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Heat capacity peculiarities of DNA at low temperatures (2–25 K)

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Abstract

This study deals with the low-temperature heat capacity (2–30 K) of DNA native fibres measured at different moisture contents with respect to the specificity of DNA hydration caused by its chemical composition (GC contents). Some peculiarities have been found for the heat capacity dependence of DNA on temperature ($C_p = f(T)$) at 2–4 K (the maximum heat capacity). The C_p for DNA reflects the redundant low-energy density of vibrational states (DVS) contributions as well as the ordinary Debye contribution. It was concluded that whereas the peculiarities of the DNA heat capacity at very low energy, below, 1 K, are well explained by the common two-level tunnelling system (TLS) model, the nature of the redundant DVS at 3–10 K is connected with the location of the vibration on the heterogeneous parts of the structure; these areas in the hydrated fibres of DNA may represent clusters of hydrate water "grown" on DNA matrix with a specific size of 1–2 nm.

Keywords: DNA; Heat capacity anomaly; Hydration; Low temperatures

1. Introduction

The study of the low-temperature heat capacity of DNA can be divided into three stages.

The first is for aqueous solutions and gels of DNA in the ice–water phase transition region (200–300 K), where the physical properties of the so-called bound-nonfreezable water in the DNA solution and the mechanisms of the hydration of the double helix have been studied [1–5].

The second stage deals with obtaining a precise knowledge of the temperature dependence of the heat capacity C_p of DNA gels for different hydrations in order to determine the limit laws for the heat capacity at low temperatures, including the liquid helium region (4–300 K) [4–7]. It is very important to compare the experimental data with the theory of heat capacity for strongly anisotropy structures [4–6]. Low temperature properties of DNA in different conformational states have been determined, namely "helix", "statistical coils", and "mechanical mixture of four nucleotides", (which is modelling a state of totally degraded chains without "linear memory", but with preservation of the chemical composition of the molecule (GC contents)) [8, 9]. A difference in specific heat capacity between native ("helix") and denatured ("coils") DNA is found. The increase in the heat capacity of the disordered forms of DNA in comparison with the double helix, all the studied intervals of tempera-

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ture (5–300 K), is mainly caused by the difference in hydration of the various conformations of DNA [8, 9].

The third stage is the study of the low-temperature thermal properties of "The Most Important Molecule" [10]; this involved overcoming two principal experimental difficulties [11]: (i) we managed to measure the specific heat of DNA below 1 K (within 0.5–5 K); (ii) for the first time, measurements were carried out in aqueous buffer solution. Fairly reliable results were obtained for the density of low-energy excitation for native DNA, and we could emphasise the analogy between the behaviour of DNA heat capacity at low temperature and those of other biomolecules and glasses [11, 12].

In the present investigation, we present low-temperature heat capacity data of native DNA fibres, with reference to the hydration of DNA, conditioned by its chemical content (GC content); this made it possible to discuss the peculiarities in the temperature dependence of the DNA heat capacity below 4 K.

The analysis of $C_p = f(T)$ takes into account the redundant low-energy density of the vibrational states (DVS) which is typical for non-crystalline solids [13,14]. The character of the heat capacity peculiarities (maxima of heat capacity) has been analysed for native DNA at 2–10 K. The conclusion states that the nature of the redundant DVS is due to the location of the vibrational excitation on the heterogeneous areas of the DNA structure which are of nanometer order. These may be the clusters of hydrate water 1–2 nm in size "grown" in the DNA matrix.

2. Experimental

2.1. DNA preparations

We used super-pure samples of NA-DNA from cow spleen (42% GC), kindly prepared and donated by D. Lando (The Institute of Bioorganic Chemistry, Minsk). Protein concentration was $< 0.1\%$; RNA; $< 0.5\%$; molecular weight, $> 10^7$ dalton (unified atomic mass units). In preparing the DNA samples, the peculiarity of the double helix hydration, caused by its GC content [5,15], was taken into account. The amount of water in native specimens of DNA was controlled by their exposure to definite relative humidities as well as by means of slow evaporation of the solvent from DNA gels in calorimetric cells followed by weighing (accuracy, 1 mg). Bidistilled water served as the solvent.

The calorimetric measurements were carried out on specimens of non-oriented DNA fibres, containing a known amount of water, calculated according to the empirical dependences [15]:

$$n_\Sigma = \{28.0 - 0.12(\%GC)\}$$

$$n_s = \{12.0 - 0.06(\%GC)\}\ M H_2O/MBP$$

(MBP is Mole Base Pairs)

where n_Σ is the total hydration of the DNA macromolecule in the B-conformation, and n_s the amount of water which makes up the so-called "inner" hydrate layer of the molecule [5,16,17]. Thus for the three samples investigated (DNA with three different water contents)

$$n_0 = 0\text{–}2\ M H_2O/MBP\ (0.050\,g\ H_2O/g\ DNA)$$

$$n_s = 10\text{–}12\ M H_2O/MBP\ (0.326\,g\ H_2O/g\,DNA)$$

$$n_\Sigma = 22\text{–}23\ M H_2O/MBP\ (0.621\,g\,H_2O/g\,DNA)$$

2.2. Calorimetric measurements

The low-temperature calorimetric equipment, working in a heat-pulse regime, has been described in detail elsewhere [4,5,18]. The main specifications are as follows: the operating temperature range was 2.0–30 K (standard germanium resistance thermometer) and 10–370 K (standard platinum resistance thermometer); the working volume of the calorimetric ampule was 0.8 cm^3; the ampule, with an inner heater and thermometer, was mechanically sealed; the temperature was measured by means of a double bridge (Automatic System Laboratory Inc., model A7,8); the measurements were in steps of 0.5–1.0 K in series; the data were processed by a computer during measurements, connected directly with the calorimeter. The maximal errors of the C_p measurements were 1% for 2–5 K, 1.5% for 5–15 K, and 6% for 20–30 K.

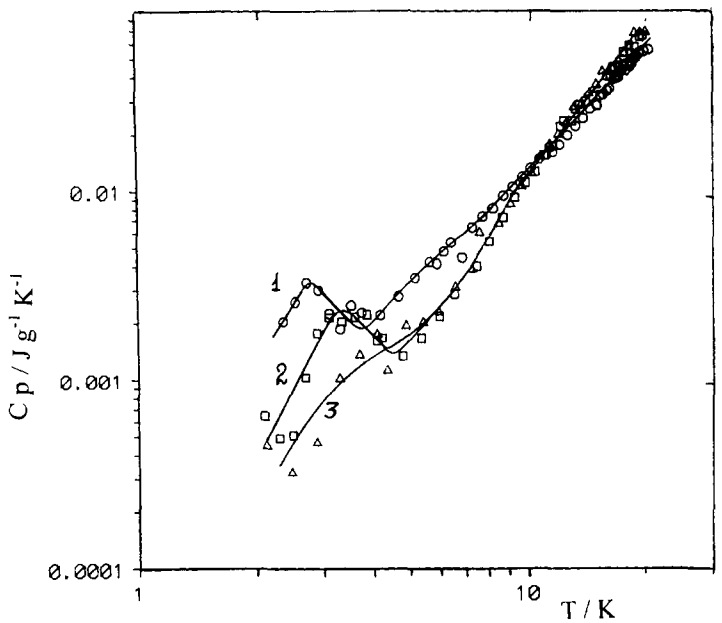

Fig. 1. Temperature dependence of the heat capacity of native fibres of DNA for different water contents: 1, $n_0 = 0$–2; 2, $n_s = 10$–12; 3, $n_\Sigma = 23$ mol water molecules per mol base pairs ($M\,H_2O$/MBP).

3. Results

3.1. Experimental data

Fig. 1 shows the temperature dependence of the heat capacity for native DNA at various degrees of hydration (n_o, n_s, n_Σ); one can see a peak in the interval 2–5 K, whose temperature maximum and width depends on a number of factors, primarily the water content; for the dehydrated fibres of DNA (n_o) (according to X-ray analysis, at this level of hydration we have a disordered state of the polynucleotide chains (see Ref. [19])), the peak maximum is at $Tn_s = 2.8 \pm 0.1$ K. The water content $n_s = 10\,M\,H_2O$/MBP corresponds to the relative humidity when an inner hydrate layer of DNA macromolecules is formed. The helix conformation at this moisture is close to the A-form [19]. The maximum of the heat capacity peak is shifted by one degree towards high temperature ($Tn_s = 3.8 \pm 0.1$ K). At a fully constructed hydrate "shell" corresponding to the humidity of the equilibrium-ordered conformation of DNA in the B-form ($n_\Sigma = 20$–25 $M\,H_2O$/MBP), the peak of heat capacity becomes a plateau. The $C_p = f(T)$ differs from the character of the specific heat for disordered chains and hydrated DNA. So the transition of DNA fibres from the disordered conformation to the ordered double helix form with the fully constructed hydrate shell (n_Σ) is directly reflected in $C_p = f(T)$. Note that the absolute values of the heat capacity of the ordered DNA samples are less than the heat capacity of dehydrated - disordered polynucleotide chains. The experimental data of $C_p = f(T)$ at low temperatures, obtained in aqueous buffer solutions [11] and gels of DNA (the present work and Ref. [5,7]) are summarised in Fig. 2. This function reflects to some extent the features of DNA thermal properties at low and extra-low temperatures:

(i) At $T < 1$ K, the linear dependence of the heat capacity is well explained by a two-level state model [11,12].

(ii) At $T \sim 2$–5 K, the heat capacity peculiarities are defined by the water content in DNA fibres (see below).

(iii) At $T \sim 5$–30 K, to reveal the deviation from $C_p = bT^3$ at low temperature and to estimate the contribution of phonons to the total heat capacity,

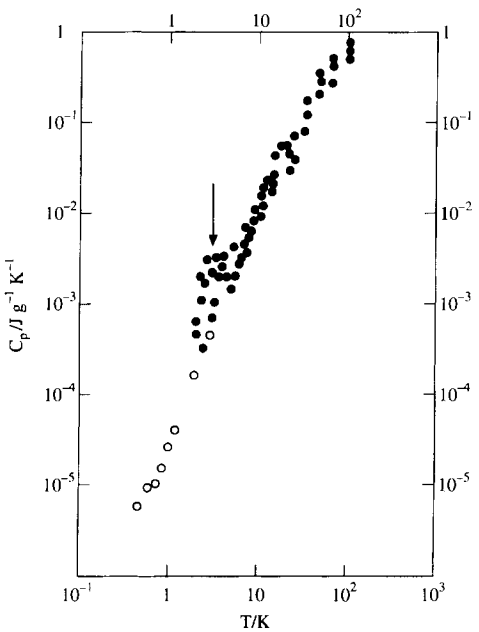

Fig. 2. Temperature dependence of the heat capacity of native DNA in solution (○) [11] and native DNA fibres for different contents of water (●) (see also [9]). The arrow shows the heat capacity anomaly.

the data are usually presented in the form of $C_p/T^3 = f(T)$ (see Fig. 3). According to this dependence, the contribution of phonons to the specific heat of hydrated fibres of DNA can be defined by the level of the horizontal line: $b = C_p/T^3 = 1.4 \times 10^{-5}$ J g^{-1} K^{-4}; this value coincides with the estimation obtained in Ref. [11]:

$$C_p/T^3 = 1.2 \times 10^{-5} \text{ J g}^{-1} \text{ K}^{-4}.$$

(iv) $T > 50$ K. For 240–273 K, despite the rather large percentage of water in DNA (n_Σ), the peak of heat absorption typical for the ice–water phase transition is absent. This indicates the formation of "aperiodic crystallohydrate" with clusters of non-freezable water in the structure (see also Refs. [4,5,9]).

Thus, the heat capacity in the range 2–5 K and the temperature maxima in the DNA heat capacity curve in this region of energy remain to be explained.

3.2. Theoretical analysis

In ordinary crystals, the density of vibrational states (DVS) at low temperature is well described by Debye's law. For glasses and other non-ordered materials in the interval 0–1 K, a constant DVS is observed and this is well explained by the two-level state model [11,12]. At 3–15 K, the model assumes a DVS maximum 2–6 times greater than the Debye maximum for different materials [13]. According to present knowledge, the DVS in the 3–15 K interval is caused by vibration excitations present in glasses

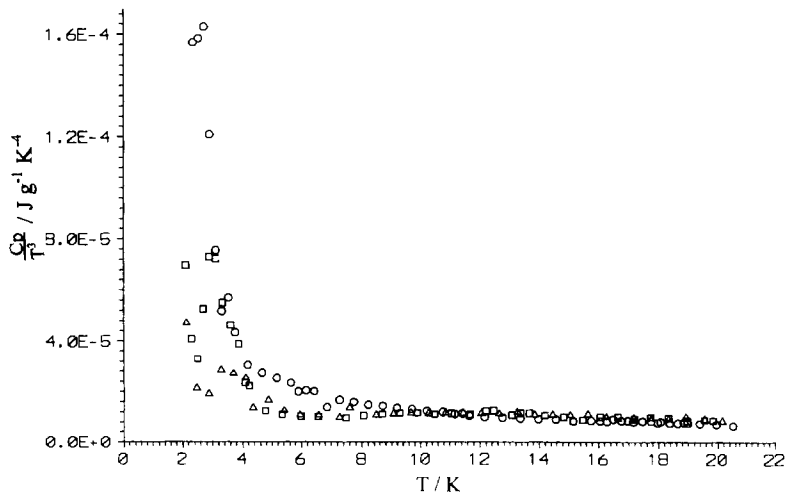

Fig. 3. DNA heat capacity divided by the cube of the temperature vs. temperature for various contents of water; ○, n_0; Δ, n_s; □, n_Σ.

and non-crystalline materials, localised in the region containing from several scores to hundreds of atoms, therefore, they provide indirect information on the structure of the material on a scale of 1–2 nm [13,14,20].

The redundant DVS is defined as $\Delta\rho(\omega) = \rho(\omega) - \rho_D(\omega)$, where $\rho(\omega)$ is the full DVS and $\rho_D(\omega)$ is Debye's DVS. The experimental spectrum of the redundant DVS is well described by a logonormal function [13]. It has only one parameter, σ, the dispersion of distribution, which is universal and equals the same number for all low-molecular weight glasses [13]: $\Delta\rho(\omega) = \exp[-\ln^2(\omega/\omega_{max})/2\sigma^2]$, and $\sigma = 0.48 \pm 0.05$ [13,21].

We use this expression to calculate the heat capacity for non-crystalline aperiodical structures with the redundant DVS taken into account.

The full energy of thermal motion for a kilogram of molecular crystal is

$$U = \int_0^{\omega_{max}} [h\omega\rho(\omega)]/[\exp(h\omega/kT) - 1]\,d\omega \quad (1)$$

The derivative with respect to temperature gives the expression for heat capacity

$$C = dU/dT = \int_0^{\omega_{max}} (h^2/4kT)[\omega^2\rho(\omega)]/Sh^2(h\omega/2kT)\,d\omega \quad (2)$$

We have noted that $\rho(\omega) = \rho_D(\omega) + \Delta\rho(\omega)$. In Debye's approximation, $\rho_D(\omega) = A\omega^2$. Hence

$$\rho(\omega) = A[\omega^2 + (\Delta\rho_{max}/A) \cdot \exp[-\ln^2(\omega/\omega_{max})/2\sigma^2]] \quad (3)$$

Taking into consideration that the full frequency integral of the spectral density is constant, we obtain

$$\int_0^{\omega_{max}} \rho(\omega)\,d\omega = \text{Const}$$

Then, for A we have

$$A = \text{Const} \Big/ \int_0^{\omega_{max}} [\omega^2 + \Delta\rho_{max} \cdot \exp[-\ln^2(\omega/\omega_{max})/2\sigma^2]/A]\,d\omega \quad (4)$$

After substitution of $\rho(\omega)$ in Eq. (2), changing the variable $\omega = 2kTx/h$ and denoting: $(h^2\Delta\rho_{max})/(4k^2A) = T_0^2$; and $2k/h\omega_{max} = 1/T_{max}$, we finally obtain

$$C(T) = \text{Const} \frac{\int_0^{h\omega_{max}/kT} [x^2 + (T_0/T)^2 \exp[-\ln^2(xT_0/T_{max})/2\sigma^2]]x^2 Sh^{-2}x\,dx}{\int_0^{h\omega_{max}/kT} [x^2 + (T_0/T)^2 \exp[-\ln^2(xT_0/T_{max})/2\sigma^2]]\,dx} \quad (5)$$

Using Eq. (5), the computer gives a qualitative picture of the heat capacity temperature dependence in the range 1–10 K. The computation was carried out in steps of 0.1 K with integration limits from 0.0001 to $10/T$. We proceeded from the reasonable assumption that for different non-crystalline materials $\omega_{max} \sim 100\ cm^{-1}$ [13]. The values were obtained by varying the T_0 and T_{max} parameters (see Fig. 4).

It is clear that in the interval 1–5 K, the change in T_{max} and T_0 leads to a shift in the heat capacity maximum and a change in peak height. Thus, in the model presented, the temperature dependence of heat capacity for non-crystalline structures for low temperatures (3–10 K) coincide qualitatively with that observed in experiments for DNA fibres. One should note that the character of $C_p = f(T)$ and the observed peculiarities depend on the degree of hydration which changes the structural parameters of the polynucleotide chains of DNA; in the theoretical model, $C_p = f(T)$ can be changed by means of three parameters (for the material structure), namely the universal σ for non-crystalline structures, the dispersion of DV, and the spectrum distribution (frequency logarithm distribution), and by two parameters with temperature dimensions: $T_0 = (h\omega_{max}/2k)\rho_{max}/A$, ~ 10–100 K, and $T_{max} = h\omega_{max}/2k$; both T_{max} and T_0 are connected with the maximum vibration frequency (ω_{max}), and T_0 carries some additional information about the state of the maximum VS density compared with the Debye density

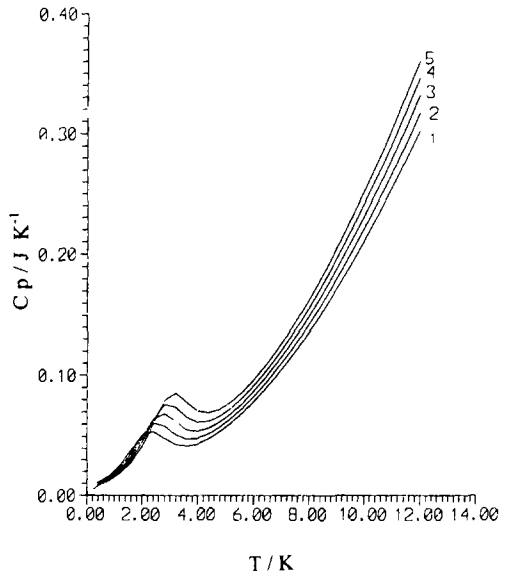

Fig. 4. Temperature dependence of the heat capacity of aperiodic structures for various T_0(a) and T_{max}(b); (see Eq. (5) and text; $\sigma = 0.48$ for both cases): (a) values of T_0: 1, 60; 2, 65; 3, 70; 4, 75; 5, 80 ($T_{max} = 10$). (b) values of T_{max}: 1, 12; 2, 13; 3, 14; 4, 15; 5, 16 ($T_0 = 75$).

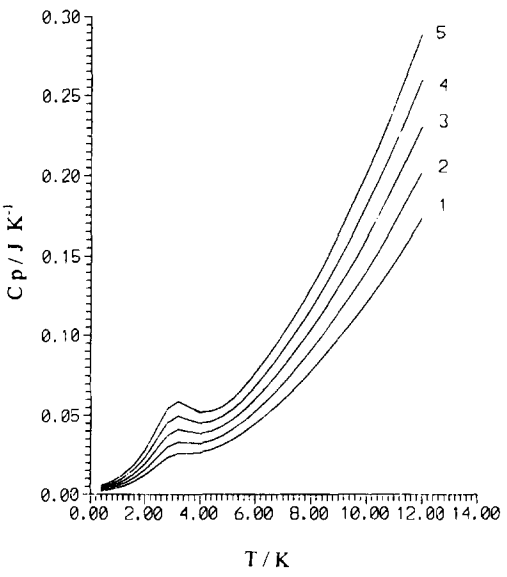

Fig. 4b. (*Continued*)

4. Discussion

Using the conclusions obtained for non-crystalline materials for biomolecules which represent "aperiodic crystals" [23], we suggest that the low-energy redundant DVS in biopolymers may also be caused by the presence of characteristic lengths on a nanometer scale. In the model we use here for 2–5 K, one can conclude that low-energy excitations responsible for the redundant DVS are also located on the heterogeneous sites of the structure. The temperature dependence of heat capacity in the 2–4 K range, the peak amplitude, and the width and temperature maximum depend on the degree of hydration of a macromolecule which we think is the principle reason for such a mechanism for DVS.

Indeed, a gradual change (increase) in the hydration of native fibres of DNA represents a technological procedure for "growing" the clusters from water molecules in the DNA structure matrix, including the grooves of the double helix. The ordered aqueous structures in "aperiodic crystallohydrate" of DNA have been revealed and studied by means of different physical and physicochemical methods (X-ray crystallography, neutronography, infrared spectroscopy, NMR, calorimetry, etc. (see Refs. [5,19,24]) and also by means of direct X-ray analysis of single crystals of oligonucleotides [16,17,19]. The size of the pentagonal aqueous clusters in major grooves of the double helix in A-DNA (at low humidity) reaches to 2 nm [16,17,19]. The size of quasi-one-dimensional water chains ("water spine") [16,17,19] in the minor grooves of B-DNA (full hydration at $n_\Sigma = 0.6$), having an ice-like tetrahedral configuration, depends on the local sequence of bases and AT-pairs content at a given site (in the native DNA, AT- and GC-base pairs are distributed quasi-randomly and contain block rich in AT or GC-base pairs [19]). For instance, the length of the clusters of water molecules ordered in this way and lining the base of minor grooves in DNA containing at least four AT-pairs, is 1.5 nm [16,17,19]. It is essential that the change in the thermal history (particularly the cooling rate of samples) which alters the size and structure of water clusters, leads to changes in the heat capacity in the interval 2–5 K. Thus, vibrations in the aggregated DNA fibres in aqueous medium take place on the heterogeneous parts of the structure defined by cluster size, including sites of polynucleotide chains and water.

In the model where the redundant DVA is caused by vibrational excitations localised on heterogeneous spots of nanometer size, the frequency of quasi-local vibrations (ω) is connected with the heterogeneity size (L) by $\omega = KV/L$ (where K is a constant of order 1, and V is phonon velocity) [13]. We estimate the size of a water molecule cluster in the hydrate shell of a double helix to be 2 nm. If $\omega = 10^{12}\,s^{-1}$ (which corresponds to $T = 10\,K$), the phonon velocity is $2000\,m\,s^{-1}$ which coincides with the usual value [25]. Thus, the connection between the low-energy excitation of DNA with the nanostructure of the double helix, including the hydrate water clusters, becomes still more convincing.

The results obtained remove all doubts that at low temperatures "The Most Important" [10] double-helical molecule of heredity, DNA, possesses a combination of the properties of crystals and glasses, in full accordance with Shroedinger's words pronounced half a century ago when the scientific world had no idea of either DNA structure or its hydration. "we suppose that a gene or perhaps a whole chromosome thread represents an aperiodic solid" [23].

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