DNA Hydration Studied by Pressure Perturbation Scanning Microcalorimetry

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ABSTRACT:

Pressure perturbation differential scanning calorimetry was used to determine thermal expansion coefficients and thus temperature-induced volume changes of DNA duplexes differing in their GC/AT content. It was shown that the temperature-induced unfolding of the DNA duplexes proceeds with a significant increase of the thermal expansion coefficient and the partial volume of the DNA. Unusually, large temperature-induced changes in the partial volume were observed for an AT-rich dodecamer, a finding consistent with previous crystallographic studies showing the presence of highly ordered water molecules hydrating the minor groove of such duplexes. The data show that the density of this ordered water is substantially higher than that of the bulk water. This ordered water cannot, therefore, be equated to ice at normal pressures but it thermodynamically resembles ice formed at high pressures. © 2008 Wiley Periodicals, Inc. Biopolymers 91: 95–101, 2009.

Keywords: pressure-perturbation; calorimetry; DNA; hydration; partial volume

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INTRODUCTION

Pressure perturbation differential scanning calorimetry (PP-DSC) is a new technique which permits determination of the thermal expansion coefficients of solute molecules in their dilute solution and thus estimates temperature-induced changes of the partial volume of the solutes.[1–4] A number of laboratories have used PP-DSC for studying aqueous solutions of various low molecular weight compounds, lipids, and proteins.[5–9] These studies led to an understanding that the thermal expansion coefficient carries information on hydration effects. However, to date this method has not been used for studying DNA, although its hydration is a matter of considerable interest. In aqueous solution, DNA is heavily hydrated and it seems that its interaction with water largely determines its conformation, stability, and ligand binding properties.[10–14] According to crystallography and NMR spectroscopy, the state of water is very different in the minor and major DNA grooves of AT-rich sequences: in the minor groove water is arranged regularly, forming an extended well-ordered cooperative spine.[15–21] The anomalously high hydration of AT-rich sequences was shown also by measuring ultrasonic velocities.[22] Surprisingly, proteins interacting with the minor groove usually choose AT-rich sequences, as though the state of water at such sequences plays an important role in binding.[23]

In this article, we present the results of a PP-DSC study of DNA duplexes differing in the content of AT base pairs, showing differences in their thermal properties, that is, thermal expansion coefficients and the temperature induced change of their partial volume, which is associated with changes of hydration. This study used a newly developed pressure perturbation scanning nanocalorimeter having capillary cells and has demonstrated the ability of this advanced technique for the detailed experimental investigation of the thermal properties of biological macromolecules in dilute solutions.

METHOD

Theoretical Basis

PP-DSC is in fact a new application of the well-known heat capacity calorimetry, particularly of its micromodification commonly referred to as differential scanning microcalorimetry. In contrast to the usual heat capacity calorimetric experiment, which is conducted under constant pressure, that is, under isobaric conditions, pressure perturbation calorimetry is carried out under changing pressure and consists of measuring the heat effect induced by compression of the sample. Compressing a liquid by increased pressure induces heat, δQ, and entropy, $\delta S = \delta Q/T$, effects.[2] With a change of pressure:

$$\left(\frac{\partial Q}{\partial P}\right)_T = T\left(\frac{\partial S}{\partial P}\right)_T \tag{1}$$

Using the Maxwell relation

$$\left(\frac{\partial S}{\partial P}\right)_T = -\left(\frac{\partial V}{\partial T}\right)_P \tag{2}$$

one obtains:

$$\left(\frac{\partial Q}{\partial P}\right)_T = -T\left(\frac{\partial V}{\partial P}\right)_P = -TV \times \frac{1}{V}\left(\frac{\partial V}{\partial T}\right)_P = -TV\alpha \tag{3}$$

where V is the volume and α is the thermal expansion coefficient.

Integration of Eq. (3) at constant temperature over a short pressure range ΔP then leads to

$$Q = -TV\alpha \cdot \Delta P \tag{4}$$

If a solution is composed of m_s grams of a solute dissolved in m_o grams of a solvent, the total solution volume V_{tot} is:

$$V_{tot} = m_o V_o + m_s V_s \tag{5}$$

Its differentiation with respect to temperature at constant pressure gives:

$$\left(\frac{\partial V_{tot}}{\partial T}\right)_P = m_o\left(\frac{\partial V_o}{\partial T}\right)_P + m_s\left(\frac{\partial V_s}{\partial T}\right)_P \tag{6}$$

Then substitution of the right hand side of Eq. (6) into Eq. (3) gives:

$$\left(\frac{\partial Q}{\partial P}\right)_T = -T\left[m_o\left(\frac{\partial V_o}{\partial T}\right)_P + m_s\left(\frac{\partial V_s}{\partial T}\right)_P\right] \tag{7}$$

Multiplying and dividing the first term in the brackets by V_o and the second one by V_s gives:

$$\left(\frac{\partial Q}{\partial P}\right)_T = -T[m_o V_o\alpha_o + m_s V_s\alpha_s] \tag{8}$$

Integration of Eq. (8) over a pressure range ΔP leads to:

$$Q = -T[m_o V_o\alpha_o + m_s V_s\alpha_s]\Delta P \tag{9}$$

In a differential experiment, when the sample and reference solutions are placed in cells with identical volumes and subjected to the same pressure ΔP, the difference heat effect would be:

$$\Delta Q = -T\Delta P[m_s V_s\alpha_s - m_s V_s\alpha_o] \tag{10}$$

And thus:

$$\alpha_s = \alpha_o - \Delta Q/[T\Delta P m_s V_s] \tag{11}$$

Instrumentation

The ideal pressure perturbation calorimetric experiment should be performed isothermally. However, it can also be performed under slow heating/cooling conditions using a heat capacity scanning calorimeter. If the pressure change proceeds much faster than the heating/cooling of the sample, the observed effect can be regarded to a first approximation as isothermal. The advantage of conducting the pressure perturbation experiment using continuous heating/cooling in the scanning calorimeter is that this permits determination of the thermal expansion coefficient over a temperature range, that is, determining its dependence on temperature, which is very important. Moreover, this yields not only the thermal expansion coefficient but also the apparent heat capacity of the studied solution over this temperature range. The simultaneous determination of these two fundamental thermodynamic parameters of the sample is essential for understanding its thermal properties.

Slow heating allows the effect of fast pressure changes to be treated using the above equations. However, the sensitivity for measurement of the apparent heat capacity of the solution decreases with reduction in the heating/cooling rate. Therefore, simultaneous measurement in a single experiment of both the thermal expansion coefficient and the heat capacity of the studied solution requires an extremely sensitive scanning calorimetric instrument. Moreover, it requires a DSC instrument with an extremely stable and reproducible baseline which does not change during experiments of several hours duration or on replacement of the sample. Furthermore, the instrument should be able to scan up and down in temperature with a chosen constant rate to check that the observed effect is reversible.

Another requirement for such an instrument concerns its operational volume: it should not change upon applying pressure, that is, the calorimetric cell should be very rigid. The most suitable for such experiments is a capillary cell (Figure 1). The other important advantage of capillary cells is the shorter thermal relaxation time when compared with cylindrical cells. TA Instruments (New Castle, DE) offers a capillary cell Nano-DSC equipped with a built-in computer-controlled pressurization system. Included software allows for both control of pressure and temperature during an experiment as well as full analysis of the data after the experiment is complete. Automation of the PPC experiment is particularly important because the temperature scanning proceeds very slowly and, together with checking the reversibility of the observed temperature induced processes, the whole experiment takes considerable time.

Experimental Procedure

Because the heat effect in a pressure perturbation experiment results from an increase or decrease of the pressure, and its magnitude is

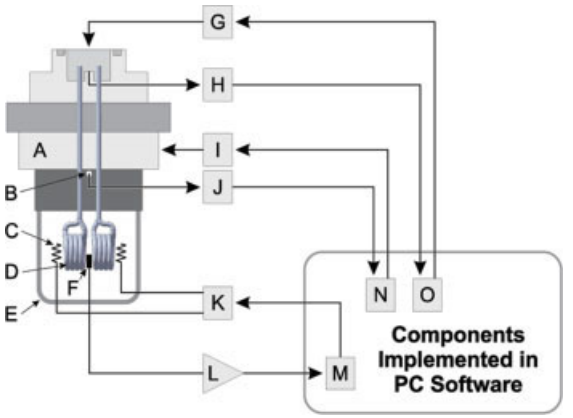

FIGURE 1 Block diagram of the automatic the pressure perturbation scanning microcalorimeter with twin capillary cells. A: Thermoelectric heating and cooling device; B: Platinum resistance temperature detector; C: Power compensation heaters; D: Capillary cells; E: Thermal shield; F: Thermosensor; G: Pressure piston driven by stepper motor; H: Pressure transducer; I: Temperature control electronics; J: Temperature measurement electronics; K: Power compensation bridge; L: Signal amplifier; M: Feedback control algorithm; N: Temperature control algorithm; O: Pressure control algorithm.

proportional to the time derivative of the pressure, it is rational to investigate pressure effect using periodic application of rapid pressure jumps between defined levels of pressure, approximating to periodic rectangular pressure pulses. As shown in Figure 2, application of a pressure pulse induces a sharp heat effect, whereas removal of the pressure results in a similar effect in magnitude but opposite in sign. The relaxation time of the pressure pulses is quite considerable and depends both on the properties of the instrument, in particular the construction of the calorimetric cells, and on the sample. Thus to investigate a system in equilibrium, the duration of each pulse and the interval between the pulses should be considerable. With a capillary cell instrument, the most practical regime is periodic pressure pulses with time intervals of about 600 s. At a scanning rate of 0.1 K/min, one pressure pulse then occurs every 1.0 K and heating over a 100 K temperature range takes 16.7 h, that is, it is a long experiment, especially if both heating and cooling are required.

The upper pressure used with the platinum capillary cells of the Nano-DSC is 5 atm. The lower pressure cannot be zero because such a pressure drop might induce the formation of bubbles in the studied solution. Appropriate pressure pulses are from 1 to 5 atm and back to 1 atm. It is important that pressure pulses are absolutely identical during the whole series of experiments with the studied solution, its solvent and the standard liquid used for calibration.

The reliability of thermal expansion coefficients determined by PP-DSC was questioned in the literature because this experiment was not conducted under isobaric conditions and the change of pressure might have induced changes in the mass of liquid in the cell, which has a fixed operational volume.[24,25] It was therefore necessary to show, using standard liquids with known thermal expansion coefficients, that results obtained from the Nano-PP-DSC are correct. Figure 3 shows the thermal expansion coefficient of ethanol as determined by the PP-DSC experiment over the temperature range from 20 to 80°C using water as the reference. One can see that the values of the thermal expansion coefficient of ethanol determined in the PP-DSC experiment are in excellent agreement with tabulated data for ethanol taken from a handbook.[26]

RESULTS

The purpose of this work is not only the demonstration of the ability of the new Nano-PP-DSC instrument to study the thermal properties of biological macromolecules but also the investigation of these properties of the DNA, particularly the thermal expansion of duplexes differing in composition. For this purpose, we studied the following 12 bp DNA duplexes differing in the number of AT bases in the middle and flanked by GC bases to stabilize their ends:

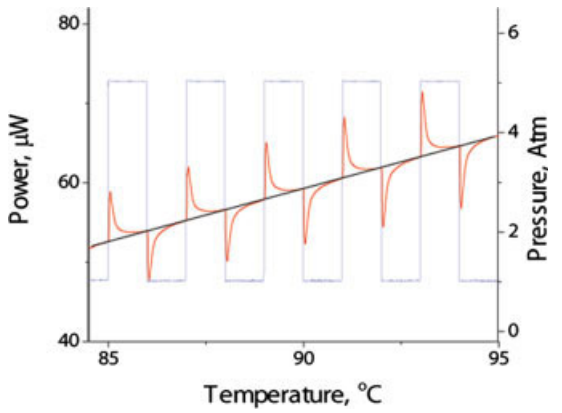

FIGURE 2 The heat effect of pressure pulses measured by PP-DSC with buffer (100 m*M* NaCl, 20 m*M* sodium phosphate, pH 7.4) in the measuring cell and water in the reference cell. The heating rate is 0.1 K/min, the time interval between pressure pulses 600 s and the pressure changes from 1 to 5 atm.

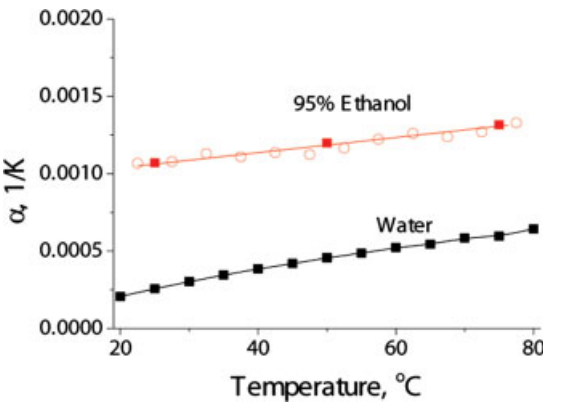

FIGURE 3 Standard thermal expansion coefficients of water and 95% ethanol (solid black and red squares, respectively)[26] and the PP-DSC measured the thermal expansion coefficients of 95% ethanol (open red circles).

i 5′-CGC*AAATTT*CGC-3′
ii 5′-CGC*AGACTG*CGC-3′
iii 5′-CGC*CGCCG*CCGC-3′

All DNA oligonucleotides were synthesized by IDT Company and additionally were purified chromatographically. To prepare DNA duplexes, equimolar amounts of the complementary strands were mixed, heated to 95°C, and slowly cooled. For the PP-DSC experiment, solutions of duplexes were dialyzed for 3 days with three changes of solvent using 1000 Da molecular mass cut-off membranes (Spectrapor). The solvent used was 20 m*M* sodium phosphate, pH 7.4, 100 m*M* NaCl, and 1 m*M* EDTA. Concentrations were determined from A260 of the free nucleotides after complete digestion of single-strand oligonucleotides and duplexes by phosphodiesterase I (Sigma) in 100 m*M* Tris-HCl, pH 8.0. In all cases, duplex concentration was determined after dialysis and repeated after the calorimetric experiments.

In the PP-DSC experiment, the DNA solution was placed in the measuring cell and solvent in the reference cell. To obtain absolute values of the thermal expansion coefficients of DNA, it is necessary first to determine this coefficient for the solvent by placing it in the measuring cell and pure water in the reference cell (Figure 4). Because the thermal expansion coefficient of pure water over the whole considered temperature range is known,[26] these data can be used to estimate the thermal expansion coefficient of the solvent used for the DNA solution (Figure 5) and then calculate the thermal expansion coefficient of DNA over the studied temperature range.

Figure 6 illustrates the original recordings of a PP-DSC experiment with a solution of the 12 bp DNA duplex I obtained upon heating at a rate of 0.1 K/min from 0 to 100°C with pressure pulses at each degree from 1 to 5 atm

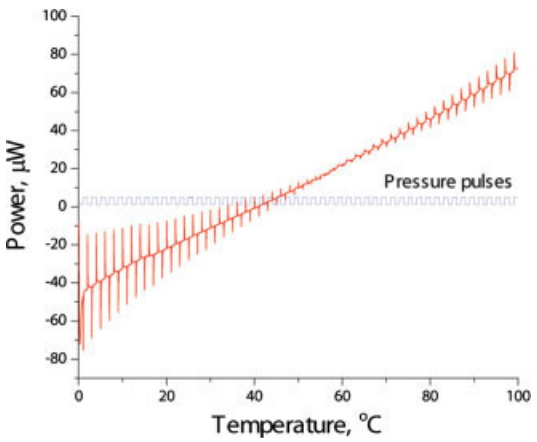

FIGURE 4 Pressure perturbation scan of the solvent used in the DNA experiment (20 m*M* sodium phosphate, pH 7.0, 100 m*M* KCl, 1 m*M* EDTA) against water in the reference cell. The pressure pulses are 1 to 5 atm, the heating rate 0.1 K/min.

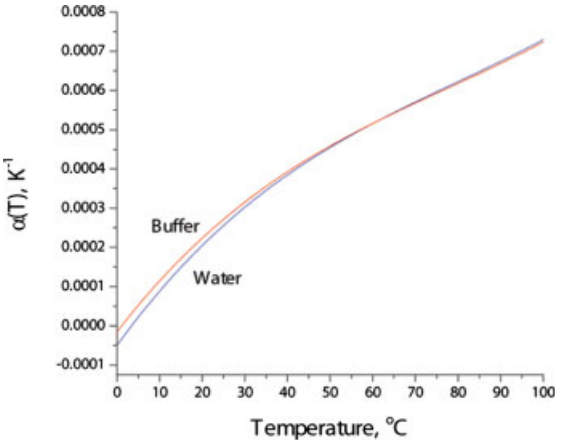

FIGURE 5 Calculated temperature expansion coefficients, $\alpha(T)$, of the buffer using the $\alpha(T)$ of pure water as a standard.

and back to 1 atm. One can see that the pressure perturbation effects are different for the solution and the solvent and they change in different ways with temperature increase. The extensive heat absorption peak observed upon heating the DNA solution is associated with the cooperative breakdown of the DNA duplex and dissociation of the complementary strands. Using the pressure perturbation effects obtained for the DNA solution and the solvent, one can calculate the thermal expansion coefficient at all temperatures over the whole considered range.

The thermal expansion coefficients of the three studied 12 bp DNA duplexes in the temperature range from 0 to 100°C are presented in Figure 7. This shows that at low temperatures, these three coefficients are rather similar and decrease significantly with temperature rise, up to the point

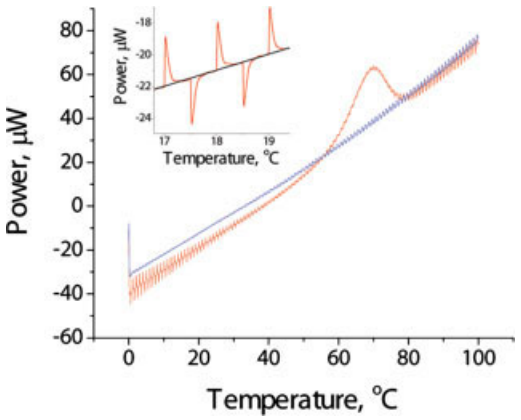

FIGURE 6 Original recording of the PP-DSC experiment with the 12 bp DNA duplex (I) (13.4 mg/ml concentration) in 100 m*M* NaCl, 20 m*M* sodium phosphate, pH 7.4 at a heating rate of 0.1 K/min. The scan of the DNA against buffer is shown in red; the scan of buffer against buffer is in blue. The inset gives an expansion of the DNA/buffer scan between 17 and 19°C.

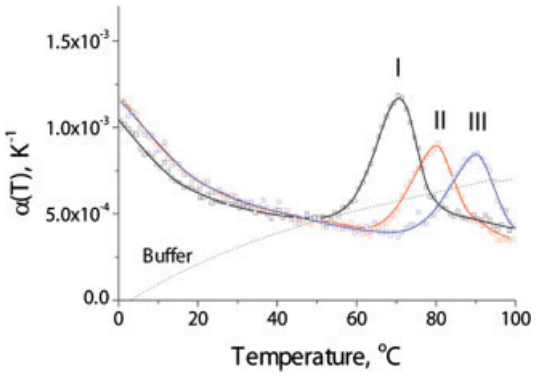

FIGURE 7 The calculated temperature expansion coefficients of the three 12 bp DNA duplexes in the temperature range from 0 to 100°C. Concentrations of DNAs: duplex I = 13.4 mg/ml; duplex II = 14.5 mg/ml; duplex III = 12.3 mg/ml (∼1.5 m*M*). Buffer: 100 m*M* NaCl, 20 m*M* sodium phosphate, pH 7.4. The PP-DSC results were obtained at a scanning rate of 0.1 K/min, with 300 s intervals between pressure jumps of 1–5 atm.

at which the duplexes start to melt, a process associated with a peak in the thermal expansion coefficient. Melting of the duplexes takes place over different temperature ranges because they differ in stability, increasing with GC content. It is most remarkable, however, that the area of the peaks of the thermal expansion coefficients is different for the three duplexes, being significantly larger for duplex I that contains the stretch of -AAATTT- bases. The initial decrease of the thermal coefficient upon heating been noted before in proteins and was assumed to be specific for the hydration of hydrophilic groups,[1] whereas the peak of this coefficients correlating with the content of A and T basses is a novel effect specific for the DNA duplexes. Therefore its understanding presents especial interest.

By integrating the temperature function of the thermal expansion coefficients, one can determine the relative change of the partial molar volume of the DNA over the considered temperature range. Because the change in the thermal expansion coefficients upon melting are very different, the increase in their partial molar volumes must be also different: in particular, melting of duplex I results in a particularly large increase of volume. To simplify comparison of the volume effects associated with unfolding the considered duplexes, we can assume that in the unfolded state their partial specific volumes are identical. This is a justified assumption if the differences in the partial specific volumes of the considered duplexes result mainly from their hydration and this difference disappears at 100°C where these duplexes are unfolded. Furthermore, for graphical presentation of these volume effects we need to choose a standard. This can be the partial molar volume of duplex III at 25°C, the advantage of this 100% GC duplex being that the partial molar volume of poly[d(G-C)] has been densimetrically measured at 25°C and found to be (152 ± 1.5) ml per mol of averaged nucleotide.[10] Although this polynucleotide differs from our duplex III in size and bases sequence, this is unessential for us because their partial volumes hardly differ significantly, whereas we are interested only in relative changes of partial molar volumes upon unfolding and not in their absolute values. The densimetrically measured partial molar volume of GC deoxypolynucleotides is therefore used only for presenting the observed relative changes in the volumes of the three studied duplexes on an appropriate scale (Figure 8).

It appears that the temperature reduction results in a decrease of the partial volumes of all three considered duplexes, especially in the temperature range of their refolding, and this volume decrease is particularly large for duplex I, which includes the contiguous -AAATTT- stretch. The decrease of the partial volume on formation of duplex I is about 20 ml/mol greater than that of the two other duplexes.

DISCUSSION

Because the volumes of the anhydrous base pairs of DNA do not differ greatly, the observed difference in partial volumes must be attributed to the differences in the volumes of the hydrating water. It follows from the above results that the volume of water hydrating the duplex containing the -AAATTT- stretch is considerably smaller than the volume of water hydrating the two other duplexes. As previously shown

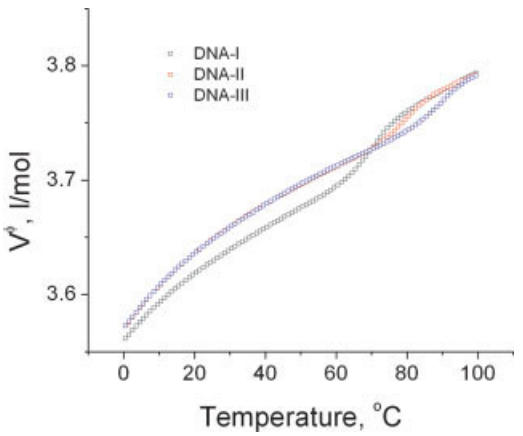

FIGURE 8 Temperature dependencies of the partial molar volumes of the three 12 bp DNA duplexes determined by integration of the thermal expansion coefficients, $\alpha(T)$, with respect to temperature and scaling them assuming that at 25°C the partial volume of the 100% GC duplex (i.e. duplex III) is 152 ml per mol of nucleotide,[10] and that at 100°C the partial specific volumes of all three samples are equal. Note that below 70°C, the partial volumes of duplexes II and III coincide.

by precise direct densimetric measurements, the density of water hydrating DNA duplexes is significantly higher than that of bulk water.[10] The decrease of the partial volume of the DNA duplexes observed by PP-DSC upon lowering the temperature shows that the folded duplexes affect the state of the surrounding water more extensively than do the unfolded oligonucleotides and this is particularly true of the duplex containing the -AAATTT- sequence. This is surprising because the water accessible surface area of the unfolded oligonucleotides is larger than that of the folded duplex. It follows that the influence of DNA on the state of the water comes mostly from its helical conformation. This is just what has been shown by structural studies of the B-DNA dodecamer CGCGAATTCGCG.[17,19,21] According to these studies, the minor groove at the AATT sequence is particularly narrow, and the water in this groove is in a highly ordered, regular state.

Thermodynamic studies of protein-DNA interactions have shown that binding of protein to the minor groove of AT-rich sequences is specified by a positive enthalpy and entropy, in contrast to protein binding to the major groove.[23] This was explained by the large enthalpy and entropy of removing the highly ordered ice-like water from the minor groove, that is, its melting. The question is now whether water in the AT-rich minor groove of DNA can be regarded as ice-like?

The PP-DSC studies show that at room temperature the duplex containing the -AAATTT- stretch occupies less volume than its dissociated oligonucleotides that do not order water. It follows that disordering the regularly ordered water in the minor groove of AT-rich DNA proceeds with an increase of its volume. However, the melting of ice at normal pressure proceeds with a decrease of volume, that is, the density of ice at normal pressure is lower than that of liquid water; it is higher only for ice formed at high pressures. It appears, therefore, that the stretch of highly ordered water in the AT-rich minor groove of DNA thermodynamically resembles ice at high pressure.

Finally, we would like to note the advantages of using the PP-DSC technique in studying the thermal expansion coefficient and temperature-induced changes of the partial molar volume of biopolymers in solution. This information could, in principle, be obtained by measuring the density of these solutions using conventional densimeters. However, even the most sensitive vibrational dosimeters need rather concentrated solutions for precise determination of the partial volume of solutes and are limited in their operational temperature range both by the difficulty of precise thermostabilization far from room temperature and by the bubbling problem, that is, the appearance of gas bubbles in the heated solution. Correspondingly, this limits the temperature range over which the partial volume and thermal expansion coefficient can be determined by this method. Using PP-DSC, one cannot determine the absolute value of a partial molar volume of a biopolymer in solution but only its change with temperature, that is, the thermal expansion coefficient, this however can be done over a broad temperature range because working under excess pressure means that this instrument does not have the bubbling problem. Moreover, PP-DSC measures the thermal expansion coefficient simultaneously with measuring the heat capacity of the solution and the integration of both with respect to temperature gives information on the temperature-induced volume and enthalpy changes of the sample, that is, of its two fundamental thermodynamic characteristics. The importance of the DSC-determined heat capacity function of temperature for understanding the thermal properties of biopolymers has been demonstrated by numerous papers published over more than the 40 years existence of this methodology (for a recent review see Ref. 27). The pressure perturbation method is much younger than DSC, and the total possibilities of this method are not yet clear, in part due to the absence of sufficiently precise instruments which could operate over a broad enough temperature range. One of the purposes of this article was a demonstration of the ability of the new Nano-PP-DSC and what one might yield by analyzing the temperature-induced changes of the volume of biopolymers, in particular DNA.

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