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THE ROLE OF BOUND WATER ON THE ENERGETICS OF DNA DUPLEX MELTING

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Abstract

A combination of common and low-temperature differential scanning calorimetry (DSC) techniques was used to detect the thermodynamic parameters of heat denaturation and of ice-water phase transitions for native and denaturated DNA, at different low water contents. We suggest that the main contribution to the enthalpy of the process of the heat denaturation of DNA duplex (35±5 kJ/mol bp) is the enthalpy of disruption of the ordered water structure in the hydration shell of the double helix (26 ±1 kJ/mol bp). It is possible that this part of the energy composes the non-specific general contribution (70%) of the enthalpy of transition of all type of duplexes. For DNA in the condensed state the ratio $\alpha=\Delta C_p/\Delta S$ ~2 is smaller than for DNA in diluted aqueous solutions ($\alpha \cong 2$–4). This means that there are other sources for the large heat capacity change in diluted solutions of DNA – for example the hydrophobic effects and unstacking (unfolding) of single polynucleotide chains.

Keywords: DNA, hydration, low-temperature DSC, melting enthalpy of DNA

Introduction

The results of the determination of apparent heat capacities of naturally occurring DNA in both native (helix) and denatured (coils) states in dilute aqueous solutions [1], and the analysis of a large amount of available experimental data (obtained also for polymeric nucleic acid duplexes by using the new generation of differential scanning calorimetry, (DSC) [2, 3]), suggests that the melting of duplexes is accompanied by positive changes in the heat capacity (ΔC_p) ~(170–400) J K^{-1} mol^{-1} ([1–3] and references therein). This has significantly changed the understanding of the origins of duplex stability [3]. This positive change in the heat capacity implies a large temperature dependence of the enthalpy and entropy of DNA duplex melting [1b]. The significant contribution to the thermodynamic parameters of helix-coil transition (ΔH, ΔS, ΔC_p, ΔG) for all duplexes is the energy accompanying of changes in hydration of duplexes [1, 4–5]; the entropic cost of transferring a single water molecule from the liquid to a site of double helical DNA is significant 58±6 J K^{-1} mol^{-1} and differs from the entropic cost for polynucleotide chains in the state of statistical coils ~15±2 J K^{-1} mol^{-1} [5]. The structure of water in grooves of the helix and

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generally - in the total structure of duplexes, strongly depends on specific types of duplexes and sites of interactions [6–11], but hydration changes (and corresponding enthalpy and ΔC_p) are predominantly really non-specific. We have determined the thermodynamic parameters of ice-water phase transitions for native and denaturated DNA, at different water contents, to evaluate the main contribution of the energy of hydrated water structure disruption to the melting enthalpy of DNA and the heat capacity increment, which characterizes the duplex conformational transition.

The electrostatic interactions, cooperativity factor of binding between water molecules in the first and other coordination spheres [4, 13, 14] and the structural factor (tetrahedral coordination of water in hydration spheres, like the water molecules in the ice-structure [6–9, 11]) determined DNA-water heat capacity behaviour at low temperatures including the temperature regions of liquid helium and ice-water phase transition (2–273 K), [12]. If the structure of DNA duplex changes, all thermodynamic parameters of ice-water phase transition also changes, and this (as demonstrated earlier [4, 12–14]) gives the possibility to reveal the water role in the energetic of the processes of the structural rearrangements of DNA duplexes.

Experimental

Materials

Super pure lyophilised samples of cow spleen Na–DNA (42 GC%), kindly prepared and donated by Lando (The Institute of Bioorganic Chemistry, Minsk, Byelorussia), with protein concentration in samples <0.1%; RNA≤5%; MW>10^7 D were used. Lyophilised samples of calf thymus DNA (SIGMA), with protein concentration in samples <3% were also used. These lyophilised materials contained 14 mass% water.

Preparation of DNA samples

DNA was 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 0.6–2.0 g H_2O/g DNA. DSC scans were recorded on heating from –100 to 120°C at scanning rate 1 K min^{-1}. The masses of the samples were between ≈10 and 20 mg.

Calorimetry

A differential scanning calorimeter (Model DSC 141, SETARAM) with a computer program 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, indium) for temperature and heat of fusion.

We designed also a new type of calorimetric cell on the basis of the stainless steel crucible of DSC-141 (Fig. 1). The new crucible has a hole sealed by a stainless steel tiny screw and with teflon stuffing. The new ampoule enables us to measure the heat of melting of water at different concentrations without change of materials inside the calorimetric cells. This gives the possibility to obtain the principal results for the correct dependence of the enthalpy of the ice-water phase transitions *vs.* the concentration of DNA (and other biopolymers) and revealed the critical concentration at which enthalpy of transitions becomes zero (Fig. 2).

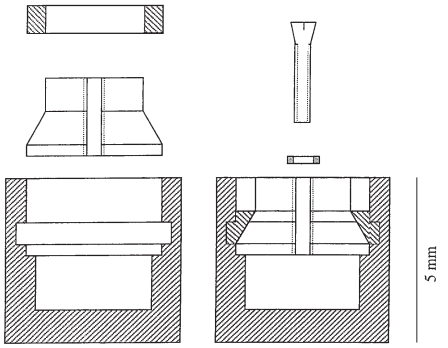

Fig. 1 Calorimetric cell (ampoule) for investigation of the process of ice-water phase transitions at different concentrations of biopolymers in gels and solutions

The bound water was calculated on the basis of the measured ice–water phase transition enthalpy difference in the gels of DNA and the enthalpy of melting of pure water (333 J g^{-1} water). The absence of heat absorption in the ice-water phase transition temperature interval indicates that all the water added to the dehydrated DNA (Fig. 2), has become bound to DNA macromolecules and became a constituent part of the double helix of DNA (more detailed [4, 13, 14]). 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 a desiccator under vacuum. (See previous section.) Since the enthalpy of freezing of water is very large (–333 J g^{-1}), this method permits the amount of bound water to be determined with very high accuracy [4, 13, 14]. We measured the heat effect of freezing/melting of the gels when DNA is in the native state and when DNA is denaturated by heating the same solution to elevated temperatures. The difference between these two heat effects of freezing/melting will correspond to the process of hydration/dehydration of DNA upon denaturation. Since throughout this experiment the DNA solution is never removed from the hermetically sealed calorimetric cell, this heat effect difference can be measured with very high accuracy. The accuracy in the determination of the bound water is ±0.01g H_2O/g DNA [4, 13, 14].

The calorimetric measurements in dilute solutions of DNA were carried out with an adiabatic capillary scanning calorimeter DASM-4 [1].

Results and discussion

Figures 2 and 3 show that the melting of the water in a native Na–DNA–H_2O system at low water content loses the character of sharp phase transition and it degenerates

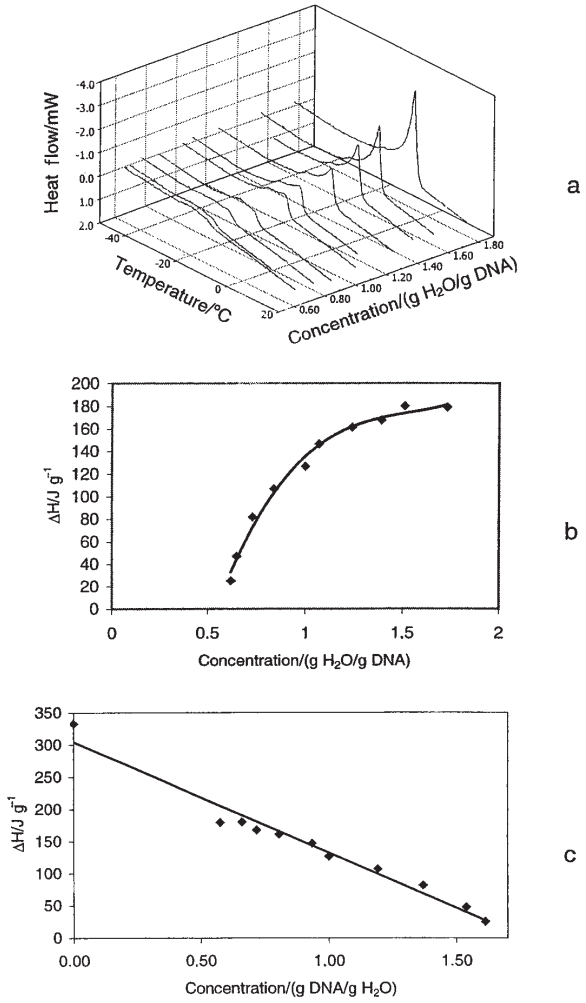

Fig. 2 Ice–water phase transition in native DNA (Na–DNA of Calf Thymus) in coordinates: heat flow (mW)-temperatures (T/°C)-concentration g H_2O/g DNA (a); Enthalpy of fusion of water as a function of water content for native DNA g H_2O/g DNA (b); Enthalpy of fusion of water as a function of concentration of DNA g DNA/g H_2O (c)

into a transition of order-disorder type that is extended in temperature [4, 14]. The maximum of the heat absorption peak is placed at –13.7°C (Fig. 3, water content 0.71±0.01 g H_2O/g DNA). The amount of bound (unfrozen) water for native DNA duplex (Fig. 3-I) is equal 0.58±0.01 g H_2O/g DNA, or 21 mol H_2O/mol bp, including the first hydration shell with the spine of water in minor grooves and cluster of ordered water network in the major grooves of helix [8, 11]. This water fraction is not crystallised in an ice structure at temperatures as low as liquid helium temperatures (2 K, [12, 15]). Figure 2c presents the enthalpy of fusion of bulk water *vs.* concentration of DNA showing a straight line (R^2=0.98) with an intercept of 312.2 J mol^{-1}. On continuing the heating of this DNA–H_2O condensed system (after ice-water phase transition

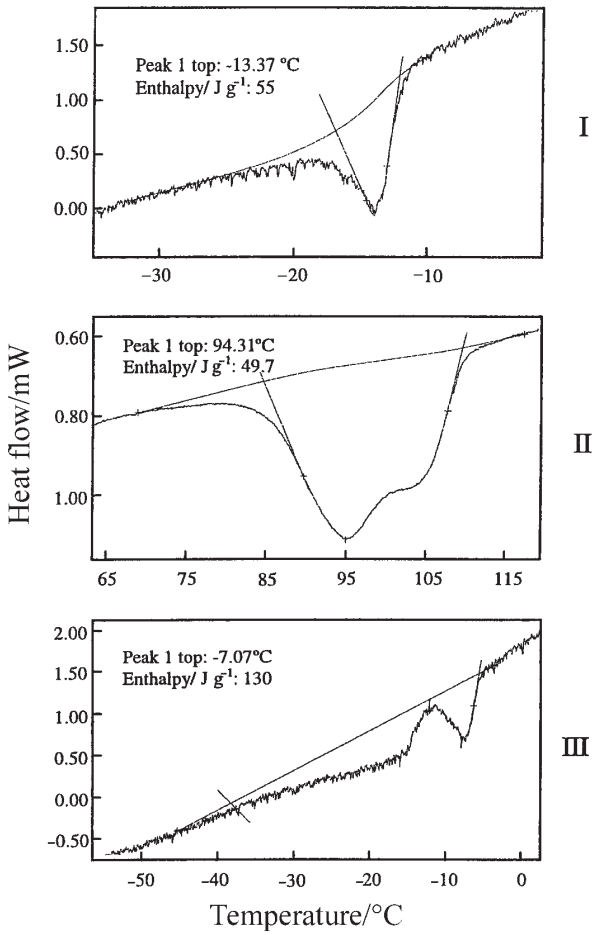

Fig. 3 Ice-water phase transition in native DNA; water content: 0.71±0.01 g H_2O/g DNA (I); Heat absorption peak accompanying of denaturation of DNA wet fibers; water content 0.71±0.01 g H_2O/g DNA (II); Ice-water phase transition in denaturated DNA; water content: 0.71±0.01 g H_2O/g DNA (III)

region) at high temperatures we detect the endothermic heat effect, corresponding to the thermal denaturation of DNA. The calorimetric melting curve profile for DNA in the condensed state (Fig. 3-II) and in the dilute solutions (Fig. 4) is different, but the energetic parameters of melting are practically equal (Table 1). We emphasise that in this condition of dense aggregates of DNA duplexes, the separated polynucleotide chains do not have the free volume to make loops, and in the final stage of denaturation, the single statistical coils. But where is the energy of transition going? The answer to this question is given by the analysis of the ice-water phase transition study for denaturated DNA. After denaturation of DNA–H_2O samples with rapid cooling (0.5 h, similar as native samples) at temperature (–150°C), a temperature scanning is repeated in the same conditions as for native DNA samples. As we see in Fig. 3-III ice-water phase transition curve for denaturated DNA is considerably altered in character as compared with native preparations: 1. the heat absorption peak is split into two peaks, 2. the temperature of the second peak maximum is shifted at high temperatures (–5–7°C) and 3. the area under these peaks is significantly increased. This means that the free water fraction is considerably increased and, in general, all bulk water obtains the properties of more free water. For denaturated DNA the bound water fraction is dramatically decreased and is equal to 0.46±0.01 g H_2O/g DNA or 17 mol H_2O/mol bp. (Fig. 3-III a, and data on Table 1). The significant part of bound water 0.12±0.01 g H_2O/g DNA is melted and replaced in the fraction of free water. The energetic cost of this transition equals: 0.12·333 J (melting enthalpy of 1 g of water) ·650, the average MW of base pairs for sodium salt DNA=26±l kJ/mol bp. This composes the 70–80% of total enthalpy of DNA denaturation in dilute solutions

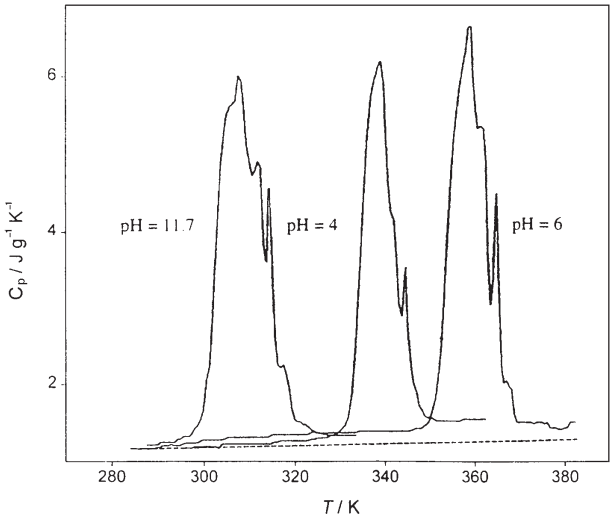

Fig. 4 Partial heat capacity as function of temperature for cow spleen Na–DNA in dilute solutions at different pH 0.1 M Na – phosphate buffer; concentration of DNA 1 mg mL^{-1}. The temperature of the maximum of peaks is determined by GC-content of satellite fractions [1, 17]

Table 1 Thermodynamic parameters of the ice-water phase transition in the Na–DNA–H_2O gels, process of DNA denaturation and bound water values in native and denaturated DNA

Na–DNA–H_2O	Ice-water phase transition		Bound water	DNA melting		Heat capacity increment
	Temperature	Enthalpy		Enthalpy	Entropy	
	T/°C	ΔH/J g^{-1}	g H_2O/g DNA mol H_2O/mol bp	ΔH/J g^{-1} kJ/mol bp	ΔS/J g^{-1} K^{-1} J/mol bp K	ΔC_p/J g^{-1} K^{-1} J/mol bp K
a) Native	–13.4±0.01	55±2	0.58±0.01 21±1	50±5 32±3	0.13±0.01 88±8	0.24±0.02 156±16
Denaturated	–7.1±0.01	130±4	0.46±0.01 17±1			
b) Native	–12±0.01	53±2	0.63±0.01 23±1	52±5 34±3	0.14±0.01 92±9	0.28±0.03 182±18
Denaturated	–6.5±0.01	110±3	0.51±0.01 18±1			

a) Hydration level 0.71±0.01 g H_2O/g DNA; amount of melting water: 0.12±0.01 g H_2O/g DNA; calculated enthalpy and heat capacity change: ΔH_w=26±1 kJ/mol bp; ΔC_p=0.24±0.02 J g^{-1} K^{-1}
b) Hydration level 0.76±0.01 g H_2O/g DNA; amount of melting water: 0.12±0.01 g H_2O/g DNA; calculated enthalpy and heat capacity change: ΔH_w=26±1 kJ/mol bp; ΔC_p=0.24±0.02 J g^{-1} K^{-1}
c) Melting enthalpy of DNA in dilute solution (pH=6) ΔH_m=37±4 kJ/mol bp; ΔC_p=0.36±0.04 J g^{-1} K^{-1}

33–37 kJ/mol bp [17]. In these calculations it was assumed that during the heat denaturation of DNA the energy of disruption of hydrogen bonds in structurally hydrating water in DNA is equal to the energy of breaking H-bonds in pure ice during the ice-water phase transition (including the presence in pure water of two types of H-bonds (weak and strong [20]) revealed in ice by using the method of inelastic neutron scattering [20, 21]). It also reflects the fact that the intramolecular H-bond is not completely lost upon melting of DNA in such a dense aggregate, but rather is replaced by a weaker bond with water (or molecular groups of neighbour chains). We must remember that intramolecular hydrogen bonding contributes little to the stability of nucleic acid structures [22].

The results of Halle and Denisov [7] support our calculations: the authors relate NMR results to structural data obtained by X-ray crystallography for oligonucleotides and shown that 5 water molecules located at the floor of the minor groove of the d[(CGCGAATTTCGCG)] duplex, have high order parameters (residence times are in the nanosecond range); similarly high orientation order parameters are found for the long-lived waters in other dodecamers [7, 8, 11]. It may be estimated that the entropy associated with transfer of these highly ordered water molecules into bulk solvent is comparable to the entropy difference between ice and bulk water [7].

It is useful to select another way for the calculation of the part of the enthalpy of DNA denaturation connected with bound water rearrangement. Expected from molecular dynamics simulation on hydration of DNA, water molecules are considered bound if they form hydrogen bonds directly with DNA [6]. In full agreement with present calorimetric data 20.6 water molecules (per mol bp) are bound to B–DNA through hydrogen bonds (H-bond) [6]. Privalov *et al.* investigating different sets of biopolymers in aqueous solution obtained a value of 6.3 kJ mol^{-1} for single H-bond [17]. The present calorimetric data indicates that a total of about 4 M H_2O/mol bp is released upon denaturation of DNA: these highly ordered water molecules transfer into bulk solvent. This means that at least 4 H-bonds are disrupted between water and DNA during thermal denaturation. The minimal energy cost of this transition is equal to 6.3·4=25 kJ/mol bp. Both calculations give similar results (Table 1).

Our results agree with the recent study of the volume and compressibility measurements [19] of DNA oligomer duplexes, according which the formation of B–DNA duplex from the mixing of their complementary strands is mostly accompanied by the uptake of structural water molecules and the overall hydration of a duplex is mainly determined by its conformation. This conclusion also agrees with the denaturation heat capacity increment ($\Delta C_p = C_p(D) - C_p(N)$). The melting of 0.12±0.01 g H_2O/g DNA gives the heat capacity increment equal to ΔC_p=0.12·2 J K^{-1}, heat capacity change during melting of 1 g of water = 0.24±0.02 J g^{-1} K^{-1}, or 156±16 J K^{-1}/mol bp. In dilute solutions the average value for ΔC_p=0.36±0.04 J g^{-1} K^{-1} 234±20 J K^{-1}/mol bp [1–3]. Experimental values directly measured from the difference in the pre- and post transition baselines of the experimental heat capacity curves (Fig. 3-III) yield ΔC_p=0.24±0.02 J g^{-1} K^{-1} or 156± 16 J K^{-1}/mol bp. Using this heat capacity increment, the average standard enthalpy, entropy and Gibbs energy of melting of DNA in the condensed state have been determined at 25°C as 24±2 kJ/mol bp, 64±6 J K^{-1}/mol bp and 5±0.5 kJ/mol bp, respectively. Interest-

ing that this ΔH_{ref} value coincides with the average value of ΔH_{ref} for different polynucleotides in dilute solutions ≅6.0 kcal/mol bp [3]. As we see the heat capacity increment value in DNA gels is larger than the DNA transition entropy per base pair. For DNA in condensed state this ratio is $\alpha=\Delta C_p/\Delta S\approx 2$ [1, 6], this value smaller than for DNA in dilute aqueous solutions ($\alpha\cong 2$–4 [16]). This means that there are other sources for the large heat capacity change in dilute solutions of DNA – for example hydrophobic effects [1, 16]. For condensed DNA–H_2O systems (low water content) these effects may be depressed because: 1. the conformation of polynucleotide chains in denatured state represent densely entangled coils, 2. The water activity is very low. It is important that total amount of bound water in dilute solutions and gels of denaturated DNA insignificantly increased on 0.06±0.01 g H_2O/g DNA [4, 13]. This means that the disrupted water molecules of the hydration shells of native DNA are replaced and with bulk water molecules proceeds an increase of hydration due to formation of hydrogen bonds with water and hydrophobic hydration of the exposed bases, since the accessible total surface is increased. Thus the total enthalpy of denaturation of DNA ($\Delta H_{melting}$, including the specific and non-specific contributions [3]) is equal for condensed DNA (semi-dilute solution, gels, crystals of DNA) and for DNA in dilute solutions (Table 1 and [18]). But the different conformation of individual polynucleotide strands in condensed states and in the dilute solutions of DNA can influence on the values of hydration enthalpy ($\Delta H_{hydration}$) and denaturational heat capacity increment (ΔC_p) in these different regions.

The main difficulty in identifying and characterizing the solvent-exposed atomic groups of DNA in their coil state is related to the uncertainty associated with the degree of unfolding of the single stranded state. It should be noted that there are a number of experimental observations suggesting that the composition and sequence, in single stranded chains of DNA may retain the so-called residual structure [23, 24].

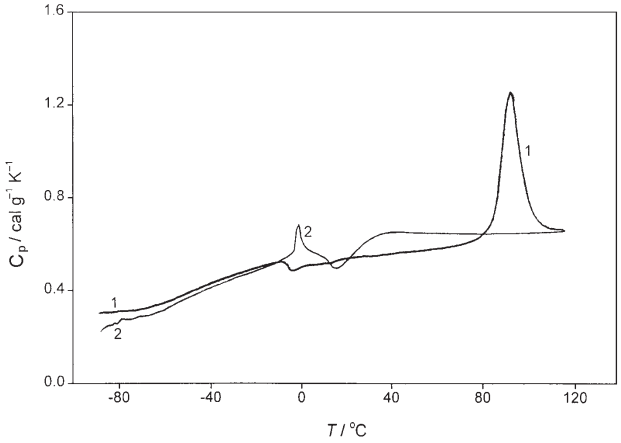

Fig. 5 Heat capacity change for native DNA gels, water content 31.2 mass% (1); Heat capacity change for denaturated DNA (recording on heating of same samples in the same temperature region, after cooling of denaturated DNA gels) (DSC111, scanning rate 5 K min^{-1}) (2)

We emphasise once more that in our denaturated samples of DNA we do not have real single stranded polynucleotide chains in the state of statistical coils; the main problem for us is: does the higher molecular DNA retain or not, the double helical residual structure after cooling the samples? The answer to this question is given by the analysis of the calorimetric recording of denaturated samples of DNA in the wide temperature interval including the ice-water and the helix-coil phase transition temperature regions (Fig. 5) [25]. We see that instead of denaturational heat capacity peak we have the jump of the heat capacity, characteristic of many biopolymers behaviour in the denaturated state: it is concluded that these properties of heat capacity reflect glass transition in the denaturated biopolymers, which depend on humidity and thermal history of the sample [26]. (See also data about enthalpy relaxation and its recovery for DNA-water system in the low temperature region obtained by using DSC [27]). Thus the denatured samples of DNA have different behaviour in the melting temperature interval and, as we see once more (Fig. 5), thermal denaturation of DNA accompanied by disruption of the ordered water structures in the double helix. As a result, this proceeds appearing of the free water fraction (see the heat absorption peak for the denatured DNA C_p-curve in the temperature interval –20/0°C, demonstrating the melting of free water. This peak is absent on the C_p-curve for native DNA (compare this result with results shown in Fig. 2 and in Fig. 5 in [12]). We underline that the jump – like destruction of the ordered water of hydration shell of double helix in dilute solutions (this means of increase of free water fraction and rearrangement of bound water quantity according from the accessible total surface area [1, 4, 12]) is confirmed by numerous NMR experiments [14, 28], IR-spectroscopy, gravimetry [18] and equilibrium centrifugation [29].

The investigation of the hydration phenomena observed in condensed wet DNA assemblies has also another sense. DNA helices in chromosomes, phage heads, and inside some cell nuclei are separated by only one to two monolayers of water [11, 30]. Crystallographic studies of even denser aggregates of natural DNA at ≤90% relative humidity revealed structural poly- and mesomorphism [31, 32], which is still poorly understood [30, 33]. We have tried to study only one aspect: revealing the role of hydrated water in the energetic of observed thermal transitions of DNA.

Conclusions

In this work we have measured the temperature dependence of the heat capacity for native and denatured DNA and the heat effects accompanying the ice-water phase transitions and the process of heat denaturation of DNA gels at different water contents. We have shown that the heat denaturation of DNA is accompanied by disruption of the ordered water structures in the hydration shells of double helix and replacing of bound water in the free water fraction. We propose that the main contribution in the enthalpy of the process of the heat denaturation of DNA duplex 35±5 kJ/mol bp is the enthalpy of disruption of the ordered water structure in the hydration shell of the double helix 26±1 kJ/mol bp. It is possible that particularly this part of energy composes the non-specific general contribution (70%) in the enthalpy of transition of

all type of duplexes [3, 18]. For DNA in the condensed state (fibres and gels at low water content) the ratio $\alpha=\Delta C_p/\Delta S\sim 2$ is smaller than for DNA in diluted aqueous solutions $\alpha\cong 2$–4. This means that there are some other sources for the large heat capacity change in dilute solutions of DNA – for example hydrophobic effects [16] and unstacking (unfolding) of single polynucleotide chains [23].

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