Folding and unfolding of the core particle DNA are processes faster than millisecond

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Core particle DNA unfolding and refolding are followed by stopped-flow circular dichroism technique. When core particles are dissociated in the stopped-flow cuvette, the high CD deviation corresponding to the dissociated state is reached in the first millisecond, which means that the dissociation process is completed within the dead time of the apparatus which is ~1 ms. The same conclusion can be drawn when core particles are reassociated, since the low CD value, typical of the associated state, is immediately reached. Similarly histone release from chromatin is a very fast process. We also include some points of discussion about core particle assembly process.

Chromatin dynamics *Core particle* *Dissociation* *Reassociation* *Circular dichroism* *Stopped-flow*

1. INTRODUCTION

Core particle may be assembled in vitro by numerous methods (review [1]) [2]. They differ from one another:

(i) In the pathway of decreasing ionic strength of DNA–histone mixture (stepwise dialysis, stepwise dilution or salt-jump);
(ii) In the core histone concentration;
(iii) In the presence or not of an assembly factor at physiological ionic strength;

and they lead to unequal core particle reconstitution yields within various times. The main event directing in vitro core particle assembly is the equilibrium existing between core histone complexes in given conditions [3]. Either DNA folding seems to be nucleated by histone octamer [4] or its intermediate products of degradation [3], or it seems nucleated by H3–H4 tetramer [5,6]. It still remains difficult to tell which process is the most relevant to biological implications.

Whatever the assembly process may be, it now appears clear that the DNA folding around the histone core must be very fast [2,4,6]. We show here by stopped-flow circular dichroism (SFCD) measurements, that folding and unfolding of core particle DNA are completed within < 1 ms.

2. MATERIALS AND METHODS

2.1. *Core particle dissociation and reassociation*

Native core particles, prepared and characterized as in [7] were suspended at 1.6 mg DNA/ml in buffer 'S' containing 20 mM Tris (pH 8) and 1 mM EDTA, then dissociated by the addition of an equal volume of buffer S–NaCl 4 M and immediately reassociated by direct dilution of the histone–DNA mixture from 2–0.25 M NaCl with buffer S.

2.2. *Static circular dichroism (CD) experiments*

CD measurements on core particles ($A_{260} = 1$) were performed with a Dichrograph III–Jobin Yvon apparatus. The results are presented in terms of molar ellipticity $|\theta|$ (in degrees $\cdot$ cm^2 $\cdot$ dmol^{-1}) based on the molar nucleotide concentration determined from an extinction coefficient of 6600 at 260 nm.

2.3. *Stopped-flow circular dichroism apparatus (SFCD)*

A SFCD system has been developed in our laboratory by combining an improved Durrum mixing system and a modified Dichrograph II-Jobin Yvon apparatus.

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At the output of the monochromator, the linearly polarized light is transformed by a 50 kHz piezo-optical modulator into alternatively left-handed and right-handed circularly polarized light. The total signal delivered by the photomultiplier is converted into an alternating signal centered at zero and directly proportional to ΔA, the difference of absorption between left and right circularly polarized light [8].

The value of the dichroism is given by the value of the peak-to-peak voltage of the signal sampled at the peak amplitude of each alternance. The modulator behaves like a $\lambda/4$ or $3\lambda/4$ wave plate at these points. Integration of the signal is obtained by a summation of the peak-to-peak voltage over a given number of cycles. The electronic interface is fast enough to register a dichroism value for each cycle since the conversion to digital form and the transfer into the memory of this value, extended in 2 words of 16 bits, requires 15 μs, a time shorter than that separating 2 successive alternances of the modulator (20 μs). The synchronisation signal delivered by the blocking system of the solution flows determines the zero time for the process to be studied. Data accumulation is then made.

Stopped-flow dead time is ~1 ms. CD resolution is $\delta\Delta A = 6.9 \times 10^{-6}$ absorbance unit.mV^{-1}. The precision of one measurement is 10^{-2} when the summation has been made over 16383 cycles. CD values (d) measured in mV can be expressed in molar ellipticity units taking $\delta\Delta A$, l and Cf into account, according to the classical formula:

$$|\theta| = 3300 \times \frac{\delta\Delta A}{lCf} \times d$$

where

$\delta\Delta A$ = the sensitivity of the SFCD apparatus;
l = the width of the cuvette;
Cf = the final molar concentration of the sample.

2.4. *SFCD experiments*

We followed DNA unfolding and refolding at 285 nm with an optical pathlength of 2 cm.

To study core particle dissociation, core particles ($A_{260} = 1.09$) in buffer S–sucrose 23.75% were mixed in the stopped-flow cuvette with an equal volume of buffer S–NaCl 3.8 M. An accumulation of 36 shots was made and, for each shot, the process was defined with 1000 points with 50 cycles (1 ms) between each point. In this case the precision of the measurement is ~3%.

To study core particle association, dissociated core particles ($A_{260} = 16$) in buffer S–NaCl 1.6 M were mixed in the stopped-flow cuvette with a 16-fold larger volume of buffer S–sucrose 10.23%. The same data accumulation was made as before.

The use of sucrose in the solvent which did not contain NaCl was necessary to match the refractive indexes of the 2 solvents and obtain correct solvent/solvent base lines.

3. RESULTS AND DISCUSSION

3.1. *Preliminary static experiments*

DNA unfolding and refolding, as followed by CD spectra registered as soon as possible after the adjunction of dissociating and reassociating buffer, respectively, are complete and very fast. In the dissociation process, we obtain in 2 M NaCl the actual CD spectrum of pure DNA in the same medium (fig. 1A). In the reassociation process (fig. 1B), we get a spectrum identical to that of the native core particle, within 3 min (i.e., the time required for mixing the solutions and registering the spectrum at 5×10^{-1} nm/s). This result clearly urges us to do fast kinetic studies.

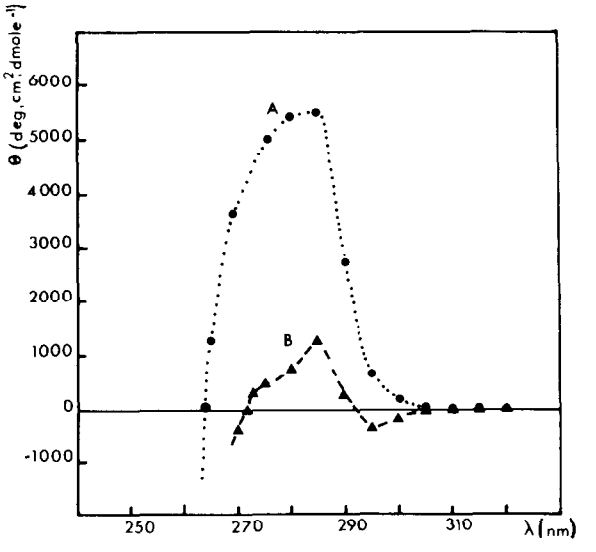

Fig. 1. CD spectra of: (A) dissociated core particles in buffer S–NaCl 2 M; (B) reassociated core particles in buffer S–NaCl 0.25 M. Experimental conditions: see sections 2.1 and 2.2.

According to CD values, the yield of reassociation is 100% since the CD spectrum of the reassociated core particle is superimposable to that of the native one in the same ionic conditions. This result gives also evidence that the 4 core histones are immediately bound to DNA. If the nucleation was driven by the tetramer H3–H4, leading to an intermediate step as in [6], this slow process would have been detected since, according to [9], such an intermediate complex exhibits much higher values of molar ellipticity ($|\theta|_{max}$ close to 5000 degrees . cm^2 . $dmol^{-1}$).

However, we cannot exclude, that such an intermediary step takes place but fast enough, in the range of concentration we were using, to be undetected.

The reassociation yield strongly depends on the core histone concentration in buffer S–NaCl 2 M as revealed in table 1. In other words, the final ellipticity values are increasing when the core histone concentration is decreasing. This increase of $|\theta|$ can be exactly correlated with the presence of free DNA (either naked or unspecifically bound to some histone molecules) in the solution, as evidenced from electron microscopy experiments (not shown). We are still unable to make a detailed analysis of the reconstitution process in our experimental conditions, but it appears likely that, in the concentration range used, the histone octamer was the predominant species and thus responsible of the high yield of reconstitution.

Finally, the reassociated core particle has not only the same morphological parameters as the native one but also exhibits the same reactivity to various histone antisera [10].

Exactly the same results were obtained when core particles at the same initial concentration were dissociated in 1.6 M NaCl and reassociated by a salt-jump up to 0.1 M NaCl, the conditions used further (see section 3.3.).

Table 1

Reassociation yields determined by CD in function of core histone concentration in 2 M NaCl

Histone (μg/ml)	$\|\theta\|$ 285 nm (degrees . cm^2 . $dmol^{-2}$)	Reassociation yield (%)
960	1300	100
240	2000	90
120	3300	70

3.2. *SFCD study of core particle dissociation*

As a preliminary precaution, we checked by electron microscopy that the rapid mixing by itself did not exert any shearing force on the core particles. Thus we could follow core particle dissociation in the experimental conditions in section 2.4. Fig. 2A shows that we do not observe the increasing exponential that we could have expected if the process could have been detected. We obtain instead in the first milliseconds, the high deviation corresponding to the dissociated state: the mean value of 37 mV (dashed line on fig. 2A) corresponds to 5560 degrees . cm^2 . $dmol^{-1}$. After the last shot, a complete spectrum had been drawn and the value measured at 285 nm is quite comparable with this

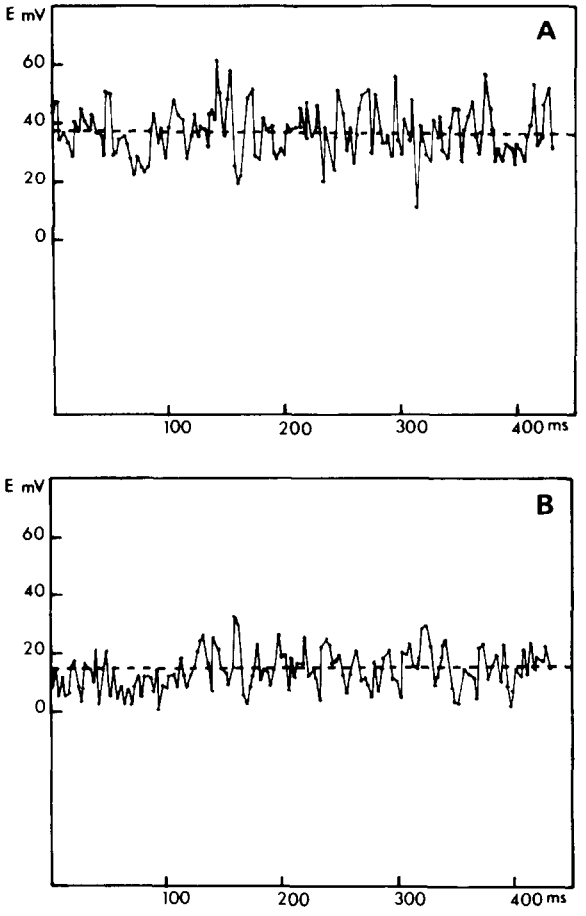

Fig. 2. SFCD experiments of: (A) core particle dissociation; (B) core particle reassociation. One point has been drawn every third millisecond. Experimental conditions: see section 2.4.

mean value. We can then ascertain that DNA unfolding process is < 1 ms.

We have wondered whether this process could be less fast when core particle is included in a more compact structure, such long chain chromatin. In this case, we observed a relaxation curve which was the actual curve of long chain DNA when it is flow-oriented. We could obtain the same curve when long chain DNA in low ionic strength was mixed in the stopped-flow cuvette with its own solvent. However, when chromatin in low ionic strength was mixed with its own solvent, we obtained the base-line. These observations constitute indirect evidence that histone release from chromatin, due to an increase of ionic strength, was also a very fast process, completed within the dead-time of the apparatus.

Although some studies (review [11]) have shown that, globally, nucleosomes do not dissociate into histones and DNA during the transcription process, we cannot exclude the possibility of very local and transient dissociation events in vivo.

3.3. *SFCD study of core particle reassociation*

Core particles pre-dissociated in 1.6 M NaCl were reassembled in the stopped-flow cuvette as in section 2.4. No decreasing exponential was observed but the low value, typical of the associated state, was reached in the very first milliseconds. The average signal value (fig. 2B, ---) is 15 mV; i.e., 1130 degrees . cm^2 . $dmol^{-1}$. Again, DNA folding process is < 1 ms.

As discussed in section 3.1., the 4 core histones are immediately bound to DNA. However, subtle histone movements may exist without significantly altering DNA folding, and then its circular dichroism [10, 12]. As suggested by Daban [6], such a rearrangement of a complex that would already contain the 4 histones is possible.

On another hand, we know that, in early embryos [13], the interphase lasts only a few minutes; it means that histone deposition must be very rapid.

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REFERENCES

[1] Laskey, R.A. and Earnshaw, W.C. (1980) Nature 286, 763–767.
[2] Daban, J.R. and Cantor, C.R. (1982) J. Mol. Biol. 156, 749–769.
[3] Jorcano, J.L. and Ruiz-Carrillo, A. (1979) Biochemistry 18, 768–774.
[4] Stein, A. (1979) J. Mol. Biol. 130, 103–134.
[5] Ruiz-Carrillo, A. and Jorcano, J.L. (1979) Biochemistry 18, 760–768.
[6] Daban, J.R. and Cantor, C.R. (1982) J. Mol. Biol. 156, 771–789.
[7] De Murcia, G., Mazen, A., Erard, M., Pouyet, J. and Champagne, M. (1980) Nucleic Acids Res. 8, 767–779.
[8] Bächinger, H.P., Eggenberger, H.P. and Hänisch, G. (1979) Rev. Sci. Instrum. 50, 1367–1372.
[9] Bina-Stein, M. (1978) J. Biol. Chem. 253, 5213–5219.
[10] Muller, S., Erard, M., Burggraf, E., Couppez, M., Sautière, P., Champagne, M. and Van Regenmortel, M.H.V. (1982) EMBO J. in press.
[11] Mathis, D., Oudet, P. and Chambon, P. (1980) Prog. Nucleic Acid Res. Mol. Biol. 24, 1–55.
[12] Erard, M., Pouyet, J., Mazen, A., Champagne, M. and Daune, M. (1981) Biophys. Chem. 14, 123–133.
[13] Blumenthal, A.B., Kriegstein, H.J. and Hogness, D.S. (1973) Cold Spring Harb. Symp. Quant. Biol. 38, 205–233.