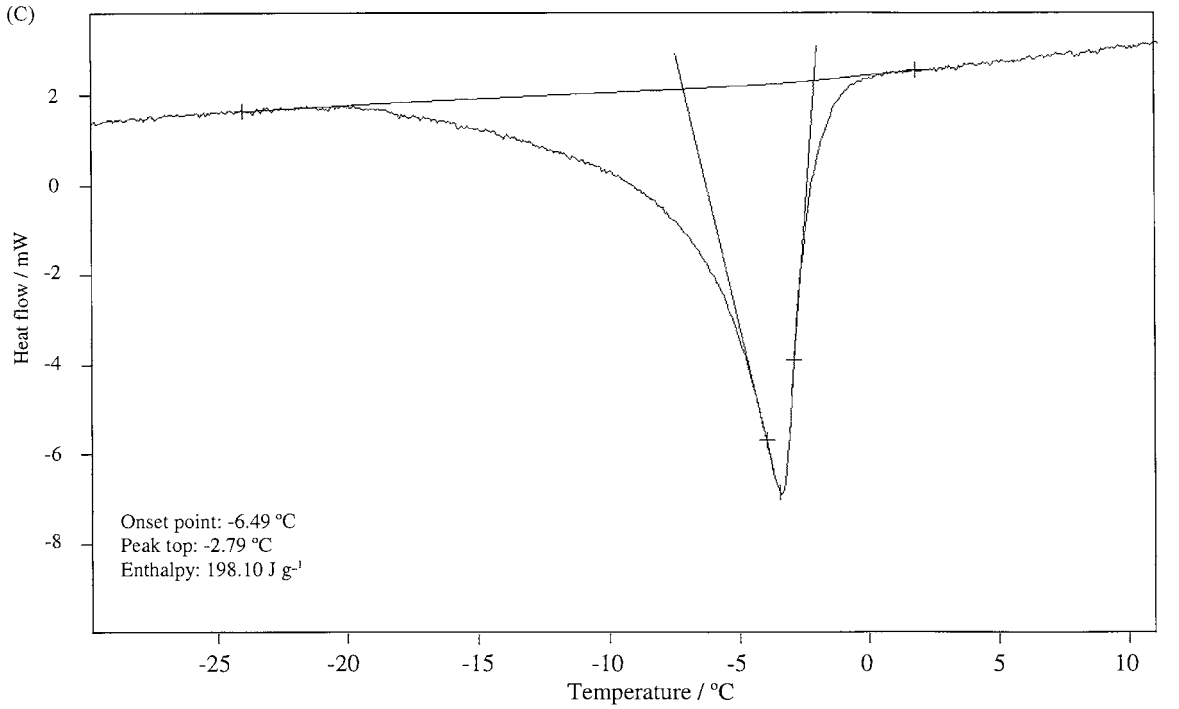

Fig. 4. (*Continued*).

in A- and B-form duplexes, are found around phosphate groups: up to seven waters per base pair lie within a 3 Å range from the O 1P and O 2P atoms [15]. It is also important that the B-DNA crystal structure confirms cyclic configurations involving either five or six water molecules, as the preferred basic component of solvent networks around DNA. It has also been established the well-known spine of hydration as a just well ordered fragment of a more extensive solvent network that fills the entire groove and creates a web around the ds-DNA [15]. Namely, these enumerated water molecules, composing the hydration water network, are *melted* upon thermal denaturation of DNA, according to the calorimetric data (this work and [2]). In ss-DNA the largest number of the hydration shell water molecules remain around the phosphate groups and in the ds-DNA the water molecules of the disordered hydration shell are replaced by less specific hydrogen bonds to the solvent.

It is also possible that the increasing of bound water quantity in ss-DNA in dilute- and semidilute solutions takes place as a result of the transition of an unpaired single strand from a random coil, in which the bases are not stacked to an ordered helical structure in which the bases are stacked (Fig. 1). It has often been suggested that stacking is also driven by classical hydrophobic interactions. In this model, ordered water is released from around the bases upon stacking, and this provides a favorable entropy term. But, the thermodynamic parameters provide no evidence that classical hydrophobic bonding is important for driving stacking ([12], p. 270).

In this respect it is interesting the DSC study of ss-DNA in a wide temperature interval, including high temperature region, for revealing the response of this temperature treatment on the character of the ice–water phase transition in the ss-DNA–water system. Fig. 4. shows the example of such experiments. We see that in semidilute solutions of ss-DNA, in the high temperature region, we do not observe heat absorption peaks (compare with heat absorption peaks on the same diagram for ds-DNA–water system); this is not surprising, because it is well known

that, in practice, it is not easy to see transitions for single-strand stacking/unstacking, since the transitions occur over a wide temperature range, even for homopolynucleotides, for example, the reported enthalpy changes, for single strand stacking in poly(A) vary from -12 to $54\,\mathrm{kJ\,mol^{-1}}$ and the enthalpy changes for single strand poly(U) are practically zero ([12], p. 263). Nevertheless, we detect some difference in the thermodynamic parameters for ice–water phase transitions after the heating of same ss-DNA solutions: ice–water phase transition temperature (peak top) for the preheated ss-DNA–H_2O solution is lowered by about $\sim$2 K, and the enthalpy of ice–water phase transition also decreases insignificantly (see inscriptions on the Fig. 4). This means that ss-DNA, (with $N_{ss} = (0.63 \pm 0.01)\,\mathrm{g\,H_2O/g\,DNA}$ in this concentration regime) after temperature treatment binds small additional quantity of water and the total value of bound water insignificantly increases: $N_{ss,heat} = (0.66 \pm 0.01)\,\mathrm{g\,H_2O/g\,DNA}$.

There is another interesting aspect of the description of the biopolymer–water system concerning the determination of the heat capacity values for bound water and macromolecules [7,16–18].

On the basis of the temperature dependence of the heat capacity of DNA–water system at wide range of temperature and water content (n) it is possible, for both ds-, and ss-DNAs, to calculate (at constant temperature, for example, at $\theta = 30\,^{\circ}\mathrm{C}$) the contribution of the heat capacity of water (C^{w}) and DNA (C^{DNA}) in the total heat capacity of the whole system, on the ground principle of additivity the latter being possible because intervals of water content (n) change by small steps [from (n_1) to ($n_2 = n_1 + \Delta n$)]; [$n = (\mathrm{g\,H_2O/g\,DNA})$ or $(\mathrm{mol\,H_2O/mol\,bases})$] [7,16–18].

$$(1 + n)C_p = C^{DNA} + n\,C^{w} \tag{1}$$

$$C^{w} = \frac{\{(1 + n_2)C_2 - (1 + n_1)C_1\}}{\Delta n} \tag{2}$$

According to the data of Maleev et al. [7] the dependence $C^{W} = f(n)$ reveals "peak" between water content n = 4–12 mol H_2O/mol bases (or between water content where all three hydration spheres of double helix are present (21 mol H_2O/mol bp, according X-ray [13] and calorimetric data (see previous sections and [2])). Peculiarities like this are absent on the dependence $C^{w} = f(n)$ for the ss-coils of DNA. This peculiarity demonstrates that on the double helix of DNA, as on the matrix, it is possible "growing the semicrystalline H-bounded water lattice" [19], with heat capacity minimum 2.9 J/g K and maximum 3.4 J/g K (we must remember that the heat capacity of pure water is equal to 4.2 J/g K and of ice is -2.1 J/g K). The area under the peak may be imagined as the energy of disordering of this water structure and is equal: $\Delta Q = T \int \Delta C^{W}(n)\,\mathrm{d}n = (30 \pm 4)$ J/g DNA $= (20 \pm 3)$ kJ/mol bp [7]. The energy cost of the destruction of the bound water fraction in the hydration shell of the duplex, upon thermal denaturation, is equal to $\Delta H_W = (26 \pm 3)\,\mathrm{kJ/mol\,bp}$ [2]. Hence, we obtain a good agreement between these two independent ways for the general characteristics of DNA–water interaction and for the description of the bound water role in the energetic of DNA double helix winding–unwinding processes. Really, accordingly to [21], by increasing the temperature from 250 to 370 K the average number of H-bonds between the oligonucleotide duplex and water molecules decreases significantly. Fig. 5 presents the temperature dependences of the heat capacity (C_P) of DNA, the average number of hydrogen bonds between the oligonucleotide duplex, $d(CGCGCG)_2$, and water molecules (according to the data obtained by using of molecular dynamics simulations [21]), extrapolated in the temperature intervals of melting, and spin–lattice (t_1) and spin–spin (t_2) proton relaxation times (s), in dilute solutions of DNA [5]. Thus, with increase in temperature, the hydrogen-bonded lattices are *melted* and the number of ds-DNA–solvent hydrogen bonds will *decrease*, giving an increasingly more endothermic contribution in total enthalpy of transition and so, positive ΔC_P to the ds-DNA $\rightarrow$ ss-DNA transition thermodynamics. Therefore, considering the physical–chemical properties of the native DNA, we should concentrate our attention at two "substructures": the DNA "aperiodic crystal", being the matrix, and the structure of H-bonded water lattice *penetrating* of the double helix [18,20]. In another words, formation of B-DNA duplex from mixing of their complementary single strands is mostly accompanied by the uptake of structural water molecules and so the overall hydration of a duplex is mainly determined by its conformation and stability.

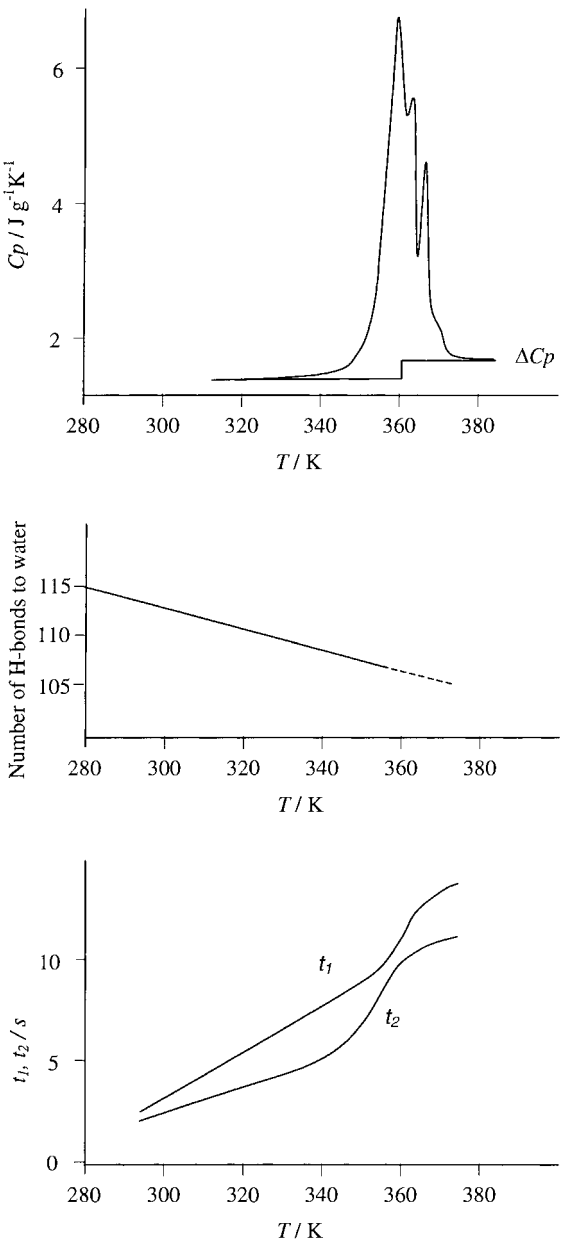

Fig. 5. The temperature dependences of the heat capacity (C_P) of DNA, the average number of hydrogen bonds between the oligonucleotide duplex and water molecules (according to data obtained by using of molecular dynamics simulations [21]), extrapolated in the temperature intervals of transition, and spin–lattice (t_1) and spin–spin (t_2) proton magnetic relaxation times (s) in dilute solutions of DNA upon duplex melting [5].

4. Conclusions

Unwinding of a ds-DNA requires that hydrogen bonds within the native double helix (hydration shell including the water spine and ordered clusters in the grooves of helix and water molecules in the duplexes around phosphate groups) be disrupted and replaced by less specific hydrogen bonds to solvent. The cooperative nature of double helix unwinding implies that most, if not all, hydrogen bonds formed in the native state will depend on conditions such as temperature. These hydrogen bonds include H-bonds forming (W–C) A·T and G·C base pairs and ordered H-bounded water lattices in the grooves and near the phosphate groups of helix. With increase in temperature, the hydrogen-bonded lattices are *melted*, the number of ds-DNA–solvent hydrogen bonds will decrease, giving an increasingly more endothermic contribution to the total enthalpy of transition and positive ΔC_P to the ds-DNA → ss-DNA transition thermodynamics. The real picture is concerned on the thermodynamics of phase transitions in hydrogen-bonded networks, in which the degree of hydrogen bonding decreases with increasing the temperature. In the results, insofar as thermodynamic properties are concerned when comparing duplexes, the temperature under consideration is as important as, if not more important than, the duplex type, the base composition, or the base sequence [8].

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