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Calorimetric investigation of DNA in the native and denatured states

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Abstract

The results of determination of apparent heat capacities of naturally occurring DNA in native (helix) and denatured (coils) states in dilute aqueous solutions have been reported. These results, supplemented by direct heat capacity measurements as a function of temperature, using a capillary scanning calorimeter, clearly show that the apparent heat capacity of polynucleotide chains of DNA in aqueous solution is strongly dependent upon the conformational state of the macromolecule. Comparison of data obtained for DNA in the "solid state", at different levels of hydration and for DNA in H_2O and D_2O solutions reveals the possibility that the heat capacity increment ($\Delta c_p = 0.36 \pm 0.04\,J\,g^{-1}\,K^{-1}$) is determined by an increase in the number of vibrational degrees of freedom, by hydrophobic effects and as a result of hydrogen bond destruction in water (including the ordered water clusters in the hydration shells of double helix of DNA).

Keywords: Capillary scanning calorimeter; DNA, denatured coils; DNA, native helix; Heat capacity

1. Introduction

Calorimetric investigations of the helix–coil transition of naturally occurring DNAs and oligonucleotides have greatly enhanced our understanding of the conformational transitions of the double helix. The investigation of conformational changes of nucleic acids has flourished due to two new developments: first, improvement of the biochemical preparation techniques of native DNAs and DNA fragments and our ability to manipulate native conformations or engineer new sequences, and, secondly, improve-

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ment of experimental techniques for determining the energy parameters of conformational transitions using highly sensitive instruments—scanning microcalorimeters.

In their excellent review Klump et al. [1] try to deconvolute individual forces that contribute to transition free-energy and to the stability of the B-DNA conformation in general: coulomb–electrostatic, stacking, and hydrophobic interaction as well as hydrogen bonds. The integral value of the transition enthalpy reported in the present work is an experimental result. Since the transition from helix to random coil is regarded as a reversible process, the thermodynamic formalisms and definitions are also valid for DNAs (only kinetically slowed down) [1–4].

In addition to the characteristic heat effects, these transitions are also accompanied by changes in the volume [5, 6] and in the heat capacity $\Delta c_p = c_p$, coil- c_p, helix [7]. The volume and absolute heat capacity properties reflect more directly the degree of hydration [5–7], an interaction found very clearly to be of critical importance for the stability of the double-helical structures in aqueous solutions. However the quantitative role of water in maintaining native DNA conformation as well as its participation in helix–coil transitions accompanying varying solution conditions remains unknown. Our general effort, therefore, has been directed toward determining variations in heat capacity (Δc_p) as well as those of the standard thermodynamic functions (ΔG, ΔH, ΔS) upon helix–coil transition of DNA in H_2O (at different pH and ionic strength) and in D_2O solutions.

2. Experimental

2.1. DNA samples

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

2.2. Calorimetry

The calorimetric measurements were carried out on an adiabatic capillary scanning calorimeter DASM-4. The principles of operation of the instrument are reviewed in the literature [8]. The heating rate was from 0.5 to 1.0 K min^{-1}. The DNA concentration in the solutions used for the experiments varied from 1.0 to 4.0 mg ml^{-1}. The c_p measurement error did not exceed $\pm$ 1–2% [8].

3. Results

When comparing the heat capacity curves with any other curves describing the change in the double helix state with temperature, we must bear in mind that heat

capacity is a temperature derivative of an internal characteristic, i.e. of an enthalpy [9]. As a derivative of a variable parameter, this function describes more precisely all the changes in the state of macromolecules with temperature. At the same time, since heat capacity is a derivative of enthalpy, no temperature-induced change in the state of macromolecule can occur without being reflected in the heat capacity curve [9]. When analysing the heat capacity curve, let us assume as a first approximation that the pre- and postdenaturational heat capacity changes in the macromolecule can be described by linear functions of temperature [9]. In this case we can easily extrapolate the heat capacity of the pure native and pure denatured macromolecules to the transition range and evaluate the heat of the denaturation process. As is seen from Fig. 1, ΔH corresponds to the peak area above the heat capacity functions extrapolated to the midpoint of the transition. The difference between the extrapolated heat capacity functions at the temperature of the midpoint of the denaturation corresponds to the difference between the heat capacity of the native and denatured state of the DNA $\Delta c_p(T) = c_p^d(T) - c_p^n(T)$. It is evident that the change in heat capacity must determine the temperature-dependence of the enthalpy of denaturation since $d(\Delta H_m)/d T_m = \Delta c_p$. The temperature-dependence of the enthalpy of denaturation can also be determined from the change in enthalpy of denaturation at changing conditions, e.g. from the

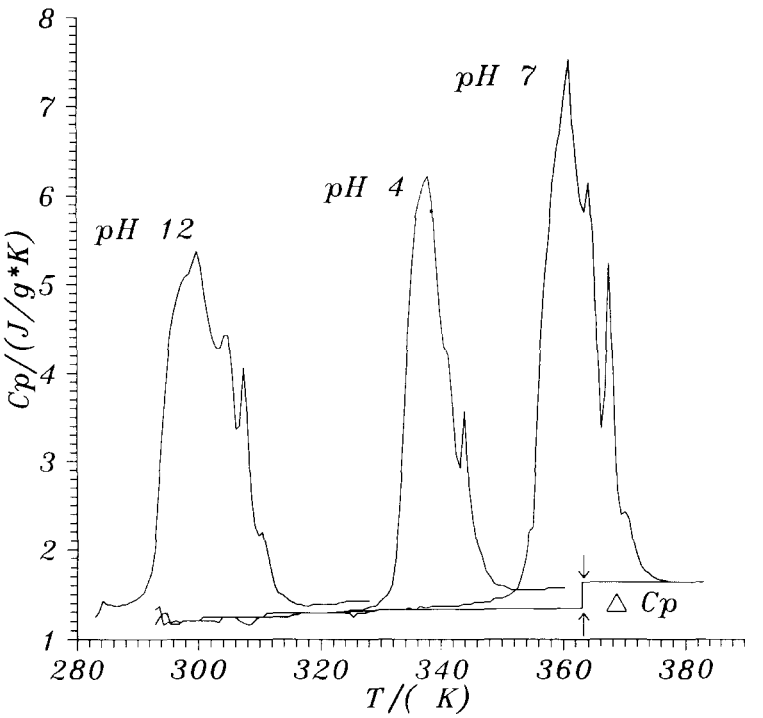

Fig. 1. Temperature-dependence of the partial heat capacity of DNA in solutions of various pH values (indicated on the curves; 0.1 M Na-phosphate buffer).

change in both—the denaturation peak area and temperature T_m, induced by changing pH and ionic strength. The temperature-dependence of the partial heat capacity of DNA in solution at three different pH values is given in Fig. 1. As can be seen, changing the pH (2.4 and 12.4) shifts the denaturation temperatures to lower values and changes the width and enthalpy of transition in agreement with well-known data [3, 10]. The most remarkable observation is that under this condition $\Delta c_p = 0.30 \pm 0.04\ J\,g^{-1}\,K^{-1}$.

The remarkable feature of the functions presented in Figs. 2 and 3 is that they are not linear. With an increase of temperature the enthalpy of the conformational transition increases over the pH interval 2.5–6.0, slightly changes at pH 6.0–7.0 and decreases in the interval pH > 7.0.

In accordance with the thermodynamic equation $\Delta c_p = d(\Delta H_m)/d\,T_m$, dependence of the transition enthalpy on temperature shows up the difference of the heat capacity of native and denatured states of DNA. In this case the absolute volume and sign of Δc_p will be determined by the properties of the solvent (ionic strength, pH, etc.). Fig. 2 shows that at neutral pH ΔH_m changes slightly. So we can consider this specific feature of $\Delta H_m = f(pH)$ and $T_m = f(pH)$ dependencies in neutral pH area to be the reflection of the enthalpy–entropy compensation effect [11], which also brings to zero Δc_p in winding–unwinding reactions. Therefore even slight changes in the chemical composition of the solvent can strongly influence the balance of forces responsible for double-helix structure (hydrogen bonds, hydrophobic interactions, etc.). In this case we must bear in mind the temperature-dependence of transition enthalpy to derive a proper equation for free energy and that compels us to take into account Δc_p. Calculated values of $d(\Delta H_m)/d\,T_m$ according to Fig. 3, for Δc_p are: $\Delta c_p = 0.6 \pm 0.06\ J\,g^{-1}\,K^{-1}$ (pH ~ 2.0–7.0) and $\Delta c_p = 0.07 \pm 0.01\ J\,g^{-1}\,K^{-1}$ (pH ~ 7.0–12.0).

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

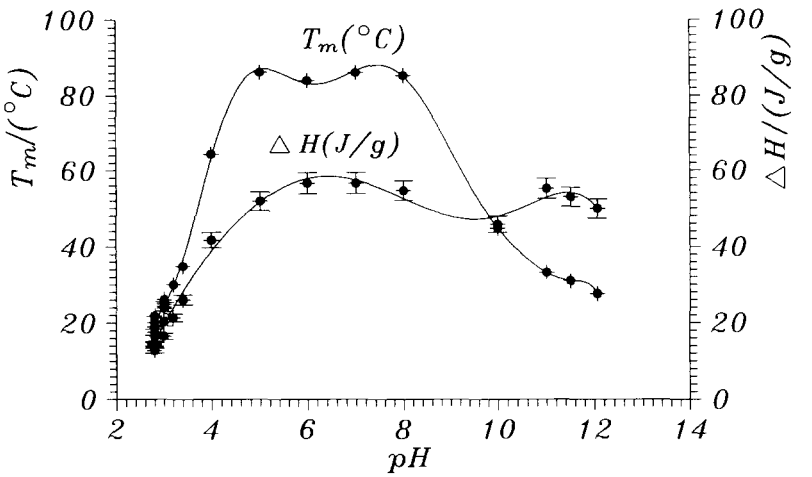

Fig. 2. Calorimetric enthalpy (ΔH_m) and denaturation temperature (T_m) plotted vs. solution pH.

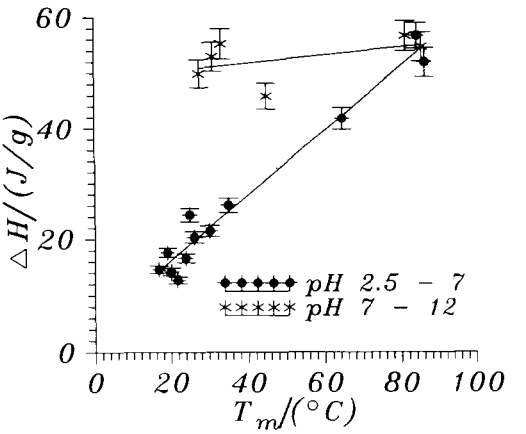

Fig. 3. Calorimetric enthalpy (ΔH_m) plotted vs. denaturation temperature (T_m).

the case of Δc_p the absolute value of the buffer ionization effect is 8×10^{-7} J K^{-1} that is up to 0.45% and is also negligibly small.

4. Discussion

There is no analysis of data on the nature of the denaturation increment of DNA heat capacity in the literature simply because it was generally considered for many years that in dilute aqueous solutions of DNA $\Delta c_p = c_p$, (coils) $- c_p$(helix) $= 0$. The discussion of the heat capacity denaturation increment for dilute ribonucleic acid solutions [12, 13] would not clarify the situation, since the direct precision calorimetric measurements are done for poly-A, poly-U, poly-(A – U) and tRNA, i.e. the compounds whose conformational specificities do not reflect the character of structural transitions of DNA double helixes. In [14] it was shown that over the range 4–300 K the heat capacity of "hydrated" coils is greater than that of "hydrated helixes" over the whole temperature range, this is explained by the change in the vibration spectrum of these two states and by different mechanisms of water–polynucleotide chains interaction in coiled and helical conformations. The latter conclusion is confirmed by the well-known fact of the general increase in the polarity of the DNA molecule as it unwinds [15]. As to hydrophobic effects, note that "exposition" of hydrophobic groups in the solvent, as has been shown for globular proteins at denaturation, can indeed lead to heat capacity increase as a result of specific hydration effects; however, it seems to us that application of the analysis designed for globular proteins to explain the effects occurring in the DNA solutions, is not only incorrect, but it will, a priori, lead to false conclusions. It was shown for globular proteins in dilute aqueous solutions that the major contribution the heat capacity increment at denaturation is made by hydration of inner groups of protein exposed to water at unfolding, and this contribution is on the order of 70% of the total increment of heat capacity (0.4–0.6 J g^{-1} K^{-1}). For DNA the situation is

opposite. As has been shown, the hydration effects in the difference between the "helix" and "coil" are also dominating, but in the process of the helix–coil transition, the heat capacity increment seems to be determined not by "hydration" of separate groups, but by "dehydration" of the double helix, resulting in destruction of the hydrated shell of DNA [14].

According to our new data from calorimetric studies of thermally induced DNA denaturation in ordinary (H_2O) and heavy water (D_2O), the energetic parameters of the helix–coil transition reaction are:

(1) in H_2O—$T_m = 76.9\,^{\circ}C$, $\Delta H_m = 54.7 \pm 6.0\,J\,g^{-1}$, $\Delta c_p = 0.3 \pm 0.1\,J\,g^{-1}\,K^{-1}$

(2) in D_2O—$T_m = 82.3\,^{\circ}C$, $\Delta H_m = 61.19 \pm 8.0\,J\,g^{-1}$, $\Delta c_p = 0.7 \pm 0.1\,J\,g^{-1}\,K^{-1}$

If we consider strengthening of hydrogen bonds in D_2O (actually D-bonds) and admit that the water clusters in DNA hydration shell are built of strongly interacting molecules, by means of "strong" hydrogen bonds (according to [16]) then we can agree that the denaturation increment of the heat capacity is caused (at least in general) by destruction of water clusters. We shall proceed from the process of helix–coil transition in which jump-like destruction of the structure of tetrahedral water spine occurs in the hydration shell of double helix which is confirmed by numerous NMR experiments [17], calorimetry [18], IR spectroscopy [19], gravimetry [20] and equilibrium centrifugation [21]. "Melting" of "water clusters" with an ice-like net of hydrogen bonds and that of additional layers "liquid-like" water should be accompanied by an increase of heat capacity as for the ordinary phase transition ice–water. Hence, it is not excluded that denaturation change of the heat capacity is caused by "melting" of the ordered water clusters together with an increase of the number of the degrees of freedom in atomic groups of polynucleotide chains in the state of statistical coils as well as hydrophobic effects.

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