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Thermochimica Acta 394 (2002) 83–88

thermochimica acta

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Effects of some metal ions on the denaturational heat capacity increments in dilute solutions of ds-DNA

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Received 12 December 2001; received in revised form 2 February 2002; accepted 2 March 2002

Abstract

The native DNA duplex may be viewed as a cooperatively-ordered H bonded structure, including well localized Watson–Crick base pairs and H-bounded networks of hydration parts of the DNA–solvent interface in the grooves of the helix. In this paper, we present the effect of different metal ions (Li^+, Mg^{2+} and Cu^{2+}) on the denaturational heat capacity increment (ΔC_p) for calf thymus DNA.

Since the contribution from the ordered hydration water fraction disruption energy to the total enthalpy and heat capacity increment values of double helix melting is significant, it must be possible to detect the effect of metal ions on the structural ordering/disordering of the H-bounded network in the hydration shell of the DNA duplex.

Experimental results suggest that Li^+, which is preferentially adsorbed in the minor groove of B-DNA and should contribute significantly to the stabilization of B-form, has a pronounced influence on the value of ΔC_p. For the system Mg^{2+}–DNA, the values of ΔC_p are also significant, which can be explained by the formation of inner hydration sphere complexes and immobilization of structural water by the grooves of the duplex, stabilizing the helix. The effect of Cu^{2+} ions is much more pronounced. The satellite peaks of calf thymus DNA become lower as the concentration of Cu^{2+} increases and for concentrations of Cu^{2+} higher than 0.010 M, they disappear and $\Delta C_p = 0$. This is indicative of the known preference of Cu^{2+} for purins and GC rich sites of DNA, binding to the N7 of guanine. As a result, disruption of the base stacking and hydrogen bounded water networks in the grooves of the double helix takes place.

Keywords: DNA–metal ions interactions; DSC; DNA unwinding; Heat capacity increment

1. Introduction

It is well known that metal ions have major effects on double helix stability and structure [1,2]. The effect on helix stability, as reflected on the thermal helix–coil transition, has been the most widely studied consequence of metal ions binding to nucleic acids. Ions will rise the transition temperature (T_m) if they bind more strongly to the double helical form than to the single-stranded form and such binding may be *specific* or *non-specific* [1,2] (and references therein).

The effect of metal ions on the denaturational heat capacity increment values (ΔC_p) in dilute solutions of ds-DNA has not been studied until now because this phenomenon (existence of ΔC_p) has been established only recently [3–6]. The results of the determination

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PII: S0040-6031(02)00241-1

of apparent heat capacities of naturally occurring DNA in both native (helix) and denatured (coils) states in dilute aqueous solutions [3] and the analysis of a large amount of available experimental data (obtained also for polymeric nucleic acid duplexes), using the new generation of differential scanning calorimetry (DSC) [5,6], suggest that the melting of duplexes is accompanied by positive changes in the heat capacity $(\Delta C_p) \sim (170\text{–}400)\,\mathrm{J\,K^{-1}\,mol^{-1}}$ (base pair) ([3–6] and references therein). This has significantly changed the understanding of the origins of duplex stability [6]. In particular, it has been suggested that the total enthalpy of denaturation of ds-DNA, $\Delta H_{\mathrm{melting}}$, includes different contributions, *specific* and *non-specific*: $\{\Delta H_{\mathrm{melting}}\ (T) = \Delta H_{\mathrm{spec}}\ (T) + \Delta H_{\mathrm{nonspec}}\ (T)\}$ [6]. The contribution of the ordered hydration water fraction disruption energy to the total enthalpy and ΔC_p values of double helix melting has been measured and discussed by us recently [7].

In this work scanning microcalorimetry was used to study the dependence of melting enthalpy (ΔH), temperature (T_{m}) and heat capacity increment (ΔC_p) on Li^+, Mg^{2+} and Cu^{2+} ions concentration, in solutions of constant DNA concentration. The thermodynamic parameters (T_{m}, ΔH and ΔC_p) of heat denaturation will be reported and the values of the heat capacity increments (ΔC_p) will be discussed in terms of DNA–metal ion interactions.

2. Experimental

2.1. DNA samples

Calf thymus DNA was obtained from Sigma as type I calf thymus DNA sodium salt (protein content <3%) and used as received with the purity checked by the absorbance ratio, A_{260}/A_{280}, of DNA [1] ($1.8 < A_{260}/A_{280} < 1.9$ for all the solutions studied).

LiCl, $MgCl_2 \cdot 6H_2O$ and $CuCl_2 \cdot 2H_2O$ (Aldrich, ReagentPlus™, 99.99%) were used without further purification to prepare the metal ion solutions.

All the solutions were prepared with ultra pure water (Millipore, Milli Q Gradient).

A DNA stock solution was diluted with the appropriate volumes of buffer solution and of the metal ions solutions to a final DNA concentration of 1 mg/ml (1.5×10^{-3} M) and different metal ion concentrations: 0.010–0.050 M for Li^+ and Mg^{2+} and 0.001–0.010 M for Cu^{2+}. For high concentrations of Cu^{2+} ($\geq$0.02 M) the formation of *aggregates* well above the micrometer size could easily be observed and, for this reason, the range of Cu^{2+} concentrations was limited to 0.010 M.

All the measurements were performed in pH 7.0 buffer solutions consisting of 0.015 M sodium chloride and 0.015 M sodium citrate.

The DNA concentration, expressed in base pairs, was determined by spectrophotometry using a molar absortivity, ε_{260}, of $20\,\mathrm{l\,g^{-1}\,cm^{-1}}$.

2.2. Calorimetry

The calorimetric measurements were carried out using a SETARAM micro DSC III calorimeter with calorimetric cell volume 1.0 ml. The heating rate was $0.2\,\mathrm{K\,min^{-1}}$.

Appropriate buffer versus buffer baselines were determined after the DNA versus buffer scans. After subtraction of the buffer scan, the DNA scan was normalized for concentration and the temperature dependence of excess heat capacity curves, $\Delta C_p^{\mathrm{e}} = f(T)$, was obtained. The heat capacity difference, ΔC_p, was determined from the difference in *pre* and *post* transition baseline, at the midpoint of the transition; the calorimetric enthalpy, ΔH, was determined by integration of the area enclosed by the transition curve and the *pre* and *post* transition baseline; the melting temperature, T_{m}, was determined as the midpoint of the melting transition.

3. Results and discussion

A typical calorimetric transition curve of calf thymus DNA (Fig. 1) shows a complicated, but remarkably reproducible, pattern of individually distinguishable peaks. As shown by Klump et al. [8] (see also [3,4]), the superstructure of the calorimetric transition curve is far from accidental and reflects the relative population of the sequence composition of the cooperative units along the calf genome [8]. This sequence reveals a large variety of thermal stabilities and the superposition of the individual sequences results in the envelope of the complete melting curve. The presence of highly repetitive short and homogeneous sequences such as the so called *satellite* DNAs, which

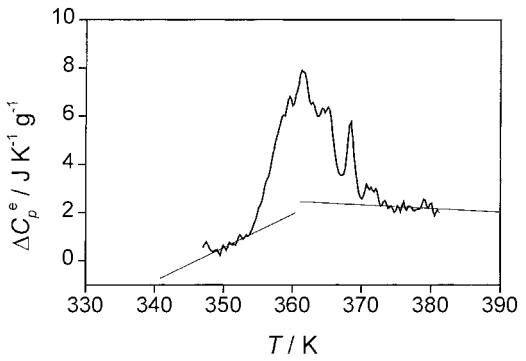

Fig. 1. Excess heat capacity as a function of temperature for calf thymus DNA. DNA 1 mg/ml, sodium citrate buffer, pH = 7.0, scanning rate = 0.2 K/min.

turn out to be related to the individual peaks sticking out from a broad transition in calf thymus DNA, is clearly demonstrated in [8] and also on Fig. 1. The temperature of the maximum of the peaks depends on the GC content of the given satellite fraction (GC-rich) and increases as the GC content increases [8].

The next important characteristic of the calorimetric transition curve of calf thymus DNA is the existence of a heat capacity change [3,4]. A positive change in heat capacity, $\Delta C_p = (195 \pm 20)\,\mathrm{J\,K^{-1}\,mol^{-1}}$, can be clearly seen on Fig. 1. Previously [7] we have shown that unwinding of ds-DNA requires the disruption of H bonds within the native double helix and its replacement by less specific H bonds to the solvent. The cooperative nature of double helix unwinding implies that most, if not all, H bonds are formed in the native state and depend on conditions such as the temperature. These H bonds include H-bonds forming Watson–Crick A–T and G–C base pairs, ordered H-bounded water lattices in the grooves (like a *water spine* covering the minor groove) [1,2] and clusters of ordered water near the phosphate groups of the helix. Increasing the temperature, the hydrogen-bonded lattice is melted and the number of ds-DNA–solvent H bonds decreases [9], giving an increasingly more endothermic contribution to the total enthalpy of transition and a positive value of ΔC_p to the ds-DNA→ ss-DNA transition. This interpretation is quite similar to A. Cooper's alternative model of protein folding-unfolding, based on the thermodynamics of phase transitions in hydrogen-bonded networks [10] and agrees with experimental data for the system DNA–water, obtained earlier, using adiabatic low-temperature calorimetry, in a wide temperature region, including the liquid helium temperature interval (2–373 K) [11,12]. According to these results, the native DNA duplex may be viewed as a cooperatively-ordered H bonded structure, including well localized H-bounded networks of hydration parts of the DNA–solvent interface [7,11], surrounded by an aqueous network of less well-ordered H-bonds, in which the degree of H bonding decreases with increasing temperature.

The effect of different metal ions on the denaturational heat capacity increment must reveal how strong is the effect of structural ordering/disordering of a given metal ion on the H-bounded network in the hydration shell of the DNA duplex. Figs. 2–4 show the temperature dependencies of the excess heat capacity for dilute solutions of calf thymus DNA in the presence of Li^+, Mg^{2+}, and Cu^{2+} ions with different concentrations.

It can be seen that in the presence of Li^+ and Mg^{2+} ions the superstructure of the calorimetric transition curves of DNA preserves all the characteristics (compare with Fig. 1): the individual peaks, sticking out from a broad transition in calf thymus DNA, are clearly seen on Figs. 2 and 3. Nucleic acid duplex melting is accompanied by a positive heat capacity change, ΔC_p, with average values of $(395 \pm 40)\,\mathrm{J\,K^{-1}\,mol^{-1}}$ for Li^+ and $(390 \pm 40)\,\mathrm{J\,K^{-1}\,mol^{-1}}$ for Mg^{2+}. The average values of melting enthalpy in solutions of Li^+ and Mg^{2+} are the same: $\Delta H = (34 \pm 4)\,\mathrm{J\,mol^{-1}}$.

It is well known that Li^+ is preferentially adsorbed in the minor groove of B-DNA [2] and this should contribute significantly to the stabilization of the B-form. This means that Li^+ ions stabilize the water spine in the minor groove of the duplex and disruption of this ordered water network contributes to the values of enthalpy [7] and denaturational heat capacity change.

High resolution X-ray analyses show that, except in direct binding of Mg^{2+} ions with phosphate groups, Mg^{2+} immobilization of structural water by the grooves of the duplex and formation of ion–nucleic acid inner-sphere complexes take place [13]. The second hydration shell incorporates five Mg^{2+} ions (three cations located at the major groove and two at the minor groove) bounded in an octahedral geometry to six water molecules [14]. So, Mg^{2+} ions stabilize

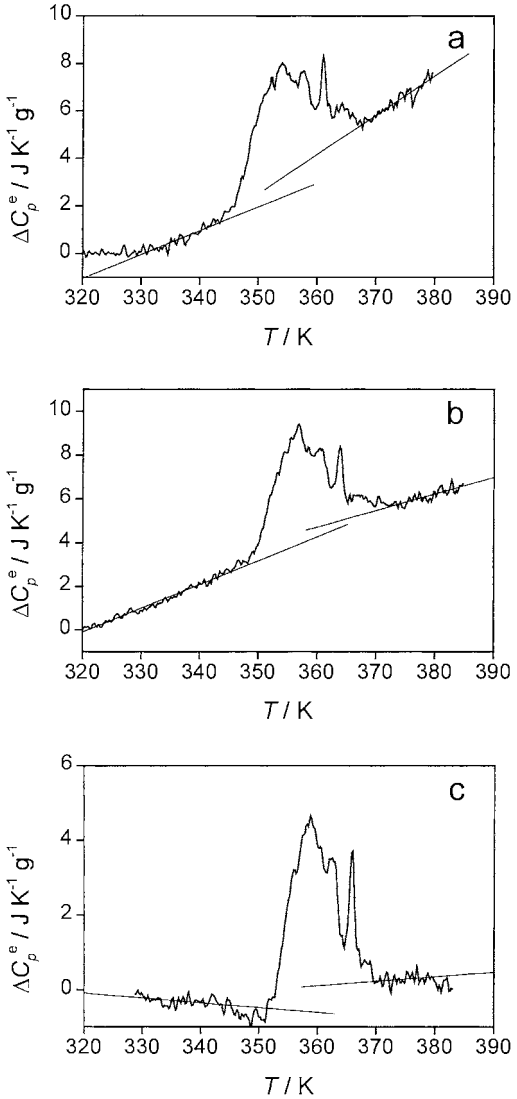

Fig. 2. Temperature dependence of the excess heat capacity for dilute solutions of calf thymus DNA in the presence of Li^+ ions with different concentrations: (a) 0.010, (b) 0.030 and (c) 0.050 M. DNA 1 mg/ml, sodium citrate buffer, pH = 7.0, scanning rate = 0.2 K/min.

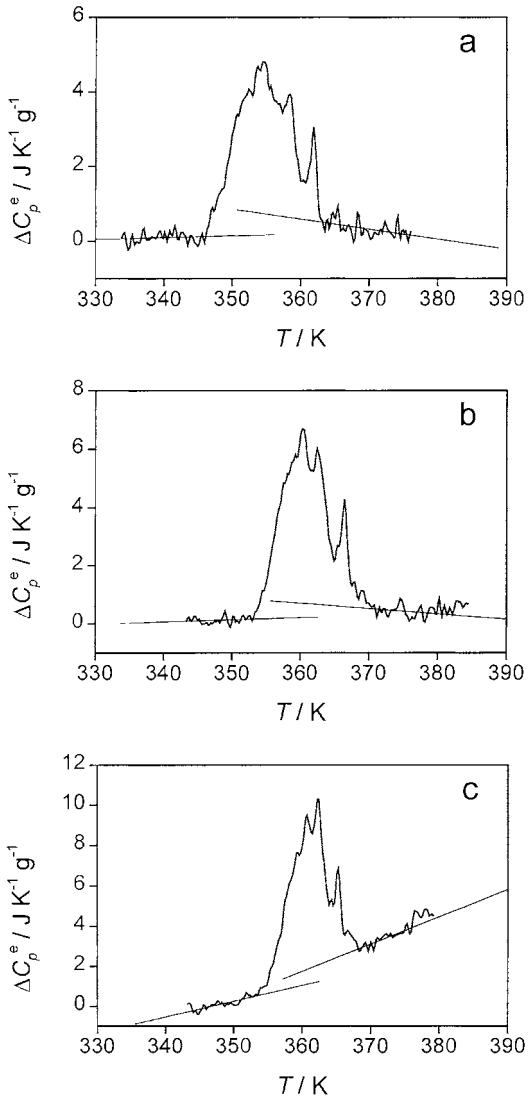

Fig. 3. Temperature dependence of the excess heat capacity for dilute solutions of calf thymus DNA in the presence of Mg^{2+} ions with different concentrations: (a) 0.010, (b) 0.020 and (c) 0.040 M. DNA 1 mg/ml, sodium citrate buffer, pH = 7.0, scanning rate = 0.2 K/min.

also the hydrogen bounded water network and, as a result, increase the denaturational heat capacity increment.

The effect of Cu^{2+} ions on the denaturational heat capacity increments is more remarkable: with increasing concentration of Cu^{2+}, the satellite peaks of calf thymus DNA become lower and for Cu^{2+} concentrations higher than 0.010 M they disappear. The average value of melting enthalpy in Cu^{2+} solutions is considerably lower than in Li^+ and Mg^{2+} solutions: $\Delta H = (28 \pm 4)\,\mathrm{J\,mol^{-1}}$. The values of ΔC_p decrease also with increasing concentration of Cu^{2+} and for

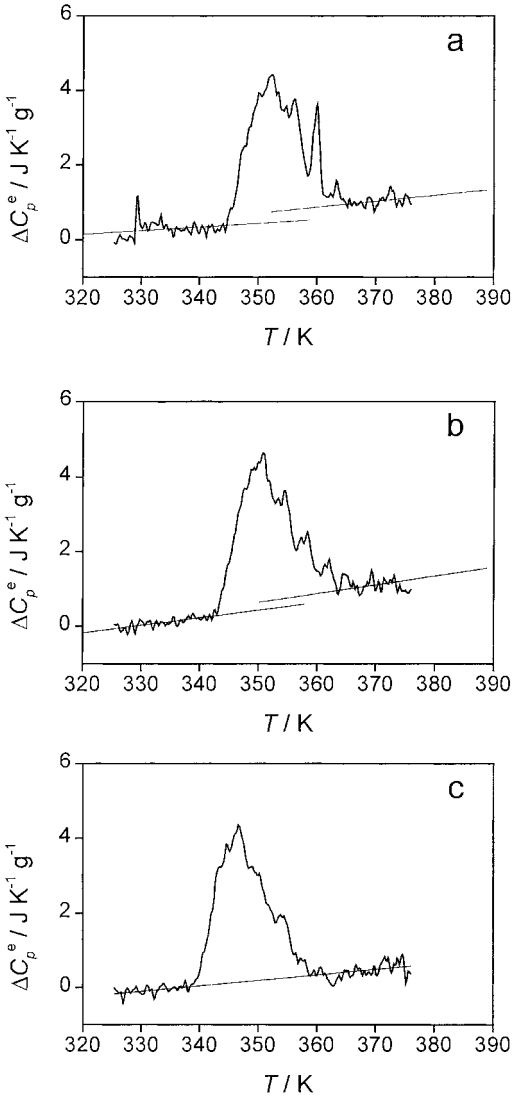

Fig. 4. Temperature dependence of the excess heat capacity for dilute solutions of calf thymus DNA in the presence of Cu^{2+} ions with different concentrations: (a) 0.004, (b) 0.008 and (c) 0.010 M. DNA 1 mg/ml, sodium citrate buffer, pH = 7.0, scanning rate = 0.2 K/min.

concentrations higher than 0.010 M, $\Delta C_p = 0$. This is indicative of the known preference of Cu^{2+} for purins and GC rich sites of DNA, binding to the N7 of guanine. As a result, disruption of the base stacking [1,2] and hydrogen bounded water networks in the grooves of the double helix takes place.

4. Conclusions

The native DNA duplex may be viewed as a cooperatively-ordered H bonded structure, including well localized Watson–Crick base pairs and H-bounded networks of hydration parts of the DNA–solvent interface and in the grooves of the helix. The denaturational heat capacity increment (ΔC_p) for calf thymus DNA in the absence of metal ions is $\Delta C_p = (195 \pm 20)\,\mathrm{J\,K^{-1}\,mol^{-1}}$. Experimental results suggest that Li^+ is preferentially adsorbed in the minor groove of B-DNA (which should contribute significantly to the stabilization of B-form) and has a more pronounced influence on the value of $\Delta C_p = (395 \pm 40)\,\mathrm{J\,K^{-1}\,mol^{-1}}$. For Mg^{2+}–DNA, $\Delta C_p = (390 \pm 40)\,\mathrm{J\,K^{-1}\,mol^{-1}}$, which is indicative of the formation of inner hydration sphere complexes, so immobilization of structural water by the grooves of the duplex, stabilizing the helix, takes place. The effect of Cu^{2+} ions is the most pronounced: the satellite peaks of calf thymus DNA get lower as the concentration of Cu^{2+} increases and for a concentration of 0.010 M Cu^{2+}, $\Delta C_p = 0$. This is indicative of the preference of Cu^{2+} for purins and GC rich sites of DNA binding to the N7 of guanine and, as a result, disruption of the base stacking and hydrogen bounded water networks, in the grooves of the double helix, occurs.

Acknowledgements

Thanks are due to *Fundação para a Ciência e Tecnologia* (FCT) for financial support given to Centro de Investigação em Química da Universidade do Porto–CIQ (UP). One of us (G.M.) thanks FCT for the award of an *Invited Scientist Grant* (Grant number PRAXIS XXI/BCC/20280/99).

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