Nucleosomes arrangement in chromatin

C.Marion and B.Roux

Laboratoire de Chimie Biologique, Université Claude Bernard Lyon I, 43, boulevard du 11 Novembre 1918, 69621 Villeurbanne, France

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ABSTRACT

The spatial arrangement of nucleosomes in rat liver chromatin has been examined using the electric birefringence technique. All chromatin subunits studied (up to 9 consecutive nucleosomes) contain their full complement of the five histone types associated with about 200 base pairs repeat length DNA.

From the relaxation times and the orientation mechanisms, the nucleosome may be assimilated to an oblate ellipsoid of dimensions about $140 \times 140 \times 70$ Å, and the DNA superhelical axis is parallel to its shorter axis.

The most important result is a sharp transition in the electro-optical properties of subunits when the number of nucleosomes in the chain is greater than 6 : the initial negative birefringence, as for DNA, becomes positive and the relaxation time is multiplied by ten. The hexanucleosome, which presents no birefringence, has an helical symmetrical structure without preferential orientation axis. This structure is approximatively spherical of about 250 Å diameter and the chromatin appears as a periodic array of such a structure.

INTRODUCTION

Enzyme digestion of chromatin has shown that this nucleoprotein complex consists of repeating subunits termed v-bodies or nucleosomes (1-5). Several studies, using a variety of physical methods, have been carried out to establish their structure (for reviews see ref. 3). They appear to be compact in structure and different models have been proposed for monomeric subunit (3, 6-12). The influence of the very lysine rich histone H1 in this organization seems to be unambiguous (13, 14). H1 is considered to be on the outside of the nucleosome and involved in higher order packaging of the subunits into a superstructure having about 6 nucleosomes per turn (15-18).

In order to contribute to explain this spatial arrangement of nucleosomes in chromatin, we have studied higher order oligomers of nucleosomes using the electric birefringence technique. Indeed, from birefringence decays, relaxation times and rotational diffusion constants can be deduced and so, molecular properties can be reached assuming some simple models (19-20).

In this paper we present the results obtained with rat liver chromatin subunits (up to 9 consecutive nucleosomes) containing their full complement of the five histones types associated with an about 200 base pairs repeat length DNA. The shapes of monomer and oligomers are determined and the compact structure of nucleosomes in tight contact or a more extended arrangement are discussed.

MATERIAL AND METHODS

Preparation of nucleosome oligomers

Tissues used in the preparation of chromatin were obtained from male Sprague-Dawley rats, weighing 200-220 g. All operations were performed at 4°C and nuclei were prepared by modifying slightly the method of Hewish and Burgoyne (21), using the classical buffers with phenylmethylsulfonyl fluoride (PMSF). The resulting extensively washed pellets were resuspended to give a final concentration of about 3×10^8 nuclei/ml. The nuclei obtained are highly purified as judged from chemical analysis and absence of cytoplasmic contamination.

Chromatin extraction was essentially performed as described by Noll et al. (22) : after preincubation at 37°C for 5 min, 1mM CaCl_2 (final concentration) was added and then digestion started by addition of 300 units of micrococcal nuclease (Worthington) per ml of nuclei suspension. The digestion was stopped 2 min 30 sec later by adding 2mM EDTA (final concentration) and chilled quickly in ice. Nuclei were then pelleted by a centrifugation at $2000 \times g$ for 5 min, rapidly resuspended in 0.2 mM EDTA, 0.2mM PMSF pH 7.0 and lysed for a 5 min incubation at 4°C. The suspension was cleared from nuclear debris by a centrifugation at $2000 \times g$ for 10 min and the chromatin was recovered in the supernatant.

Fractionation of the chromatin subunits was performed as described by Finch et al. (23), using isokinetic sucrose gradients (Figure 1). Fractions corresponding to each peak were pooled and dialysed overnight against 0.2mM EDTA, 1mM phosphate buffer pH 7.4.

DNA and histones analysis

DNA was purified from the chromatin fractions by an overnight digestion with 100 µg/ml of proteinase K (Merck) at 37°C in a buffer containing 1M NaCl, 1 % SDS, 3mM EDTA. The incubation was followed by two treatments with chloroform-isoamyl alcohol (24:1, v/v). DNA was finally extracted with phenol-chloroform-isoamyl alcohol mixture (50:48:2, v/v/v) and precipitated with 2 volumes of absolute ethanol at -20°C. Electrophoresis was performed on a 3 %

acrylamide gel, as described by Loening (24) with bromphenol blue as a mobility marker and scanned at 260 nm using a Gilford spectrophotometer.

Histones were extracted twice with 0.25 N HCl (final concentration) and recovered by sedimentation at 10 000 x g of precipitated DNA. The supernatants were dialysed overnight against distilled water and lyophilysed. Histones then dissolved in 0.9 N acetic acid, 15 % sucrose were analysed by electrophoresis according to Panyim and Chalkley (25).

Thermal denaturation curves

Absorbance melting curves of chromatin subunits were recorded at 260 nm on a Beckman DU spectrophotometer modified in our laboratory as previously described (26). A XY recorder (Luxytrace Sefram) allowed exactly absorbance-temperature profiles recording. The heating rate was 0.5°C/min and 1.0 to 1.2 A₂₆₀/ml was used.

Electro-optical measurements

The general principles of the electric birefringence studies of macromolecules have already been reviewed elsewhere (27-29). To describe dynamic birefringence, Benoit (30) and O'Konski and Zimm (31) have set up the basic equations, generalized by Tinocco and Yamaoka (32).

An optically anisotropic macromolecule, placed in an electric field, $\vec{E}$, will have an average degree of orientation if these molecules carry a permanent or an induced dipole moment. This orientation gives rise to a positive or negative birefringence, Δn .

Before onset of the field, the birefringence is zero because the particles are randomly oriented. When the single pulse of duration θ is applied, Δn increases till the steady state birefringence Δn_{eq} is reached. θ must last long enough : significantly greater than the longest relaxation time (in all experiments presented here, the pulse duration varies between 40 and 300 μ s). According to the Kerr's law Δn_{eq} is proportional to E^2 . A saturation may be observed for higher applied electric field.

When the field is switched off, Δn decreases with a typical time dependence, $\Delta n(t)$, which is given whatever the orientation mechanism is, by the Benoit's equation (30) :

$$\Delta n(t) = \Delta n_{eq} e^{-t/\tau} \quad (1)$$

τ is the relaxation time which is related to the rotational diffusion constant D_r , $D_r = 1/6\tau$.

For revolution ellipsoids where a and b are respectively the long and the short semi-axes, D_r is given by Perrin's formula (33) :

$$D_r = \frac{kT}{8\pi\eta ab^2} r(p) \quad (2)$$

T is the absolute temperature, k the Boltzmann constant and η the absolute viscosity of the solvent. The values of $r(p)$ which is a function of the axial ratio $p = a/b$, are reported by Daune et al. (34) and Broersma (35).

The birefringence apparatus, already used for measurements on DNA solutions (19, 36) has been built in our laboratory and described elsewhere (37). It uses a very sensitive optical device including a quarter-wave retarder and a powerful stable and noise-free He-Ne laser (Spectraphysics model 120) supplied by a high performance power supply. The low noise solid state optical detector is a photo-diode followed by a linear amplifier.

For the experiments presented here, the signals are displayed on a fast storage oscilloscope (Tektronix) then photographed. The decay curves so recorded, for example see Figure 6, are sampled. If the solution is monodisperse, a straight line is obtained from which the relaxation time can be determined.

According to the required sensitivity, different Kerr cells are used with length varying from 2 to 5 cm. The electrodes are made of gold and are either 1 or 0.5 cm spaced.

RESULTS

Fractionation of chromatin

The micrococcal nuclease digested rat liver chromatin was fractionated by an isokinetic (5-28.2 %) sucrose gradient centrifugation in a SW 27 rotor. For the electro-optical study described below, the nuclease concentration was 300 units/ml with 3.10^8 nuclei/ml and the digestion lasts always 2 min 30 sec. Under these conditions, monomeric and oligomeric nucleosomal components can be completely separated from each other. A typical fractionation pattern is shown in Figure 1. The prominent peak (I) contains monomer particles and we will refer to the others (reading from the right to the left) as "dimer", "trimer" ... etc. Only the fractions from each peak providing a correct purity are collected, dialysed against 1mM phosphate buffer, pH 7.4, 0.2mM EDTA and then concentrated for physical experiments.

The purity of the final fractions was verified by an electrophoresis of the extracted DNA in polyacrylamide gels. The patterns obtained were found similar to those previously published and the densitometry of the gels allowed to determine if there was some cross-contamination by neighbouring fractions : then, the purity of nucleosome mono- and oligomers studied always varied from more than 95 % for the monomer to 65 % for the octamer and nonamer.

Figure 1 : Isokinetic sucrose gradient fractionation of micrococcal nuclease digested rat liver chromatin.

The chromatin solution was layered on gradients containing 1mM EDTA, 1mM phosphate buffer pH 7.4 with $C_t = 5\%$, $V_m = 33$ ml, $V = 36$ ml and $C_v = 28.2\%$.

Centrifugation was performed at 27 000 rpm for 20 hours in a SW 27 rotor (Beckman) at 4°C. Digestion time was 2 min 30 sec with 300 units of micrococcal nuclease per ml of nuclei suspension.

The gradients were collected from the top of the tube using a Gilford density gradient scanner.

Protein analysis

On the other hand, integrity of proteins in chromatin subunits prepared by micrococcal nuclease digestion is a necessary requirement for a comparative study of their electro-optical properties.

For all the nucleosome oligomers, the A_{230}/A_{260} ratio was in the range 0.72-0.78 (0.80 for intact chromatin) and this result was in agreement with the protein estimation according to Lowry.

The protein/DNA ratio was always about 1.62 for all samples and this value is lower than the 1.70 g of protein per g of DNA found for intact chromatin. This last ratio corresponds to the following chemical composition (w/w) DNA-histone protein- non histone protein : 1/1.15/0.55. Indeed both fractionated and unfractionated chromatin have a total histone/DNA weight ratio of 1.15 and it can be seen that the decrease in protein/DNA ratio of oligomers originates in a loss of some of the non histone chromosomal proteins which are easily dissociable during digestion (38, 39). With the mild digestion conditions

Figure 2 : Gel electrophoresis scanning of histones extracted from mononucleosome.
Polyacrylamide gel (15 %) were stained with Coomassie blue and scanned at 550 nm.

used in our experiments, the chromatin subunits have preserved most of their proteins (about 90 %).

The full complement of histone proteins in each subunit was confirmed by the electrophoresis analysis. **Figure 2** displays the gel electrophoresis pattern of monomer nucleosome histones, which is found strictly similar for all fractions (up to nonamer).

These analysis indicate the presence of all the five histones which run in the order H1 (the slowest), H3, H2B, H2A and H4 (the fastest) with an identical proportion to that observed in unfractionated chromatin.

We can note also that the four last components are present in comparable amounts. On the other hand, the degree of loss of histone H1 appears to be related to the extent of digestion (40-41). So, it seems that the mild digestion conditions used in these experiments allow chromatin subunits fractionation with histone H1 always present in the same proportion (up to nonamer).

Physico-chemical properties

Thermal denaturation profiles were recorded for mono and oligomers : the melting points and hyperchromicities determined are comparable with other reported values and are given in **Table I**. No difference in T_m was observed ($T_m=79.5^\circ\text{C}$)

Table 1. Physico-Chemical properties of chromatin subunits

Nucleosomes	T_m ^{a)} (°C)	Hyperchromicity ^{b)} (%)	A_{230}/A_{260}	Protein/DNA ^{c)}	s ^{d)} (Svedberg)
Monomer	79	40.7	0.78	1.66	10.8
Dimer	79.5	40	0.74	1.60	14.9
Trimer	79.5	41.5	0.75	1.62	18.1
Tetramer	79	41	0.74	1.61	21.1
Pentamer	80	41.8	0.73	1.59	23.8
Hexamer	79.5	40.6	0.74	1.60	26.2
Heptamer	80	41.1	0.78	1.66	28.1
Octamer	79	41	0.72	1.59	30.1
Nonamer	80	41.3	0.73	1.61	32.0

^{a)} T_m is the temperature corresponding to half the final increase of hyperchromicity.

^{b)} The percent hyperchromicity is defined as the normalized absorbance increase after treatment of the sample with heat :

$$h = 100 \times (A_{260}^{\max} - A_{260}^{20}) / A_{260}^{20}$$

^{c)} Determined by the Lowry's method and optical density measurement at 260 nm.

^{d)} Calculated using the Fritsch's equation (42).

whatever the nucleosome fraction was. However, the unfractionated chromatin behave slightly differently : the melting point is 3°C higher than for the subunits and a somewhat broader transition is observed. Hyperchromicity is similar for all chromatin samples, about 41 % for fractionated ones and 42 % for unfractionated ones.

For all chromatin subunits, the melting curves are found to be monophasic and these results are in a good agreement with recent works of Lawrence et al. (43) and Wittig and Wittig (44). These curves show a very high cooperativity explained by an electrostatic screening of the associated proteins on the phosphate backbone of DNA.

The data reported by several authors (45-48) showing biphasic or multiphasic thermal denaturation profiles for monomer, oligomers or native chromatin reflect a rearrangement of proteins which then, stabilize no more uniformly the DNA molecule. Such looking like free DNA regions appear in the H1 depleted chromatin (49) but multiphasic profiles are also observed in the presence of urea (50), when the subunits are aged (44), sheared (51) or prepared in high ionic strength (47). So, the multiphasic melting curves seem originate

from the presence of denatured nucleosomal material.

Approximate sedimentation coefficients could be calculated using Fritsch's equation for isokinetic gradients (42) and reported in Table I. It was found that s values were in accordance with those found previously but perceptibly lower, except for monomer (10.8S). This difference is essentially due to the sucrose gradient medium in which subunits particles are centrifuged and these s values cannot be considered as $S_{20,w}$ values.

Another explanation could be the exclusion of non histone proteins during the chromatin digestion but the chemical analysis of the subunits allows to dismiss this hypothesis. A log-log plot of s versus multimer number could show that s is proportional to about $M^{0.5}$ as precendently reported (13,39).

Steady state birefringence

These electro-optical measurements have been carried out in the 0.2mM EDTA 1mM phosphate buffer medium and performed in a non-thermostated cell, at about 20°C. This temperature is much lower than the chromatin subunits denaturation temperature range and electric pulse heating has never been found to be higher than 1°C, even when the longest pulses are applied. On the other hand, it has been verified that the solutions, stored at 4°C for 5-6 days, exhibit identical values of their electro-optical parameters. The electric field range used varied from 250 to 5 000 V/cm.

The typical oscillograms of electric birefringence of chromatin monomer and nonamer are shown in Figure 3 (b and c). The three parts of signal, the rise curve (I), the steady state (II) and the decay curve (III) are described in "Methods".

Birefringence is proportional to c for all the range studied (1 to 8 A_{260}/ml) and Δn could be expressed in term of specific birefringence.

The steady state specific birefringence, $\Delta n_{eq}/A_{260}$ versus E^2 is reported in Figure 4. It shows a linear dependence and then that Kerr's law is obeyed for all chromatin subunits. These results allow to be sure that there are no deformation of molecular structures due to the applied electric forces.

For the mononucleosome and up to pentamer, Δn is negative ; hexamer exhibits no birefringence signal even for the highest electric fields, and the birefringence becomes positive for the longest chains of nucleosomes ($n \geq 7$). This change of sign is obtained whatever the applied electric field and the chromatin subunits concentration are.

To follow this transition between pentamer and heptamer, subunits with large contamination have been studied and the oscillograms are shown in Figure 3 (d-e).

Figure 5 shows the variation of steady state specific birefringence as a

Figure 3 : Oscillograms of electric birefringence of chromatin subunits with an electrical rectangular pulse (a). The electrical field applied was 1500 V/cm. The typical pulse response obtained with the mononucleosome (b) is described in "Methods". The birefringence is positive with the nonamer (c) and intermediate signals are showed for contaminated subunits : pentamer (d) and heptamer (e). Pulse length : 1 div. = 10 μ s.

function of the number of subunits in the multimer (n) for an electric field applied of 3 KV/cm. The birefringence is maximum for the dinucleosome and then a continuous decrease is observed when n increases. There is a 2.5 ratio between the values of $\Delta n_{eq}/A_{260}$ for di- and mononucleosome.

Transient birefringence

The relaxation times for all chromatin subunits have been determined from the birefringence decays obtained on a storage oscilloscope as described in

Figure 4 : Verification of Kerr's law : field dependence of steady state specific birefringence.
(1 esu = 300 V/cm).
No birefringence was observed for hexamer (VI).

"Methods". This study has shown no effect of concentration and electric field applied on τ , in the ranges used.

Figure 6 presents the logarithmic plot of normalized birefringence versus time : **6a** for the oligomers up to $n = 5$ and **6b** for $n \geq 7$. The straight lines obtained show a good fitting with a single exponential : so, these subunits are monodisperse. The results are summarized on **Table II** where rotational diffusion coefficients D_r and electro-optical parameters are also reported.

For the more birefringent fractions, the linearity observed on approximately 3 neperian units shows that the birefringences are recorded down to 4-5 % of their initial values. For the other fractions, this percentage is only 13-15%.

Through the experimental precision (52), the relaxation time may be considered as constant for dimer to pentamer. A sharp increase appears when n is greater than 6 subunits. This transition is obtained in the same time than the change of birefringence sign.

On the other hand, the comparison of the rise and decay birefringence curves allows to determine the contribution of permanent (P) and induced (Q) moments to

Figure 5 : Variation of steady state specific birefringence as a function of n , number of subunits in the fractions.
Applied field $E = 3000$ V/cm.

the orientation mechanism. This contribution is appreciated by the ratio $r = P/Q$ (30). The rise of birefringence is more complicated than the decay because it depends on r . For pure induced moment, $r = 0$ and the rise curve is symmetrical to the decay curve.

DNA presents negative birefringence and it is now admitted that the orientation is essentially due to an induced electric dipole produced by polarization of ionic atmosphere (29). The macromolecule is oriented with its long axis in the direction of the applied electric field and the plane of base pairs roughly perpendicular to the length of the molecule.

If there is contribution of a permanent moment, an asymmetry appears and the value of r can be estimated using the equation of Benoit (see Figure 7). A graphical example is given for the rise curve of mononucleosome : a set of theoretical rising functions has been constructed for various values of r and the best fitting curve is obtained with a ratio of about 0.5.

For all oligomers the curves are almost symmetrical and r about 0.5 : then their orientation seems mainly due to an induced dipole interaction with the field. However, the existence of a small permanent dipole cannot be disregarded.

Figure 6 : Determination of relaxation time for nucleosomes : logarithmic plot of normalized birefringence versus time for an applied electric field $E = 3 \text{ KV/cm}$.

a- for $n < 6$
b- for $n \geq 7$

Table II Hydrodynamic and electro-optical parameters.

Nucleosomes	Relaxation Time τ (μ s)	Rotational Diffusion Coefficient D_r (s^{-1})	Kerr Constant* B ($esu^{-1}.g^{-1}.cm^2$)
Monomer	0.6	28×10^4	-3.5×10^{-3}
Dimer	1.8	9×10^4	-9×10^{-3}
Trimer	1.8	9×10^4	-7×10^{-3}
Tetramer	1.6	10×10^4	-5×10^{-3}
Pentamer	1.4	12×10^4	-2.5×10^{-3}
Hexamer	-	-	-
Heptamer	12	1.4×10^4	$+4 \times 10^{-3}$
Octamer	18	0.9×10^4	$+7 \times 10^{-3}$
Nonamer	28	0.6×10^4	$+9.5 \times 10^{-3}$

* $B = \Delta n / c \lambda E^2$ with $\lambda = 6328$ nm.

Figure 7 : Fitting of the rise curve of mononucleosome birefringence with theoretical rising functions, for various values of r . The rise curve is in full line with circles. The normalized rise curve is given by the equation of Benoit (30) :

$$\Delta B(t) = 1 - \frac{3r}{2(r+1)} e^{-t/3\tau} + \frac{r-2}{2(r+1)} e^{-t/\tau}$$

DISCUSSION

The most significant result is the change of sign of the birefringence when the number of subunits increases. At the same time, a sharp transition in the

relaxation time appears for $n > 6$. The fact that no birefringence is observed for the hexanucleosome supposes the existence of a symmetrical structure without preferential orientation axis.

From the relaxation time, subunits molecular dimensions can be calculated, assuming simple geometrical shapes. Because the appearance of the nucleosome in the electron microscope, a compact roughly spherical structure, about 100-130 Å diameter has been suggested (1,2,23,60-62). However, recently numerous models have been also proposed such as ellipsoids, discs, cylinders or wedge-shaped (6-12, 63-68), with dimensions ratio equal to about 2. Taking into account experimental precisions, the proposed models may be assimilated respectively to oblate (with $p=0.5$) or prolate (with $p=2$) revolution ellipsoids.

For a prolate model, according to Perrin (33) and Small and Isenberg (69), the ellipsoid dimensions are 160 x 80 x 80 Å (with $\tau = 0.6\mu s$). The oblate ellipsoid dimensions could be 70 x 140 x 140 Å. Precisions may be obtained, considering the properties of higher oligomers.

The measured relaxation time for the dinucleosome (1.8 μs) is too small to be the presented one by a end to end association. So, in all proposed nucleosome array, the particles must be in contact by their longer side and shape a symmetrical structure for a six subunits array. Considering theoretical calculations, the proposed model for this hexanucleosome structure is an helical array of nucleosomes in tight contact as shown by the very small decrease of relaxation times from $n = 2$ to $n = 5$. This structure is approximatively spherical with a diameter about 250 Å as calculated using eq. (2) (where $a=b$ and $r(p)=1$) with τ about 1.4 μs . These results are in good agreement with the hydrodynamic data reported by Wittig and Wittig for nucleosome mono, di, tri and tetramer, which measure translational diffusion constants (44).

With regard to its electro-optical properties, the nucleosome is constituted of two components with opposed sign birefringences : DNA exhibits a negative birefringence and the proteins a positive one, like reported previously for different histone species (52).

The change of birefringence sign can be explained either by a more important contribution of the positive birefringence component (proteins) in the complex when n increases, or by an evolution to a tight structure with a modification of the orientation axis for $n = 7$.

As, in absolute value, the DNA Kerr constant is about twenty times greater than the histone complex one (53) and as the DNA/histone ratio is constant for all studied fractions, it seems that the first explanation can be to dismiss.

On the other hand, Rill and Van Holde (54, 55), Houssier et al. (56) and

Klevan et al. (57, 58) have also studied electric dichroism and birefringence of chromatin : they observe always negative signals for nuclease-resistant fragments of the chromatin as for whole and H1-depleted chromatin. However these fractions are most probably constituted of various lengths nucleosome chains (56). To propose an explanation of this behaviour, these authors calculate the dichroism of chromatin superhelical arrangements using a relation derived by Rill (59) for a complete orientation of the particle with DNA superhelical axis parallel to the applied orientation force. In all cases, obtained signs must to be positive. Thus, they envisage two hypothesis to explain the measured negative signs : (i) the DNA superhelical axis in the nucleosome would be perpendicular to the long axis of the nucleosome array in the chromatin, (ii) the presence of extended internucleosome fragments with free DNA which, present in sufficient amount, could reverse dichroism or birefringence sign.

In our preparation, the superstructure of chromatin is conserved as showed by the protein composition, the DNA/histone ratio and the monophasic melting curves for all oligomers : so, there are no free DNA fragments. As discussed above, the compact structure is also verified by the variation of the relaxation time with the number of nucleosomes in the chains. Negative birefringence signals observed for lower oligomers may be explain by the first hypothesis proposed above (54-58) with the DNA superhelical axis in the nucleosome perpendicular to the long axis of nucleosomes array.

As the birefringence of mononucleosome has the same sign than DNA, one can suppose that the axis of larger polarizability of nucleosome particles coincides with the axis of DNA, which is wrapped around the histones core : so, the birefringence is essentially due to the nucleic part of the complex and DNA is oriented with its long axis in the direction of the electric field.

Then, the four different types of models which can be considered are prolate or oblate ellipsoids with the DNA superhelical axis parallel or perpendicular to the long axis of the equivalent ellipsoid (Figure 8).

Among these models, just one is consistent with the orientation mechanism (Figure 8). Indeed, considering the negative birefringence sign, the models with the DNA superhelical axis parallel to the long axis of nucleosomes array must be eliminated (Schemes A and C). On the other hand, as the nucleosomes are in contact by their longer side and as the birefringence decreases when n increases, it seems that the models in which the path followed by DNA is longer along an ellipsoid axis than another can be also dismissed (Scheme B).

Finally, the nucleosome model which fits with our results is an oblate ellipsoid or disc-shaped particle of dimensions about $140 \times 140 \times 70 \text{ \AA}$, which

Figure 8 : Orientation of the nucleosome assimilated to a prolate (Schemes A and B) and oblate (Schemes C and D) revolution ellipsoid. The thick gray thread corresponds to DNA. The axis is the DNA superhelical one.

is quite in good agreement with other reported values (6,7,9,11,58,63,66). In this model, the DNA superhelical axis is parallel to the short axis of the equivalent ellipsoid (Scheme D), as recently proposed (12).

The change of birefringence sign obtained with the seventh nucleosome may be explain, as proposed (54-58), by its DNA superhelical axis parallel to the hexamer structure axis. A relaxation time transition is also observed which cannot be explain only by a nucleosome chains unfolding since the birefringence is positive. Finding a single τ for the octa- and nonanucleosomes seems confirm this fact. So, it can be imagine that the higher oligomer structure is constituted of two consecutive hexanucleosomes structures with approximatively perpendicular long axes.

Then, in conclusion, the results presented here show a sharp transition

in the electro-optical properties of nucleosome chains when they contain six subunits. The existence of such a hexanucleosome structure, as a periodic array in the chromatin structure, which has been postulated by many authors (15-18) seems now clearly confirmed.

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REFERENCES

- (1) Olins A.L. and Olins D.E. (1974) *Science*, 183, 330-332
- (2) Oudet P., Gross-Bellard M. and Chambon P. (1975) *Cell*, 4, 281-300
- (3) a- Kornberg R.D. (1977), *Ann. Rev. Biochem.* 46, 931-954
b- Felsenfeld G. (1978), *Nature*, 271, 115-122
- (4) Shaw B.R., Herman T.M., Kovacic R.T., Beaudreau G.S. and Van Holde K.E. (1976) *Proc. Natl. Acad. Sci. USA*, 73, 505-509
- (5) Barrett T. (1976), *Nature*, 260, 576-578
- (6) Pardon J.F., Worcester D.L., Wooley J.C., Cotter R.I., Lilley D.M.J. and Richards B.M. (1977), *Nucl. Acids Res.* 4, 3199-3214
- (7) Suau P., Kneale G.G., Braddodk G.W., Baldwin J.P. and Bradbury E.M. (1977) *Nucl. Acids Res.* 4, 3769-3786
- (8) Hjelm R.P., Kneale G.G., Suau P., Baldwin J.P., Bradbury E.M. and Ibel K. (1977), *Cell*, 10, 139-151
- (9) Finch J.T., Lutter L.C., Rhodes D., Brown R.S., Rushton B., Levitt M. and Klug A. (1977), *Nature*, 269, 29-36
- (10) Gordon V.C., Knobler C.M., Olins D.E. and Schumaker V.N. (1978), *Proc. Natl. Acad. Sci. USA*, 75, 660-663
- (11) Bram S., (1978), *Biochem. Biophys. Res. Comm.*, 81, 684-691
- (12) Trifonov E., (1978), *Nucl. Acids Res.*, 5, 1371-1380
- (13) Noll M. and Kornberg R.D., (1977), *J. Mol. Biol.*, 109, 393-404
- (14) Thoma F. and Koller Th., (1977), *Cell*, 12, 101-107
- (15) Carpenter B.G., Baldwin J.P., Bradbury E.M. and Ibel K., (1976), *Nucl. Acids Res.*, 3, 1739-1746
- (16) Finch J.T. and Klug A., (1976), *Proc. Natl. Acad. Sci., USA*, 73, 1897-1976
- (17) Renz M., Nehls P. and Hozier J., (1977), *Proc. Natl. Acad. Sci. USA*, 74, 1879-1883
- (18) Worcel A. and Benyajati C., (1977), *Cell*, 12, 83-100
- (19) Bernengo J.C., Roux B. and Herbage D., (1976), *Ber. Bunsen Ges. Phys. Chem.* 80, 246-249
- (20) Bernengo J.C., Herbage D., Marion C. and Roux B., (1978), *Biochim. Biophys. Acta* 28, 562-566
- (21) Hewish D.R. and Burgoyne L.A., (1973), *Biochem. Biophys. Res. Comm.*, 52, 504-510
- (22) Noll M., Thomas J.O. and Kornberg R.D., (1975), *Science*, 187, 1203-1206
- (23) Finch J.T., Noll M. and Kornberg R.D., (1975), *Proc. Natl. Acad. Sci. USA*, 72, 3320-3322
- (24) Loening V.E., (1967), *Biochem. J.*, 102, 251-257
- (25) Panyim S. and Chalkley R., (1969), *Arch. Biochem. Biophys.*, 130, 337-346

- (26) Marion C., (1974), Thesis, University C. Bernard, Lyon, France.
- (27) Yoshioka K. and Watanabe H., (1969) in *Physical Principles and Techniques in Protein Chemistry*, Part A, pp 335-367, Academic Press. New York
- (28) Hanss M. and Roux B., (1972), *Ann. Phys. Biol. Med.*, 3, 133-163
- (29) Fredericq E. and Houssier C., (1973), *Electric Dichroism and Electric Birefringence*. Clarendon Press. Oxford
- (30) Benoit H., (1951), *Ann. Phys.*, 6, 561-587
- (31) O'Konski C.T. and Zimm B.H., (1950), *Science*, 111, 113-115
- (32) Tinocco I. and Yamaoka K., (1959), *J. Phys. Chem.*, 63, 423-432
- (33) Perrin F., (1934), *J. Phys. Radium* 5, 497-511
- (34) Daune M., Freund L. and Spach G., (1962), *J. Chim. Phys.*, 59, 485-493
- (35) Broersma S., (1960), *J. Chem. Phys.*, 32, 1626-1631
- (36) Roux B., Bernengo J.C., Marion C. and Hanss M., (1978), *J. Colloid and Interface Science* in press
- (37) Bernengo J.C., Roux B. and Hanss M., (1973), *Rev. Sci. Instr.*, 44, 1083-1086
- (38) Augenlicht L.H. and Lipkin M., (1976), *Biochem. Biophys. Res. Comm.*, 70, 540-543
- (39) Gottesfeld J.M. and Butler P.J., (1977), *Nucl. Acids Res.*, 4, 3155-3173
- (40) Axel R., (1976), In *Progress in Nucleic Acid Research and Molecular Biology* Davidson I.N. and Cohn W.E., Eds., Vol. XIX, pp. 355-371, Academic Press
- (41) Gaubatz J.W. and Chalkley R., (1977), *Nucl. Acids Res.*, 4, 3281-3301
- (42) Fritsch A., (1973), *Anal. Biochem.*, 55, 57-71
- (43) Lawrence J.J., Chan D.C.F. and Piette L.H., (1976), *Nucl. Acids Res.*, 3, 2879-2893
- (44) Wittig B. and Wittig S., (1977), *Nucl. Acids Res.*, 4, 3901-3917
- (45) Sahasrabudhe C.B. and Van Holde K.E., (1974), *J. Biol. Chem.*, 249, 152-156
- (46) Woodcock C.L.F., Safer J.P. and Stanchfield J.E., (1976), *Exp. Cell. Res.*, 97, 101-110
- (47) Woodcock C.L.F. and Frado L.L.V., (1975), *Biochem. Biophys. Res. Comm.*, 66, 403-410
- (48) Mandel R. and Fasman G.D., (1976), *Nucl. Acids Res.*, 3, 1839-1855
- (49) Weischet W.O., Tatchell K., Van Holde K.E. and Klump H., (1978), *Nucl. Acids Res.*, 5, 139-160
- (50) Whitlock Jr. J.P. and Simpson R.T., (1976), *Nucl. Acids Res.*, 3, 2255-2266
- (51) De Murcia G., Das G.C., Erard M. and Daune M., (1978), *Nucl. Acids Res.*, 5, 523-536
- (52) Roux B., (1976), Thesis, University C. Bernard, Lyon I, France
- (53) Martet C., (1975), Thesis, University C. Bernard, Lyon I, France
- (54) Rill R.L. and Van Holde K.E., (1973), *J. Biol. Chem.*, 248, 1080-1083
- (55) Rill R.L. and Van Holde K.E., (1974), *J. Mol. Biol.*, 83, 459-471
- (56) Houssier C., Bontemps J., Emonds-Alt X. and Fredericq E., (1977), *Ann. N. Y. Acad. Sci.*, 303, 170-189
- (57) Klevan L. and Crothers D.M., (1977), *Nucl. Acids Res.*, 4, 4077-4089
- (58) Klevan L., Hogan M., Dattagupta N. and Crothers D.M., (1978), *Cold. Sprg. Harb. Symp. Quant. Biol.* in press
- (59) Rill R.L., (1972), *Biopolymers*, 11, 1929-1941
- (60) Van Holde K.E., Sahasrabudhe C.G., Shaw B.R., Van Bruggen E.F.J. and Arnberg A., (1974), *Biochem. Biophys. Res. Comm.*, 60, 1365-1370
- (61) Pardon J.F., Worcester D.L., Wooley J.C., Tatchell K., Van Holde K.E. and Richards B.M., (1975), *Nucl. Acids Res.*, 2, 2163-2176
- (62) Bustin M., Goldblatt D. and Sperling R., (1976), *Cell*, 7, 297-304
- (63) Langmore J.P. and Wooley J.C., (1975), *Proc. Natl. Acad. Sci. USA*, 72, 2691-2695
- (64) Hyde J.E. and Walker I.O., (1975), *Nucl. Acids Res.*, 2, 405-421
- (65) Varshavsky A.I. and Georgiev G.P., (1975), *Mol. Biol. Rep.*, 2, 255-262
- (66) Wooley J.C. and Langmore J.P., (1977), *J. Supramol. Structure Suppl.*, 1. Abstr. 103

- (67) Sussman J.L. and Trifonov E.N., (1978), Proc. Natl. Acad. Sci. USA, 75, 103-107
- (68) Bakke A.C., Wu J.R. and Bonner J., (1978), Proc. Natl. Acad. Sci. USA, 75, 705-709
- (69) Small E.W. and Isenberg I., (1977), Biopolymers, 16, 1907-1928