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THERMAL TRANSITION OF CORE PARTICLE IS NOT A TWO-STATE PROCESS

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Thermal transition of core particle which occurs before melting of DNA and can be followed by circular dichroism is not a two-state process; it is the result of two processes which cannot be dissociated in static experiments: unfolding of core particles is immediately followed by their aggregation. It is thus impossible to get thermodynamic parameters of core particle unfolding from its thermal transition monitored by circular dichroism. Thermal denaturation kinetics of core particles gives some information about their stability. Finally core particle structure is more stable in chromatin than in its isolated state.

1. Introduction

In the core particle, the energy of interaction between the histone octamer and the 145 base pair DNA can be decomposed into an electrostatic energy and a so-called "folding" energy [1]. Both aspects are due to the nature of histones: these polycations contain a central hydrophobic region, organized in α helix, which allows histone–histone interactions [2] and the creation of a spherical surface of ionic interaction with the DNA. During thermal denaturation, the hydrophobic interactions are partially destroyed, but the electrostatic ones remain intact: one must thus obtain unfolded core particles where histones are still attached to DNA by electrostatic forces, but with a different shape of interacting area. In line with this, one should be able to describe core particles thermal transition, which takes place before DNA melting and can be followed by circular dichroism, as a simple two-state process from native core particles to unfolded ones. The activation energy of the unfolding process could therefore be determined from kinetic measurements at different temperatures. Our results show that it is not the case. Core particle transition is the result of two events which are almost concomitant in our experimental conditions: the unfolding of core particles is immediately followed by their aggregation. This double process which has been studied at different temperatures in function of time gives some information about core particles stability.

2. Material and methods

2.1. Preparation and characterization

Long chain chromatin was extracted from chicken erythrocyte nuclei after a short Micrococcal nuclease digestion [3] and core particles were prepared from the same material according to Lutter's method [4] except for the last step: a 5–30% sucrose gradient in a zonal rotor was always used instead of chromatography in a sepharose 4B column that destabilizes core particle as it will be shown elsewhere (manuscript in preparation). Both preparation and characterization have been described in detail for chicken erythrocyte chromatin and core particles [5].

The core particles obtained are totally deprived of H1 and H5 and the length of their DNA is 145 ± 3 base pairs [5].

2.2. Physical measurements

2.2.1. Circular dichroism

CD melting measurements were made with a Mark III Jobin Yvon Dichrograph in a jacketed quartz cuvette with 1 cm optical pathlength. Temperature was recorded with a platinum resistance connected to a digital thermometer the signal of which was applied to the X-way of a Sefram TGM 164 X—Y recorder which is also equipped for kinetic measurements. The temperature control was ensured by a digital temperature programmator which will be described elsewhere [6]. In our experiments, temperature was linearly increased at a rate of 0.25°/mn.

All the solutions contained 10 mM Tris pH 7.4 and 0.2 mM EDTA and had an optical density of 1 at 258 nm. We used a molar extinction coefficient of 6600 at this wavelength.

2.2.2. Electron microscopy

After being kept at a given temperature for a certain time, the core particle solutions were either quickly chilled or slowly cooled, then diluted to 0.5 μg/ml with a buffer containing 5 mM NaCl, 5 mM Triethanolamine, pH 7.4, and 0.2 mM EDTA. It was checked that no fixation was required. Spreading on positively charged carbon coated grids according to Dubochet's method [7], staining and shadowing of the specimen were performed as previously described [8]. The grids were examined in a Siemens 101 Elmiskop.

Sampling was different with chromatin: 2 μl of chromatin solution were taken from the cuvette at various temperatures during melting and transferred into 200 μl cold buffer containing 0.1% glutaraldehyde, 5 mM NaCl, 5 mM Triethanolamine and 0.2 mM EDTA; fixation in this medium lasted 1 hour at 0°C.

3. Results and discussion

3.1. Thermal transition of core particles

During melting of a histone—DNA complex, denaturation of the DNA and denaturation of the histones could be followed separately by recording the circular dichroism in function of temperature at two appropriate wavelengths, 280 and 227 respectively [9, 10]. For these two wavelengths, the two curves obtained with core particles (fig. 1) show quite parallel and simultaneous transitions between 20° and 78° which have their inflexion points at the same temperature (75°C) and occur before melting of the DNA. It is a priori easy to correlate both transitions. Most of the α helix regions of histones are denatured, as indicated by the increase of the negative molar ellipticity at 227 nm. After this disruption of hydrophobic interaction between histones, DNA-histones contact area loses its globular shape and the specific constraint of DNA is released, as indicated by the increase of the molar ellipticity at 280 nm.

It is important to notice that the initial θ_{280nm} value is very low (850°-cm^2-dmole^{-1}), which shows that the precise folding of DNA around the histone kernel has been well preserved during the preparation of core particles. This is confirmed by the monophasic aspect of the melting transitions followed both by absorbance (result not shown) and by circular dichroism at 280 nm (fig. 1). The discrepancy with previous results [11] could arise from the last step of the core particle preparation as it has been mentioned in section 2.

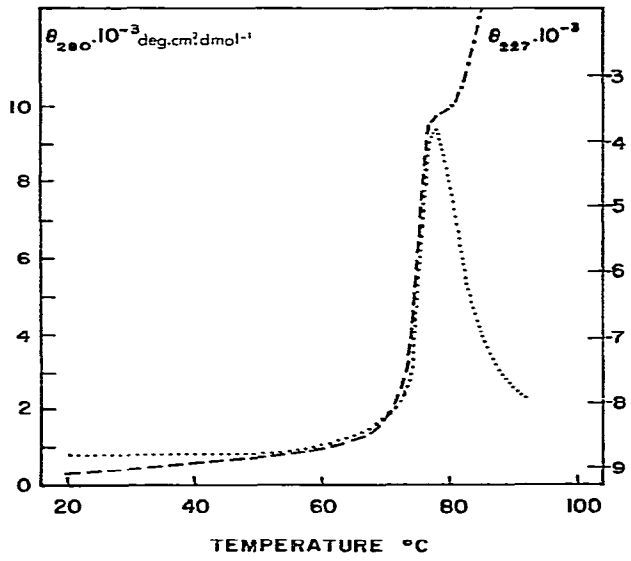

Fig. 1. Thermal denaturation of core particles followed by circular dichroism at 280 nm (...) and at 227 nm (———). The end of the curve at 227 nm (—·—) is uncertain because of turbidity

Since electrostatic interactions between histones and DNA are maintained, one should be able to describe thermal transition of core particles between 20° and 78° as a simple two-state process from a native core particle to an unfolded one. Yet a careful analysis of the state obtained at 78° is necessary. Assuming histones behave only as counterions and offers to DNA an equivalent ionic strength of 0.1 M NaCl, core particles have to melt around 82° [12]. This is indeed the case as seen either from the absorbance melting curve (not shown here) or from the θ_{280} melting curve (fig. 1). In consequence, CD spectrum of core particles at 78°, from 320 nm to 260 nm, should be similar to that of DNA alone at 78°, in 0.1 M NaCl and in the same wavelength range, but this is not at all the case (fig. 2). This CD spectrum rather looks like that of DNA $\bar{A}$ form which appears in presence of both polyamines and ethanol [13]. Furthermore, this peculiar secondary structure obtained at 78° is stable since its CD spectrum does not change either after a quick chilling or after a slow cooling. It means that denatured histones besides their role of counterions are also able to create another type of hydrophobic environment; now denatured histones are likely to be aggregated [14]. This is largely confirmed by electron microscopy: fig. 3d shows the aspect of core particles gradually heated to 78° and then quickly chilled. It is reasonable to think that it well represents the state at 78°, since, according to CD, the cause of the DNA transition — i.e. histone aggregation— already existed at 78°. Similar types of aggregates have been previously described by Olins [15] when guanidine-

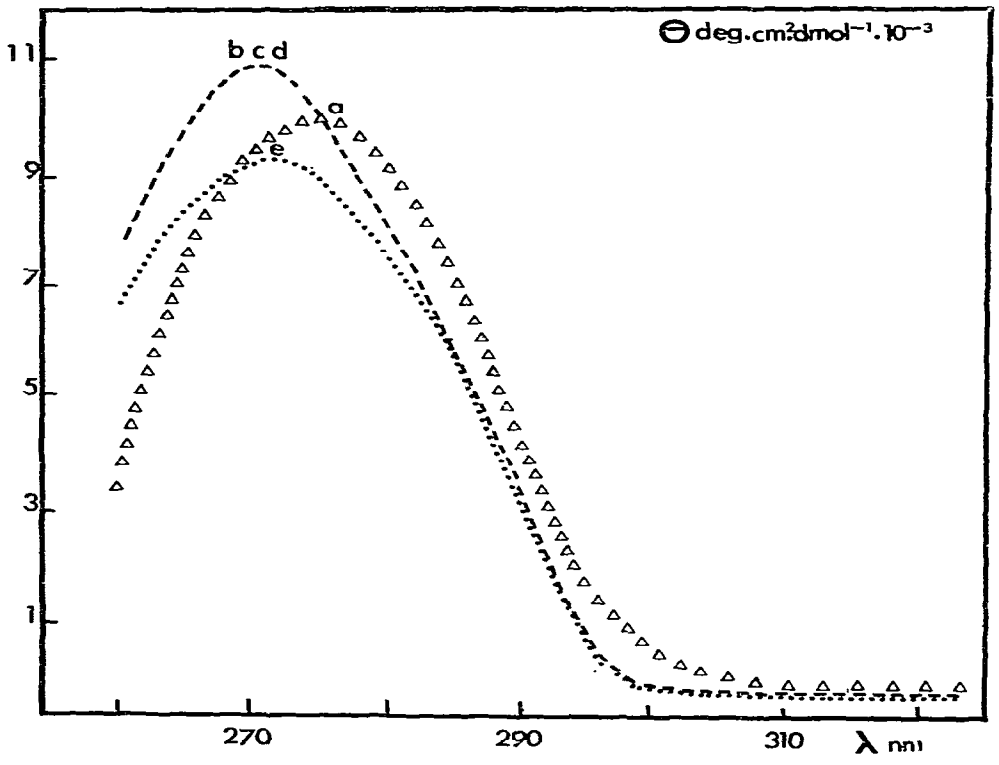

Fig. 2. CD spectra of DNA in 0.1 M NaCl at 78° (△ a), of core particles heated to 78° (—— b), of core particles heated to 74°5 and maintained 30 mn at this temperature (—— c), of core particles heated to 75°7 and maintained 15 mn at this temperature (—— d) and of core particles maintained at 73° for several hours (... e).

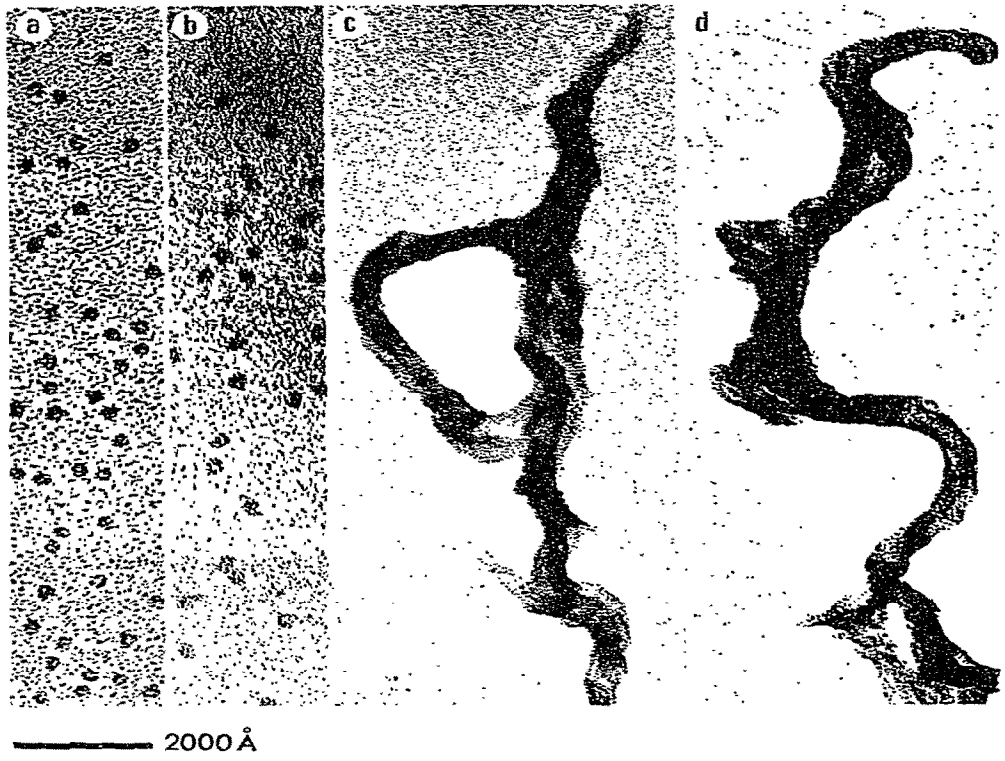

Fig. 3. Electron microscopy of native core particles (a), core particles maintained at 66° 8 for several hours and slowly cooled (b), core particles maintained at 73° for several hours and slowly cooled (c) and core particles heated to 78° and quickly chilled (d).

denatured H4 is reassociated with T_7 DNA.

In consequence the thermal transition of core particles which occurs before DNA melting is not a two-state process but a complex process, the kinetics of which is governed by the denaturation and the aggregation of histones.

3.2. Thermal denaturation kinetics

We followed thermal denaturation kinetics by circular dichroism at 280 nm at different temperatures. The solutions of core particles were heated at a rate of 0.25°/mn up to a preselected temperature and then maintained at this temperature for several hours (fig. 4). Three domains can be distinguished experimentally:

(1) Below a certain temperature the core particles remain globular and do not aggregate. If the core particles are heated to 57.5° or 66.8° there is only a slight modification of θ_{280} and no further significant variation when the temperature is kept constant for several hours (fig. 4). It is to be correlated with a previous observation on mononucleosomes [16] the shape of which is not seriously altered till 70°. However the precise folding of DNA around the histone octamer has been irreversibly perturbed since the original θ_{280} is not recovered after a slow cooling to room temperature.

(2) If core particles are heated to 73°, they reach, if maintained one hour at this temperature, a very stable equilibrium state (fig. 4) without any further reorganization of DNA after a slow cooling to ambient temperature, since its CD spectrum remains identical (fig. 2). Electron microscopy (figs. 3c and 5) shows that this equilibrium state is actually a mixture of an aggregated form – as obtained at 78° – and apparently intact globular core particles. This explains respectively the blue shift of λ_{max} to 270 nm and the lower value of θ_{max} than that obtained after heating to 78°

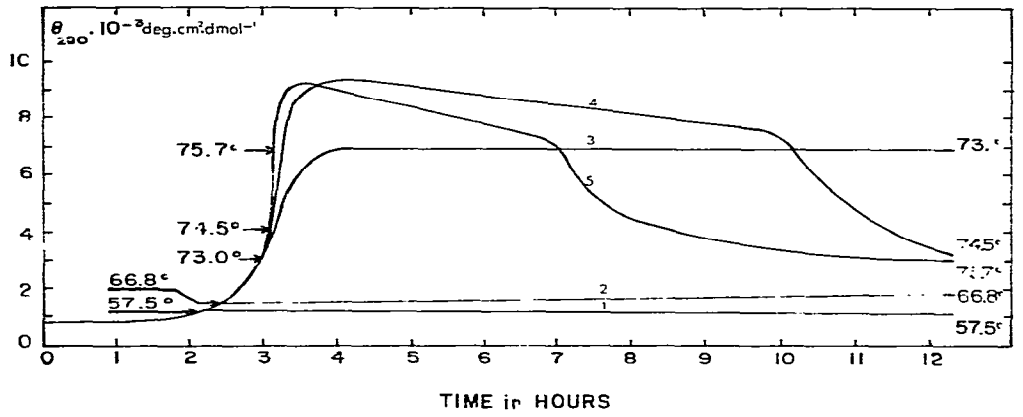

Fig. 4. Core particle thermal transition kinetics followed by circular dichroism at 280 nm, when temperature is linearly increased (0.25°/mn) and then maintained at 57°5 (1), 66°8 (2), 73° (3), 74°5 (4) and 75°7 (5).

(9.200° instead of 10.900 degrees cm^2 $decimole^{-1}$ (fig. 2). Presence of globular core particles is confirmed by the lower α helix denaturation after several hours at 73° than after a continuous heating up to 78° (fig. 6). It means that core particles have not the same activation energy: when maintained at 73° some

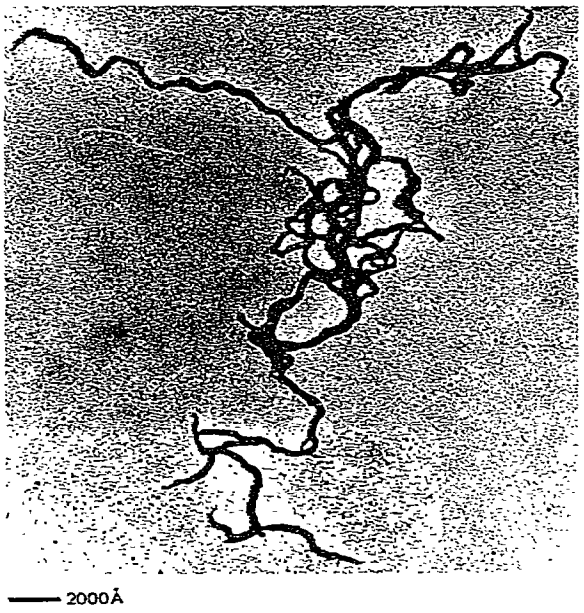

Fig. 5. Large field electron micrograph of core particles maintained several hours at 73° and slowly cooled. No fixation is employed (see section 2).

are able to unfold and to aggregate while the others remain globular. This heterogeneous behaviour has to be compared with the differential answer of core particles to ethidium bromide as previously studied [5]. It could hint that some core particles are rendered particularly stable by a very precise interaction between the histone core and a few terminal base pairs which might have been cleaved by the nuclease in the other core particles [5].

(3) If core particles are heated to 74°5 or 75°7, DNA as well as histones rapidly reach a state similar to that following the continuous heating to 78°, as judged by a comparison between figs. 1 and 6 (θ_{227}) and figs. 1 and 4 (θ_{280}). If the system is maintained few hours at this temperature, α helix denaturation slowly increases (fig. 6), followed by a non cooperative melting of DNA (fig. 4). In other words, it is possible to melt DNA at a temperature lower than 82°C, without changing the ionic medium or the histone content but likely the type of electrostatic interaction between histones and DNA.

3.3. Histone core stability

We have compared histone core stability in the isolated core particle with that in chromatin (fig. 7). They are different, since in chromatin at 75° (fig. 7c) there are still histone octamers while most of the isolated core particles have begun to unfold and aggregate at this temperature (as seen in table 1) by θ_{270} and

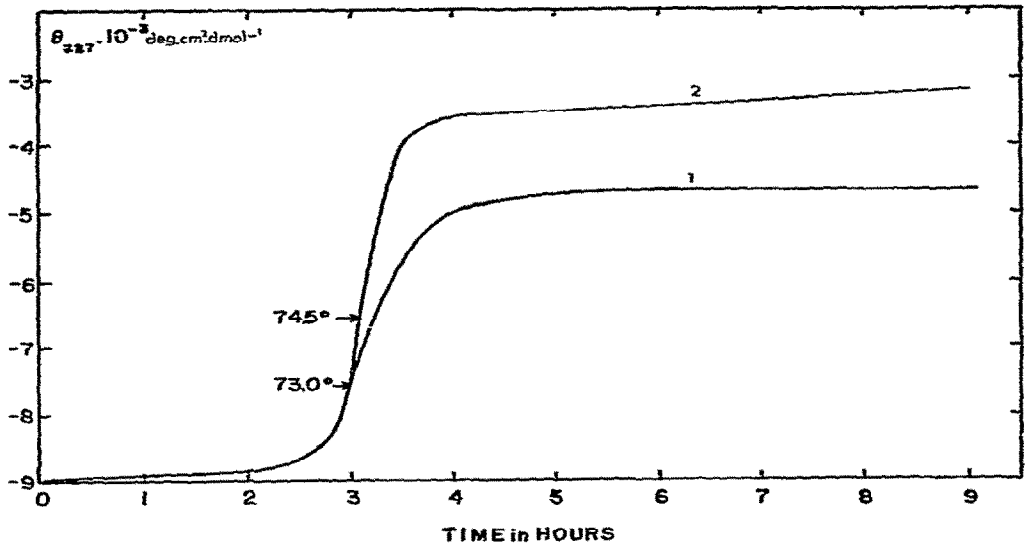

Fig. 6. Core particle thermal transition kinetics followed by circular dichroism at 227 nm when temperature is linearly increased (0.25°/mn) and maintained at 73° (1) and 74°5 (2).

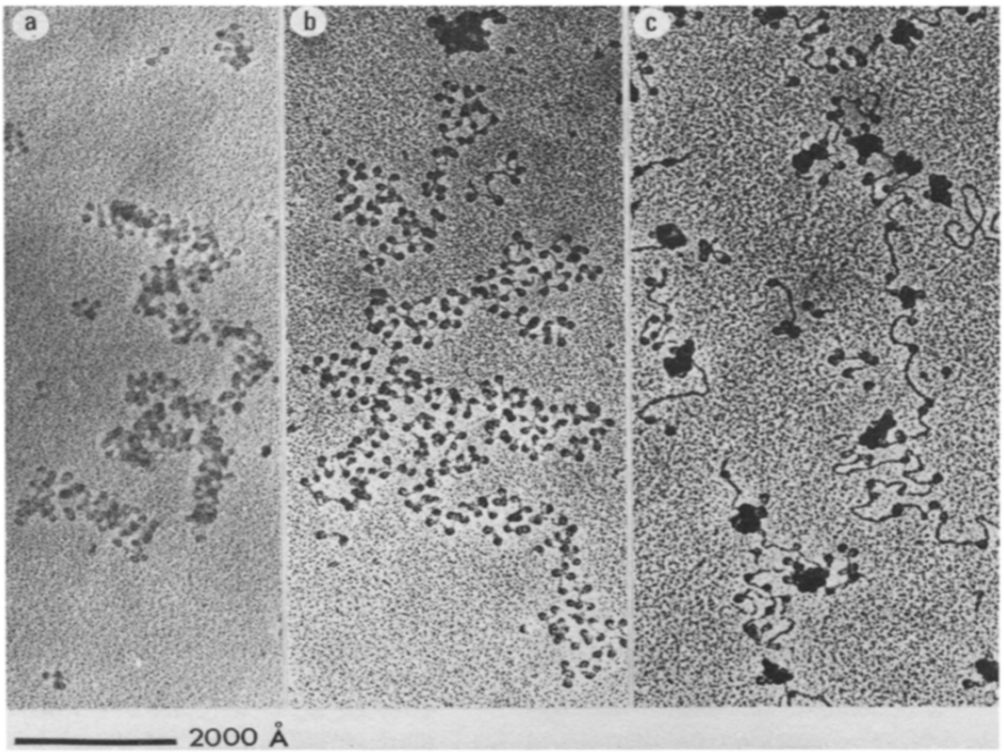

Fig. 7. Electron microscopy of native chromatin (a), chromatin heated to 60° (b) and chromatin heated to 75° (c). Glutaraldehyde fixation has been used (see section 2).

Table 1
Some molar ellipticity values of core particles for different temperatures and times where the blue-shift of λ_{max} has occurred

	t^0, t_{mn}				
	74°5	74°5	75°7	75°7	78°
λ_{nm}	0 mn	30 mn	0 mn	15 mn	0 mn
270 nm	5700	10900	8600	10900	10900
280 nm	4000	8200	6400	8200	8200

θ_{280} values obtained for $t = 0$ mn at 74°5 and 75°7). Furthermore the temperature of the inflexion point of chromatin thermal transition followed by CD at 227 nm (result not shown) is greater than that of isolated core particles, i.e. 75°. Thus core particle structure is more stable in chromatin than in its isolated state.

Finally one has to point out the peculiar aspect under electron microscope of chromatin heated at 75.7°C (fig. 7c), in which clusters of denatured histones together with large pieces of apparently naked DNA can be clearly seen. It is to be noticed that the structural effect of temperature increase on chromatin is comparable with that of ethidium bromide at the same ionic strength [5]: it first generates an unwinding of the linker DNA and then a sliding of core histones into clusters.

4. Conclusion

These different heating experiments were made in an attempt to determine the thermodynamic parameters describing the thermal stability of core particles and to follow their thermal unfolding kinetics.

Actually such an analysis is only possible if a simple two-state process is taking place, but this is obviously not the case.

In order to summarize our experimental data we can distinguish two types of behaviour during the heating of core particles in 10 mM buffer.

(1) Up to around 67°C, structural and morphological changes are negligible. As it was stated above, a small increase of θ_{280} could be detected but the original value is not recovered after cooling and thus an equilibrium between two conformational states of the core particle cannot be considered. On the other hand, changes are very fast and cannot deserve a type of slow kinetic analysis.

(2) When the core particle is heated to 73°C a completely different picture is observed since large conformational and morphological perturbations are occurring in this small range of temperature between 67°C and 73°C (and more likely in a smaller range). A kind of threshold is thus appearing which seems to be related to some precise but so far unknown conformational changes of the histone molecules.

However this sudden disruption of the core particle, i.e. unfolding of DNA can never lead to a single type of denatured particle as observed for example in the case of ethidium bromide binding [5]. As soon as a given part of the helical structure of histones is destroyed between 67°C and 73°C, two processes are occuring simultaneously: (a) a release of the constraint which was exerted upon DNA by the histone assembly, (b) an aggregation of histone molecules leading to very large fiber structures in which DNA is no more organized in the B-form. In the type of experiments which were performed, it is impossible to find experimental conditions for which a decoupling between the two processes would be observed. Any thermodynamic interpretation is therefore meaningless. It is also the case with kinetic measurements on a scale of seconds (unpublished results). Only fast kinetic studies should be able to analyze the complex phenomena taking place in this range of temperature, and we are presently doing research in this direction. We must point out the role played by electron microscopy to visualize such new structural features which cannot be detected from spectroscopic studies (UV absorbance or CD spectra).

Finally it is important to notice that such aggregated structures were not observed after heating of nucleosomes [16] or reconstituted material (unpublished results). In both cases, however, the length of the DNA was greater than 145 bp. The peculiar instability of histone moiety in the core particle could be tentatively ascribed to the absence of a piece of DNA (may be the whole linker) bound to histones and able to prevent any further aggregation of the proteins following the partial disruption of their secondary and tertiary structure.

Acknowledgements

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