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Stability of DNA in nucleosomes

(calorimetry/chromatin/histones/protein–DNA interactions)

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ABSTRACT **Heats of thermal denaturation of chromatin core particles and core particles with covalently crosslinked histones were measured by differential scanning calorimetry. The additional stabilization of the nucleoprotein complex by crosslinking is not reflected in the transition enthalpy. The contribution of protein denaturation to the total heat was estimated by comparison of core particles with core particle DNA in a high-salt solution. By taking into account the temperature dependence of the transition enthalpy of DNA, we conclude that the enthalpy change for denaturation of DNA in core particles is nearly the same as that for naked DNA in solution.**

The nature of the forces responsible for maintaining DNA in a condensed state in the cell nucleus is not yet understood. It is established that the first order of DNA compaction is achieved by the wrapping of from 145 to about 200 base pair length of DNA around histone cores containing two molecules each of the four smaller histones (1–3). These structures, called nucleosomes, are the fundamental repeating units of chromatin (1–3). As a first step toward understanding the DNA compaction process, it is of interest to know the stability of nucleosomal DNA relative to that of naked DNA, as well as the enthalpic and entropic contributions to the free energy of stabilization. In this paper we report the results of a thermodynamic study of the stability of nucleosome core particles, employing differential scanning calorimetry.

MATERIALS AND METHODS

Preparation of Core Particles. Chicken erythrocyte nuclei were prepared as described by Hymer and Kuff (4). The nuclei were swollen in 0.25 mM EDTA and the very lysine rich histones (H1, H5) were removed by repeated suspension of the chromatin in 0.7 M NaCl/0.01 M Tris·HCl, pH 8.0/1 mM EDTA (5). The chromatin was subsequently digested with micrococcal nuclease (Worthington) and the digest was fractionated by sucrose gradient centrifugation as described (5). Core particles thus purified contain the four smaller histones in equal amounts, approximately 145 base pairs of DNA, and no H1 or H5 histones.

Preparation of Crosslinked Core Particles. Purified core particles were crosslinked with dimethylsuberimidate (Pierce) in 0.01 M sodium borate, pH 10.0, for 40 min at 25°C (6). The crosslinked particles were further purified by fractionation on sucrose gradients. The histones from these particles appear on electrophoresis entirely as octamers (6).

Preparation of DNA. The 145-base-pair DNA was extracted from core particles with 3 M NaCl/0.05 M sodium phosphate buffer, pH 7.0, and purified with hydroxylapatite in a batch procedure (6).

Calorimetric Measurements. Calorimetric measurements were made in a scanning microcalorimeter (7) purchased from Mashpriborintorg, Moscow, USSR.

RESULTS AND DISCUSSION

The total enthalpy change upon thermal denaturation, ΔH_d, of the core particle nucleoprotein complex can be measured with good precision by means of differential scanning calorimetry. If, in addition, ΔH_d for the DNA and histone components is measured under appropriate conditions, information can be obtained on the enthalpic contribution to the histone–DNA interactions that occur in chromatin.

Fig. 1 shows the excess apparent heat capacity as a function of temperature for core particles. The curve can be approximately resolved into two two-state transitions as outlined in the legend for the figure, although there is a small shoulder on the high-temperature side of the main transition. The heats of the transitions are reported in Table 1. These data are in close agreement with those reported by Weischet *et al.* (10). These authors showed that these transitions roughly parallel those observed optically, and suggested that the low-temperature transition corresponds primarily to the melting of about 40 base pairs of DNA from the ends of the core particles. Also, Simpson (11) has shown that core particles denature by the extension of DNA from the histone core with subsequent fraying of the ends. The main transition appears to be a cooperative breakdown of the nucleoprotein complex (10).

At the low ionic strength at which thermal denaturation studies are generally performed (to avoid precipitation of the nucleoprotein), core particles exist in an expanded, partially unfolded conformation (12, 13). This reversible expansion at very low ionic strength is probably due to electrostatic repulsion between DNA phosphates (13). In contrast to native core particles, core particles with covalently crosslinked histone cores remain compact at very low ionic strength (13). We have shown previously (6) that core particles with crosslinked histone cores exhibit a low-temperature optical transition similar to that of native core particles but that the main transition is shifted to a higher temperature. This is consistent with fraying of DNA from the ends of the particle and a strong coupling of the stability of the central region of the DNA to the stability of the histone octamer, as postulated for native core particles (10, 11). Therefore, it is of interest to compare the enthalpy change for denaturation of fully folded crosslinked particles with that found for partially unfolded native particles.

Fig. 2 shows the temperature dependence of the excess heat capacity for crosslinked core particles. The denaturation profile parallels that obtained optically (6), as in the case of native particles. Here, the curve is most simply resolved into three transitions, the enthalpies of which are reported in Table 1.

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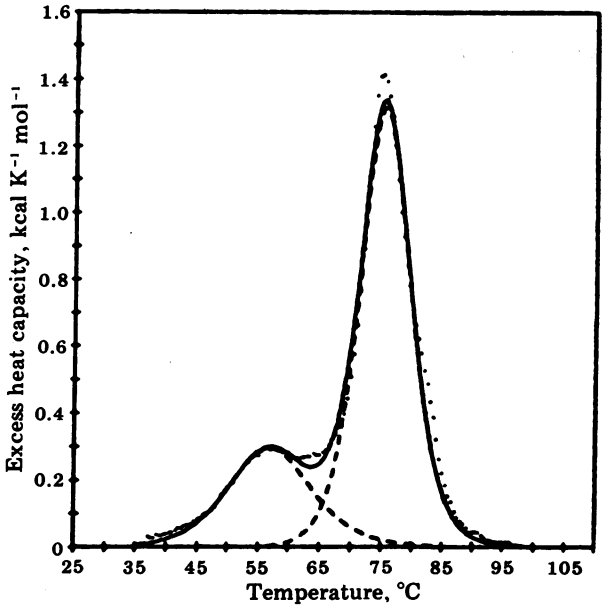

FIG. 1. The temperature dependence of the apparent excess heat capacity of nucleosome core particles in 0.25 mM EDTA, pH 7.0 (·····). The data were digitized by means of a Tektronix display-tape-tablet system and read into a PDP 10 computer (program written by Jane Small). The data were analyzed by using the MLAB interactive system designed by Gary Knott (5, 8, 9). The experimental curve (·····) was decomposed into two transitions (- - -), assuming each to be a two-state process. The height, the width, and the temperature of maximal heat capacity of each transition were used as adjustable parameters. The solid curve (——) denotes the sum of the two transitions.

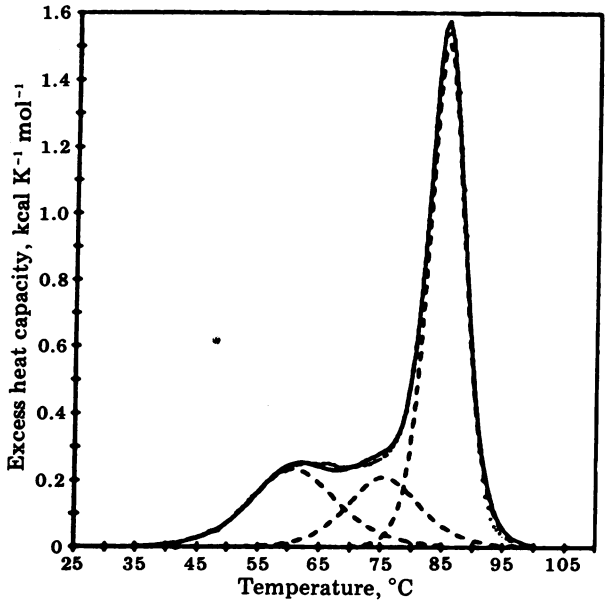

FIG. 2. The temperature dependence of the excess heat capacity of crosslinked nucleosome core particles in 0.25 mM EDTA, pH 7.0. The experimental curve (·····) was decomposed into three transitions (- - -) by assuming a two-state approximation for each transition. The solid curve (——) denotes the sum of the three transitions. The data were digitized and analyzed as for Fig. 1.

Significantly, the total heat is the same, within experimental error, as for native particles. This suggests that the enthalpy change for the low ionic strength unfolding transition is small, a conclusion consistent with the measurements of others (13, 14) using noncalorimetric techniques.

We next measured the protein contribution to ΔH_d. In 2 M NaCl/0.25 mM EDTA, pH 7, core histones associate to form octamers (15–19). Moreover, high salt concentrations may provide screening for the highly charged histones similar to that provided by DNA in chromatin. There is experimental evidence that the conformations of core histones in 2 M NaCl are similar to those that exist in chromatin (20). Also, at this salt concentration histones are completely dissociated from DNA (21). Consequently, we have performed differential scanning calorimetry on core particles in 2 M NaCl (Fig. 3) and also on the DNA extracted from the core particles (Fig. 4). In 2 M NaCl, the t_m of the DNA is 93°C, and no change in excess heat capacity was observed at temperatures lower than 80°C (Fig. 4). With core particles, a complex but reproducible series of transitions occurred over the whole temperature range (Fig. 3). The total heat for core particle denaturation minus that measured for DNA should be a reasonable approximation for the contribution of the protein component. This value, 4.83 cal g^{-1} (526 kcal mol^{-1}) is less than that found for the unfolding of an average globular protein (≈8 cal g^{-1}) (22). However, this value is probably a lower limit because: (*i*) histones, as do many other proteins, aggregate upon thermal denaturation under these conditions, and protein aggregation generally releases heat (22); (*ii*) ΔH_d for proteins generally increases with temperature (22), and histone denaturation in core particles at low ionic strength appears to occur predominantly at higher temperatures (10).

Weischet *et al.* (10) have compared the ΔH_d values found for core particles and purified core particle DNA, each measured in 1 mM cacodylate buffer, pH 7.2. They found that the enthalpy change for core particle denaturation is substantially greater than that for purified DNA, but cautioned that part of this difference might be due to a contribution from the protein component. However, they also suggested that this large difference in transition enthalpies between DNA and core particles was consistent with a negligible contribution from the heat of histone denaturation and a maximum coverage of DNA by histones, as concluded from the studies of DNA–polylysine interactions by Klump (23). This therefore implies that there may be a large enthalpic contribution to the interaction of DNA with the histone core. In contrast with this study, we provide evidence here that the enthalpic contribution to the stabilization of DNA by histones in core particles is zero within experimental uncertainty.

Table 1. Transition properties of chromatin core particles and derivatives

	ΔH_T, kcal mol^{-1}	$t_{1/2\,1}$, °C	ΔH_1, kcal mol^{-1}	$t_{1/2\,2}$, °C	ΔH_2, kcal mol^{-1}	$t_{1/2\,3}$, °C	ΔH_3, kcal mol^{-1}
Core particle (0.25 mM EDTA)	1831 ± 50	57.5	465 ± 25	75.5	1366 ± 60	—	—
Crosslinked core particle	1827 ± 50	61.0	407 ± 20	75.6	310 ± 25	85.4	1110 ± 55
Histones (2 M NaCl)	526 ± 20						
DNA (0.25 mM EDTA)	889 ± 25	51.5					
DNA (1 M NaCl)	1653 ± 46	93.0					

Subscripts T, 1, 2, and 3 refer to the overall and component transitions shown in the figures. ± indicates SD. One kcal = 4.184 kJ.

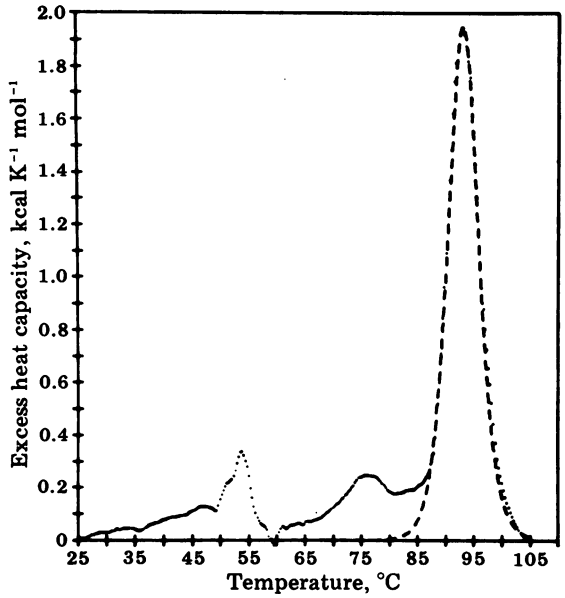

FIG. 3. Temperature dependence of the excess heat capacity of core particle (·····) and purified 145-base-pair-length DNA (- - -) in 2 M NaCl/0.25 mM EDTA, pH 7.0.

In order to obtain a meaningful comparison between uncomplexed DNA and core particles, it is necessary to measure the various transition enthalpies under appropriate conditions. On the basis of a compilation of calorimetric data from the literature on the enthalpy change accompanying base pair formation for DNA and double-stranded polynucleotides, Bloomfield *et al.* (24) concluded that the heat of melting increases with temperature and that it is not a function of salt concentration. This was confirmed by Breslauer *et al.* (25, 26) in a detailed study of the helix–coil transition of $rA_7 \cdot U_7$ upon comparison with results reported for poly(A)·poly(U). This same conclusion was also reached by Suurkuusk *et al.* (27) for the poly(A)·poly(U) $\rightleftarrows$ poly(A) + poly(U) transition on the basis of direct heat capacity measurements of the single- and double-stranded polynucleotides, as well as by scanning calorimetry. The temperature dependence of ΔH_d in the case of these synthetic polynucleotides corresponds to a heat capacity change, ΔC_p, of approximately 100 cal K^{-1} (mol of base pairs)$^{-1}$. Additionally, Shiao and Sturtevant (28) have suggested that the denaturation of DNA is accompanied by an increase in the apparent heat capacity and have found that the value of ΔC_p is similar to that observed with synthetic polynucleotides. Consequently, the transition enthalpy of core particles should be compared with that for DNA at the same temperature, rather than at the same ionic strength, where the t_ms differ substantially.

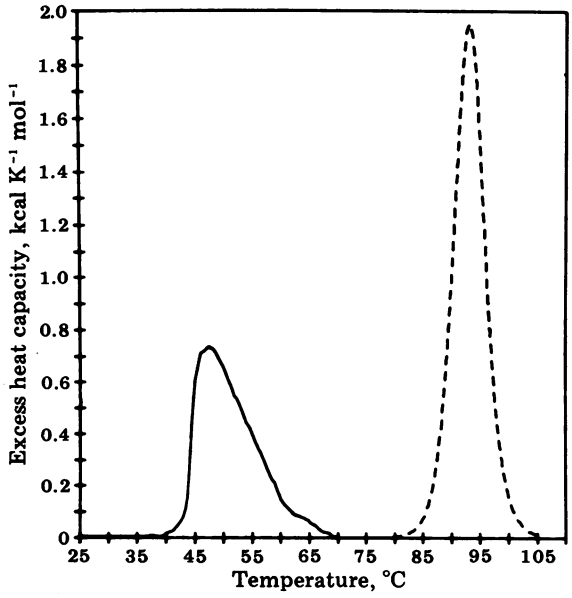

FIG. 4. Temperature dependence of the excess heat capacity of 145-base-pair-length DNA in 0.25 mM EDTA (—) and 2 M NaCl/0.25 mM EDTA, pH 7.0 (- - -).

We have therefore measured ΔH_d for purified core particle DNA in both 0.25 mM EDTA, pH 7.0 (Fig. 4), and in 1.0 M NaCl/0.25 mM EDTA, pH 7.0. The latter transition curve was similar to the one shown in Fig. 4 for 2 M NaCl. Interpretation of the low-salt transition curve is complicated by its pronounced asymmetry. In accordance with the procedure that is appropriate for a two-state process, we have arbitrarily assigned the observed enthalpy to the temperature at which half the enthalpy has been absorbed. On this basis, the two transitions give 6.1 kcal (mol of base pairs)$^{-1}$ at 51.5°C and 11.4 kcal mol^{-1} at 93°C. Clearly, ΔH_d for core particle DNA is quite temperature dependent with ΔC_p = 128 cal K^{-1} (mol of base pairs)$^{-1}$. Linear interpolation gives values of 6.9 and 9.2 kcal (mol of base pairs)$^{-1}$ at 57.5 and 75.5°C, respectively, the t_ms of the core particle transitions in 0.25 mM EDTA, pH 7.0. Because approximately 40 base pairs melt at 57.5°C and 105 at 75.5°C (10, 11), we estimate that the enthalpy change as it occurs in core particles is 40 × 6.9 + 105 × 9.2 = 1242 kcal mol^{-1}.

Using this value for the DNA component of core particles and our value of 526 kcal mol^{-1} for the histone component gives 1768 kcal mol^{-1} for the total transition enthalpy expected for core particles if there were no heat of interaction between DNA and protein. The value measured for core particles was 1831 ± 50 kcal mol^{-1} and the value for fully compact crosslinked core particles was 1827 ± 50 kcal mol^{-1}. The difference between the mean of these values and the value calculated above, −61 kcal mol^{-1}, is of questionable statistical significance. Moreover, as discussed above, the value of 526 kcal mol^{-1} for the histone component should probably be regarded as a lower limit, and only a 12% increase in this value is required to account for the total measured transition enthalpy for core particles. We conclude that the transition enthalpy of DNA folded around the histone core is *nearly the same* as that of DNA in solution.

This conclusion is based upon total heats in order to avoid assumptions about what fraction of the protein heat appears in the first transition. However, we can now estimate this fraction, using the data in Table 1. For 40 base pairs of DNA melting in the first transition, the enthalpy change is 11.6 kcal (mol base pairs)$^{-1}$, compared with the value 6.9 kcal (mol base pairs)$^{-1}$ for purified DNA at the same temperature. This suggests that about 36% of the heat of protein denaturation occurs during this transition.

By using the data in Table 1 and assuming that ΔC_p is not a function of temperature and taking $\Delta G^\circ = 0$ at the temperature of half completion of the enthalpy absorption, we calculate that in 0.25 mM EDTA, pH 7.0, ΔG° (25°C) is 117 ± 10 kcal mol^{-1} for the DNA component of core particles compared to 51 ± 3 kcal mol^{-1} for naked core particle DNA. Therefore, strand separation of nucleosomal DNA requires about 66 kcal mol^{-1} additional free energy compared to naked DNA. We found essentially no increase in enthalpy for this process. It thus seems possible that the additional stability of nucleosomal DNA is primarily of entropic origin.

Entropic stabilization of the DNA in chromatin core particles

is consistent with histone–DNA interactions being primarily electrostatic in nature (29). In the thermodynamic analysis of protein–nucleic acid interactions developed by Record *et al.* (30), entropic stabilization arises from the release of counterions into solution from both the DNA and protein components. Because DNA in the core particle is folded around the histone core, this reaction may be more complex than reactions in which proteins interact without substantial alterations in DNA structure. Conceivably, any heat required to bend the DNA may be compensated by heat released upon interaction. However, a number of recent findings give a consistent picture for the DNA folding process: (*i*) DNA can collapse around a crosslinked histone octamer, for which histone–histone interactions cannot provide the energy for DNA folding (6); (*ii*) recent calculations by Manning (29) and Riemer and Bloomfield (31) indicate that the stiffness of DNA may arise primarily from electrostatic repulsion between DNA phosphates, and that DNA should spontaneously fold upon interaction with molecules possessing sufficiently high positive charge density; (*iii*) protein–nucleic acid interactions can be driven entropically by counterion release (30); and (*iv*) the similar transition enthalpies for nucleosomal and naked DNA reported here suggest that the additional stabilization of DNA in nucleosomes could be primarily entropic. These findings taken together indicate that electrostatic interactions may play a primary role in bringing about the folding of DNA in chromatin.

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