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ELECTRIC BIREFRINGENCE OF DNA AND CHROMATIN. INFLUENCE OF DIVALENT CATIONS.

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The effects of divalent cations on the DNA and chromatin conformation have been investigated by electric birefringence and birefringence relaxation measurements at low and constant ionic strength (0.001). An important decrease of the intrinsic optical anisotropy of DNA has been found in the presence of Mn^{2+} and Cu^{2+}, but not with Mg^{2+}. A complex variation of the mean relaxation time with the ratio *I/P* of ion to DNA-phosphate molar concentration has been evidenced in the presence of Mn^{2+} and Cu^{2+}, while the mean relaxation time monotonously decreased in the presence of Mg^{2+}. These observations are interpreted in terms of a specific organization of DNA in a compact, rigid structure, in the presence of Mn^{2+} and Cu^{2+}, and a non-specific coiling in the presence of Mg^{2+}. Drastic conformational changes encountered by chromatin in the presence of Mg^{2+} and Mn^{2+} cations have also been evidenced through electric birefringence measurements. They are interpreted by the formation of a superhelical compact arrangement of nucleosome strings which yielded a reversal of the birefringence sign with respect to the negative anisotropy observed in the presence of Na^{+} ions. The removal of the histone H1 prevented the appearance of this quaternary structure. More extended fragments of the chromatin chain obtained by ECTHAM chromatography of sonicated chromatin could not afford such compact arrangements.

1. Introduction

The presence of metal cations strongly bound to native samples of DNA, RNA and chromatin has been demonstrated by radiochemical methods and emission spectroscopy. The most abundant are Mg^{2+}, Ca^{2+}, Mn^{2+}, Zn^{2+} and Cu^{2+}, their relative proportion in the analysed samples depending on their origin. They play an essential and functional role in many enzymic reactions; they are involved in the transfer of genetic information and in the degradation of the nucleic acids. They have also stabilizing effects on the structure of nucleic acids and of their complexes with proteins (chromatin, ribosomes). These different aspects have been discussed in several recent reviews [1–3].

Mg^{2+} ions interact with the phosphate groups of DNA by electrostatic attraction. In addition to this process, cations of transition metals such as Mn^{2+} and Cu^{2+} can form a coordination complex with nucleic acids bases; this coordination predominantly occurs at guanine sites in DNA [1–15]. Mn^{2+} and Cu^{2+} form bidentate complexes with a phosphate group and the N_7 of guanine (chelation) [1–4, 6–10,13–15]. In the case of Cu^{2+}, the binding takes also place inside the double helical structure and involves either a single G base [13–15], two bases of adjacent G-C planes (intercalation) [7,8] or two bases of the same G-C pair (insertion) [13–15]. In chromatin, the binding of divalent cations, especially Mg^{2+} and Mn^{2+}, is influenced by the presence of histones; their number of binding sites is reduced but their binding constant does not seem to be appreciably affected as compared to DNA [16–18].

Recent physico-chemical studies on the interaction of metal ions with nucleic acids aimed to determine the conformational changes induced in macromolecular structure upon their binding, with the hope of elucidating their mechanism of action in biological processes.

An alteration of the circular dichroism spectrum indicative of a conformational change in the DNA

structure has been reported in the case of transition metal cations [4–7,9]; this conformational change seems to be related to a B to C transition of the secondary structure of DNA.

In electron microscopy, chromatin fibers at low ionic strength consists of strings of spherical particles (nucleosomes), 80 to 100 Å diameter, connected by fibrils sometimes as thin as 15 Å (for a review, see refs. [19–22]). At physiological salt concentration and in the absence of chelating agents, this linear arrangement of beads condenses into fibers of 200 to 300 Å diameter, which seems to be the native structure in nuclei [18–22]. There is good evidence from electron microscopy studies to support the proposal that divalent cations [18,22,23] and histone H1 [23–25] are involved in the stabilization of this condensed, thick chromatin fiber. It seems also that the presence of histone H1 is always required in order to observe such a change in the diameter of the chromatin fiber, even in the presence of divalent cations [23,26]. These results must be correlated with the studies of the ionic condensation of chromatin in solution which intimately depends on the presence of histone H1 [27–30]; in this process, divalent cations also appear to be about fifty times more effective than monovalent cations [29,30]. All these results point out the dominant role played by divalent cations and histone H1 in the structural organization of condensed chromatin; it seems also plausible that divalent cations and histone H1 act jointly.

In the present work, we describe results of electric birefringence and birefringence relaxation which will give us some further insight into the conformational changes induced in the DNA and chromatin structure upon binding of divalent cations. We also studied the effect of divalent ions on H1-depleted chromatin and on two chromatographic fractions of chromatin.

2. Material and methods

Calf thymus DNA was prepared using the detergent method [31]. Native samples having molecular weights of the order of 12×10^6 were obtained. Stock solutions at about 2 g l^{-1} were dialysed against double distilled water (pH adjusted at 6.5 by addition of a dilute NaOH solution) in order to eliminate the excess of ions precipitated with the DNA at the end of its purification.

After isolation of cell nuclei following the method of Chauveau et al. [32], the chromatin from calf thymus was extracted according to Fredericq [33], dialysed against double distilled water at pH 7 and stored at −15°C. Prior to their use, the solutions of the gel-forming chromatin (at a concentration of about 50 mg dl^{-1} in DNA) were sonicated either during 15 min at 20 kHz or during 30 min at 800 kHz as already described [34]. Sonicated chromatin had a sedimentation coefficient of about 37 S in 1 mM NaCl by band centrifugation. Its protein to DNA weight ratio was about 1.5–1.7. Chromatin depleted from histone H1 was obtained using the procedure of Bolund and Johns [35]. The chromatography of sonicated chromatin on ECTHAM cellulose was performed as described by Simpson and Reeck [36]. Elution by 0.01 M tris-0.01 M NaCl solution yielded a broad peak of which the early-eluted (CH-I or condensed fraction) and late-eluted (CH-II or extended fraction) fractions corresponding to the first and last 10% in volume, were separated. These eluates were dialysed against double distilled water and in some cases concentrated by evaporation under vacuum. The CH-I and CH-II fractions showed in 1 mM NaCl, thermal denaturation temperatures of 83 and 77°C, and protein to DNA weight ratios of 2–2.2 and 1.3–1.6, respectively. In circular dichroism, the $\Delta\epsilon$ (280 nm) of the CH-I and CH-II fractions were 0.65 and 1.5, respectively. These characteristics are comparable to the differences between the condensed and extended fractions obtained by Simpson and Reeck [36].

Sample solutions of DNA or chromatin were obtained by direct dilution of the stock solutions in the unbuffered medium of appropriate ionic composition. All the cations studied (Na^+, K^+, Mg^{2+}, Mn^{2+} and Cu^{2+}) were introduced in the form of chloride salts. The various ratios of divalent cation to DNA-phosphate molar concentrations (I/P) were made at constant I (3.33×10^{-4}) and variable P (ranging from 6.2×10^{-5} to 6.2×10^{-4}), except at low I/P (below 0.5) where P was maintained constant (3.1×10^{-4}) while I varied (1.6×10^{-5} to 1.6×10^{-4} M). The total ionic strength of the solutions was always kept constant at 10^{-3} by addition of a dilute NaCl solution if necessary (at low I/P). The pH of chromatin solutions was kept at about 7 ± 0.3 by addition of a dilute NaOH solution while the pH of DNA solu-

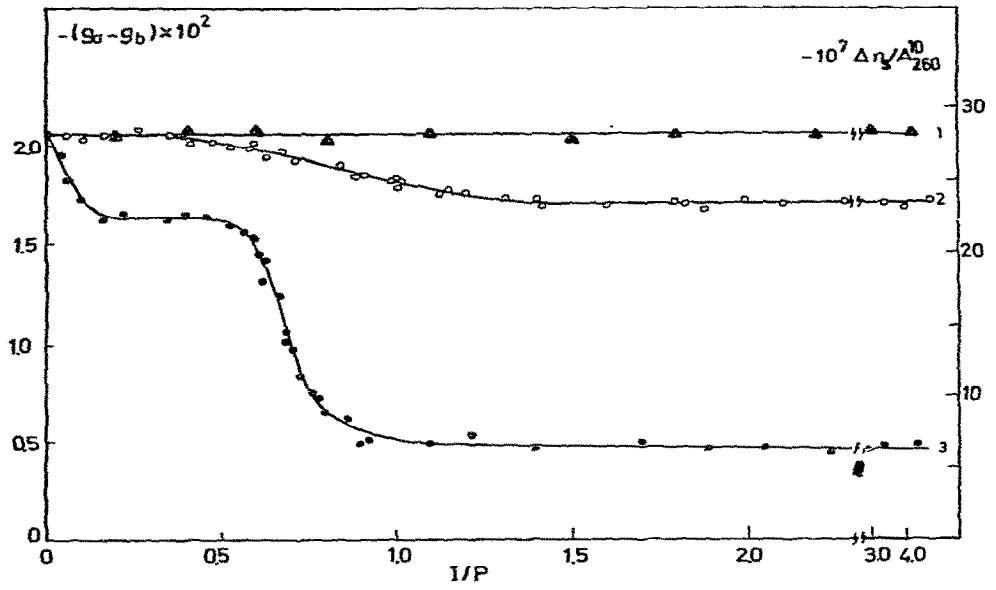

Fig. 1. Variation of intrinsic optical anisotropy of native DNA with increasing ratio I/P; (1) Mg^{2+}, (2) Mn^{2+}, (3) Cu^{2+}. Ionic strength: 10^{-3}; pH 6–6.5. The optical anisotropy factor $(g_a - g_b)$ is obtained from the experimental data $\Delta n_s/A^{10}_{260}$ by the relation $(g_a - g_b) = (\Delta n_s/A^{10}_{260})nE^{1g/ml}_{260}/2\pi\bar{v}$, where n is the solution refractive index (1.33 for dilute aqueous solution), $E^{1g/ml}_{260}$ the extinction coefficient in the (ml/g)cm^{-1} scale at 260 nm (20 000 for DNA); the partial specific volume $\bar{v}$ is equal to 0.55 cm^3/g in 1 mM NaCl, and has been considered constant under all our experimental conditions. This approximation does not introduce any appreciable error in the values of $g_a - g_b$ since it has been shown that the variations of $\bar{v}$ in the presence of Mg^{2+} and Mn^{2+} are very small [7]. It seems reasonable to consider that these variations are also small in the presence of Cu^{2+}.

tions ranged from 6 to 6.5. The DNA concentrations of the solutions was determined spectrophotometrically, taking $\epsilon(260) = 6450$ or 6800 M^{-1} cm^{-1} for purified DNA or chromatin, respectively [33].

Electric birefringence and birefringence relaxation measurements were performed using the technique previously described in detail [34,37]. An appreciable improvement of the experimental setting has been achieved by use of a very fast transient recorder (Biomation model 8100) interfaced to a minicomputer (Modular computer systems, II/10) with a 10^6 words disc unit. A general description of the whole system and an indication of the stability and reproducibility of the measurements have been presented elsewhere [38].

The analysis of the field strength dependence of the steady-state anisotropy (Δn) yields the orientation function Φ which gives access to the permanent and induced dipole terms, while the saturation value of the birefringence Δn_s yields the intrinsic optical anisotropy factor of the particles $(g_a - g_b) = \Delta n_s \cdot (n/2\pi c\bar{v})$, n is the refractive index of the solution, c the polymer concentration and $\bar{v}$ the partial specific volume of the polymer [34,37,39]. This analysis was performed with a general multiparametric program for non-linear least-square fittings (available from Meites [40]).

For all the results presented in this work, the orientation function used was that of a pure induced dipole orientation mechanism, since the small contribution of permanent dipole or slow polarizability detected in some cases by the reversing pulse technique has no significant influence on the shape of the field strength dependence of the birefringence [34]. In order to obtain a satisfactory fitting of the data, three terms with different polarizabilities had to be introduced and the birefringence curve was represented by the general equation [37,39]:

$$\Delta n = \Delta n_s \sum_{i=1}^{3} \phi_i \Phi(\Delta\alpha_i) \qquad (1)$$

where $\Delta\alpha_i$ is the electrical polarizability anisotropy and ϕ_i the weighting factor. The use of a maximum of

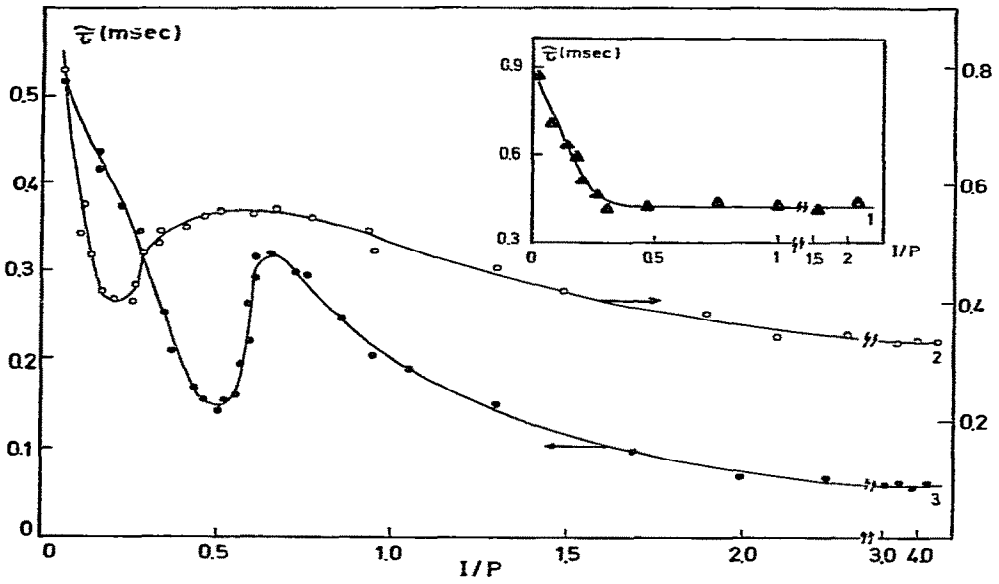

Fig. 2. Dependence of the mean relaxation times $\bar{\tau}$ of DNA on the ratio I/P; (1) Mg^{2+}, (2) Mn^{2+}, (3) Cu^{2+}. Ionic strength: 10^{-3}; pH: 6–6.5. The mean relaxation time of DNA in the presence of Na^+ or K^+ ions is equal to 870 ± 20 μs. All the relaxation times are determined at E = 13 kV/cm.

three terms is imposed by the limit of accuracy of such a fitting procedure for which the relative uncertainty on the mean polarizability values $\Delta\bar{\alpha} = \Sigma\phi_i\Delta\alpha_i$ may reach more than 20 to 30 percents. We recognize that such a procedure is only a provisional empirical approach in the absence of appropriate treatment of the electro-optical properties of flexible polymer chains.

The relaxation of the birefringence after the sudden removal of the electric field is given, for a polydisperse solution, by [37]:

$$\Delta n_t = \sum_i \Delta n_{0,i} \exp(-t/\tau_i). \qquad (2)$$

The relaxation times τ_i were determined from the birefringence decay curve with the aid of the same multiparametric curve-fitting program as used for the birefringence versus field strength curves. Generally, three relaxation times had to be taken into account in order to obtain a satisfactory fitting of the data. The mean relaxation time $\bar{\tau} = \Sigma_i \Delta n_{0,i}\tau_i / \Sigma_i \Delta n_{0,i}$ will be considered in the discussion of the results.

All the electro-optical measurements were made at room temperature (20°C) and at a wavelength of 550 nm.

3. Results and discussion

3.1. Electro-optical behaviour of DNA

Strong evidence for binding effects and conformational changes in the double helical structure of native DNA upon interaction with metal ions was obtained from the determination of the intrinsic optical anisotropy in the presence of Mg^{2+}, Mn^{2+} and Cu^{2+} cations at various I/P values (fig. 1).

In the presence of Mg^{2+} cations, the $(g_a - g_b)$ value of DNA is equal to $-(2.05 \pm 0.03) \times 10^{-2}$, independently of the ratio I/P. This value is the same as that determined in the presence of monovalent cations, irrespective of their nature, in agreement with flow birefringence observations [41]. No influence of the concentration of DNA (in the range of 2 to 20 mg%) nor of the nature of other buffered media used (cacodylate, citrate, phosphate or tris-HCl, in the pH range

5 to 9) on the $(g_a - g_b)$ value has been noticed. The absence of modification of the intrinsic optical anisotropy upon Mg^{2+} binding as compared to monovalent cations, was also observed in flow birefringence [41] and electric dichroism [42] studies in ionic strength conditions quite different from those used in the present work. On the contrary, in the case of Mn^{2+} and Cu^{2+} cations, the optical anisotropy of DNA, which remained negative, decreased with increasing I/P, in a two-phases process for the Cu^{2+}-DNA interaction (fig.1). We observed the same general trend for the variation of $\Delta n/C$ with I/P at high and low fields which reflects the small variation of polarizability (see below) also evidenced by the small modification of the shape of the orientation function. These modifications cannot simply be explained in terms of changes in the DNA conformation from a B to C-like structure as the circular dichroism observations suggest [4–7,9] since this kind of conformational change would produce a tilting of the base planes with respect to the helical axis of less than 8°. We rather think that the observed decrease of anisotropy reveals the organization of the DNA in a more compact structure as a result of the coordination of Mn^{2+} and Cu^{2+} ions to electron-donating sites of the bases.

The formation of a more compact structure is clearly evidenced by the distinct shape of the decrease of the mean relaxation time $\bar{\tau}$ in the presence of Mn^{2+} and Cu^{2+} cations. At low I/P (below 0.2 and 0.5 for Mn^{2+} and Cu^{2+}, respectively) the mean relaxation time decreased sharply; an increase of $\bar{\tau}$, corresponding to an extension of the macromolecule then follows at higher I/P, with a subsequent decrease characterizing a recondensation of DNA at I/P above 0.7–0.8 (fig. 2). This particular biphasic behaviour indicates that the DNA condensation in the presence of Mn^{2+} and Cu^{2+} ions proceeds by a very complex mechanism. On the contrary, $\bar{\tau}$ monotonously decreased in the presence of Mg^{2+}, reaching a constant value at I/P above 0.5. The relative decrease of the relaxation times is to be related to the sedimentation behaviour previously reported for DNA in the presence of Mg^{2+}, Mn^{2+} and Cu^{2+} cations [6].

It should however be emphasized that, in our opinion, the relaxation times are related in a complex way to the flexibility and overall dimensions of the molecules investigated so that it is not possible to derive quantitative estimations of changes of their overall size or persistence length.

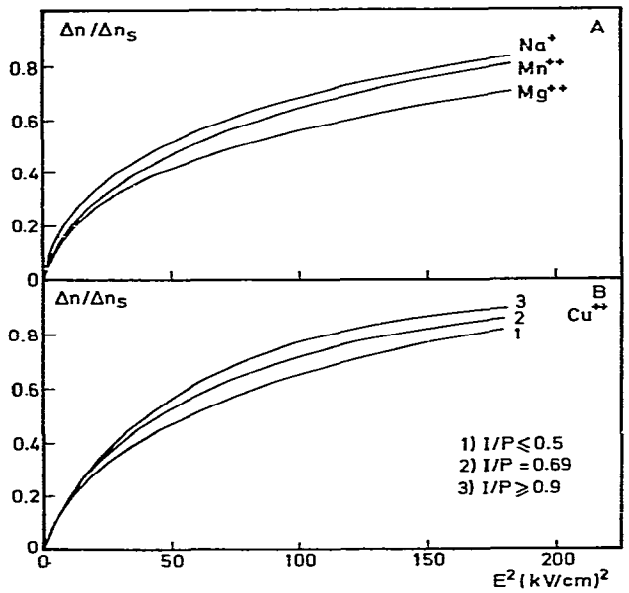

Fig. 3. Influence of Mg^{2+}, Mn^{2+} and Cu^{2+} ions on the field strength dependence of the DNA orientation function $\Phi = \Delta n/\Delta n_S$. Ionic strength: 10^{-3}; pH: 6–6.5.

The field strength dependence of the orientation function $\Phi = \Delta n/\Delta n_S$ is presented in fig. 3. It is difficult to realize a quantitative study of the variation of polarizability with the nature of the cation and with the ratio I/P because of the low accuracy of the polarizability determination from the fitting of the function Φ, which implies to take into account three terms of polarizability (see sect. 2). Consequently, we have preferred to make a qualitative comparison of the curves $\Phi = f(E^2)$.

The following general features were evidenced in this way (fig. 3):

i) No significant difference was found between Na^+-DNA and K^+-DNA ($\Delta\bar{\alpha} \approx 22 \times 10^{-32}$ F m^2) in agreement with the observations of Hornick and Weill [41]; in the work of these authors, however, the optical anisotropy factor was deduced from flow birefringence measurements and the electric polarizability subsequently obtained from the Kerr constant.

ii) While the polarizability was found only slightly lower with Mn^{2+} ($\Delta\bar{\alpha} \approx 18.5 \times 10^{-32}$ F m^2) com-

pared to Na^+ and K^+, a more important decrease of $\Delta\bar{\alpha}$ occurred in the presence of Mg^{2+} ($\Delta\bar{\alpha} \approx 15.5 \times 10^{-32}$ F m^2) as previously reported for sonicated DNA [41]; no regular variations of the electric polarizability with *I/P* were detected for Mg^{2+} and Mn^{2+}.

iii) In the case of Cu^{2+}-DNA, the $\Delta\bar{\alpha}$ value at low *I/P* ($\approx 13 \times 10^{-32}$ F m^2) was smaller than in the presence of Na^+ and K^+, increased with *I/P* (fig. 3), reaching a plateau at *I/P* above 0.9 with values of the same order or slightly higher than those of Na^+-DNA or K^+-DNA (data not shown).

The interpretation of the electrical polarizability changes observed in the presence of divalent cations is rendered difficult by the concomitant conformational change of the DNA occurring. According to the theory, the electrical polarizability is dependent on the square of the charge of the counterion and on the third power of the length of the polyion [41,43,44]. In particular, the molecular flexibility of native DNA which alters the effective length of the polyelectrolyte could greatly influence its electrical polarizability. If the polarizability was following the changes in dimension reflected by the decrease of the mean relaxation times (fig. 2), then it should decrease by a factor of about 10 in the presence of Cu^{2+} at high *I/P*, in opposition with the experimental findings.

A general interpretation of the decrease of polarizability can be found in the increase of the electrostatic repulsion between counterions as a result of the increase of their charge and of the decrease of the thickness of the divalent counterion layer [41,43, 44]. In the case of Mg^{2+}-DNA, an increase of flexibility of the DNA chain would also occur as a consequence of the decrease of electrostatic repulsion between the phosphate groups, while for the Mn^{2+} and Cu^{2+} interaction, a stiffening of the DNA structure associated with the conformational change suggested above, seems to us the best explanation compatible with the decrease of relaxation times.

3.2. Electro-optical behaviour of chromatin

The electro-optical observations on chromatin (CH), on its chromatographic fractions (CH-I and CH-II) and H1-depleted chromatin in Na^+-salt solutions are summarized in table 1 and fig. 4 (part A). The lower value of the intrinsic optical anisotropy ($\Delta n_s/A_{260nm}^{10mm}$) compared to Na^+-DNA evidences a significant change in the orientation of the DNA bases with respect to the orientation axis in the electric field, when DNA is incorporated into chromatin. It results from the folding of chromatin DNA into a tertiary structure [34,45], especially in the nucleosome subunits. H1-depleted chromatin is very similar to whole chromatin in regard to its optical ($\Delta n_s/A$), hydrodynamical ($\bar{\tau}$) (table 1) and electrical ($\Delta\alpha$) properties (table 1 and fig. 4); this is in good agreement with the observations of Frisman et al. [46] using viscosity and flow birefringence measurements. This may be explained on the basis of X-ray diffraction studies which have shown that histone H1 is not involved in the stabilization of the tertiary structure of chromatin [28,47,48]. The birefringence decay curves of whole chromatin and H1-depleted chromatin were not monoexponential; they could be fitted with a minimum of three exponential terms, giving mean relaxation time values of the order of 12—13 μs. On the contrary, CH-I and CH-II fractions showed monoexponential relaxation decays with $\bar{\tau}$ values of about 30 μs (table 1). They had very similar electrical polarizabilities as well as relaxation times while they both showed lower optical anisotropy than whole chromatin.

Strong evidence for conformational changes in the chromatin structure in the presence of divalent cations was also obtained from this electro-optical study. Complex variations of the optical anisotropy and of the relaxation times with *I/P* were evidenced, with a distinct behaviour for the various samples (figs. 4 and 6, table 1). The general trends of the observations may be summarized as follows.

While the electric birefringence of DNA in the presence of Mg^{2+} and Mn^{2+} cations remained negative, a change of its sign was observed in the case of chromatin, at high *I/P*. The birefringence sign is negative at low *I/P* either in the presence of Mg^{2+} or with Mn^{2+} ions but becomes positive at *I/P* above 0.8 in the presence of Mn^{2+}. Composite signals with positive and negative birefringence contributions were observed at $0.3 < I/P < 0.8$ and $I/P > 0.5$ for Mn^{2+} and Mg^{2+} respectively. The particular shape of the electro-optical signals observed was perfectly reproducible and remained unchanged upon consecutive pulsing of the same sample or under pulses of longer duration; the effect is thus not due to an artefact. The

Table 1
Electro-optical behavior and parameters of whole chromatin, its chromatographic fractions and H1-depleted chromatin in the presence of Na^+, Mg^{2+} and Mn^{2+} cations. The symbol neg/pos indicates that the sample solution shows a composite electro-optical signal

Sample	Cation	*I/P* range	Birefringence sign	Optical anisotropy $(\Delta n_s/A_{260}^{10}) \times 10^7$	Mean electric polarizability $\Delta\bar{\alpha} \times 10^{32}$ ($F\,m^2$)	Mean relaxation time $\bar{\tau}$ (μs) at $E = 13$ kV/cm
CH	Na^+	–	neg	−7.4	14	13 ± 1
	Mg^{2+}	0–0.5	neg	decrease with *I/P* (fig. 2A)	11	decrease with *I/P* (fig. 4A)
		>0.5	neg/pos	neg. comp.: decrease with *I/P* (fig. 2B); pos. comp.: 0.28 (constant)	neg. comp.: 11 pos. comp.: 25	neg. comp.: 10–15 pos. comp.: ≈200
	Mn^{2+}	0–0.3	neg	decrease with *I/P* (fig. 2A)	11	decrease with *I/P* (fig. 4A)
		0.3–0.8	neg/pos	neg. comp.: decrease with *I/P* (fig. 2C,B); pos. comp. 0.77 (constant)	neg. comp.: 11 pos. comp.: 25	neg. comp.: 10–15 pos. comp.: ≈200
		>0.8	pos	increase with *I/P* (fig. 2C)	≈80	decrease with *I/P* (fig. 4A)
H1-depleted CH	Na^+	–	neg	−7.7	14	12 ± 1
	Mn^{2+}	0–5	neg	decrease with *I/P* (fig. 2D)	11	decrease with *I/P* (fig. 4A)
CH-I	Na^+	–	neg	−3.5	14	29 ± 1
	Mg^{2+}	>0.5	pos	+0.9 (constant)	25	210 ± 11 (constant)
	Mn^{2+}	>0.5	pos	+2.7 (constant)	25	180 ± 10 (constant)
CH-II	Na^+	–	neg	−5.6	14	31 ± 1
	Mg^{2+}	0–5	neg	−5.6 (constant)	11	31 ± 2 (constant)
	Mn^{2+}	0–5	neg	decrease with *I/P* (fig. 2E)	11	decrease with *I/P* (fig. 4B)

occurrence of composite electro-optical signals with solutions of biological or synthetic macromolecules under pulsed electric fields has been reported in a number of cases [37,49], and the procedure for their decomposition has been described [49].

Since the electric polarizability corresponding to the positive birefringence component was much larger than that of the negative component, saturation of the former one occurred at lower field so that the composite signals were observed at low fields as in previous studies. The origin of this behaviour was believed to be in the presence of two entities, orientable in the electric field, which in the case of chromatin, could be thought as being the extended and condensed fragments CH-I and CH-II. The electric birefringence of H1-depleted chromatin, on the other hand, always remained negative, even in the presence of high Mn^{2+} contents.

The field strength dependence of the orientation function $\Delta n/\Delta n_s$, which is directly correlated to the electric polarizability, is presented in fig. 5 for the different experimental conditions studied. The following general features were found: (i) no significant difference was found between the effect of Mg^{2+} and Mn^{2+} ions on the electric polarizability; (ii) no regular dependence of the electric polarizability on *I/P* was observed, except in the case of whole chromatin at *I/P* above 0.8, in the presence of Mn^{2+} where it slightly increased

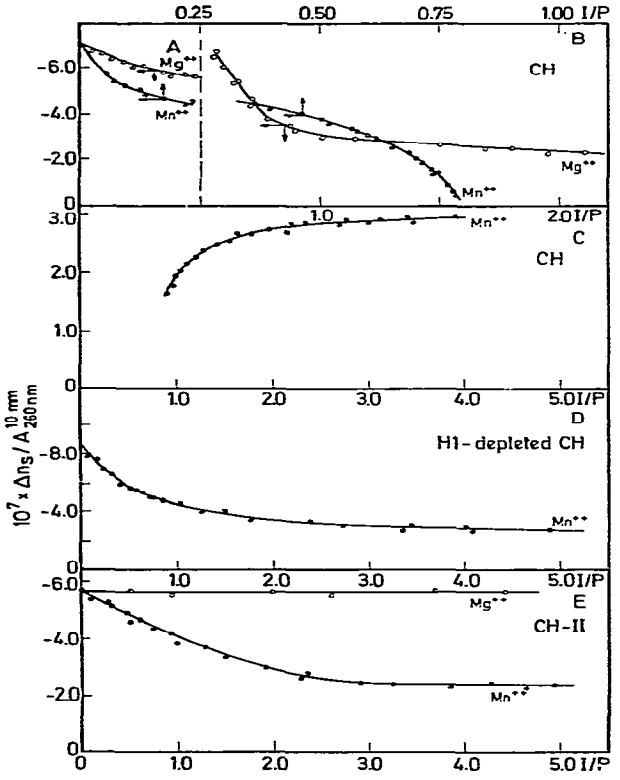

Fig. 4. Intrinsic optical anisotropy of whole chromatin (CH), H1-depleted chromatin and of the CH-II chromatin fraction in the presence of Mn^{2+} and Mg^{2+} as a function of *I/P*. Part A: negative birefringence of CH at low *I/P*; Part B: negative component of complex electro-optical signals of CH (slightly higher anisotropy of this sample in the absence of divalent cations, as compared to the sample used for the measurements of part A); part C: positive birefringence of CH in the presence of Mn^{2+} at high *I/P*.

with the ratio *I/P*; (iii) an important increase of electric polarizability was always noted when the birefringence became positive, while its value was of the same order as that determined in the presence of Na^+ when the birefringence was negative, independently of the nature of the chromatin fraction considered. As for DNA, we believe that the absence of variation or the increase of electric polarizability observed here reflects an important increase of rigidity of the chromatin structure which counterbalances the decrease of polarizability due to the counterion charge effects. This would be related to the formation of a compact and more rigid quaternary structure as we shall discuss below.

We shall now consider in more detail the distinct behaviour of optical anisotropy and of relaxation, displayed by whole chromatin, H1-depleted chromatin and by the CH-I and CH-II fractions, as a function of *I/P*.

(i) Whole chromatin. In the case of whole chromatin, a decrease of the intrinsic optical anisotropy was observed at low *I/P* (where the birefringence is negative) in the presence of Mg^{2+} and Mn^{2+} ions (fig. 4, part A); this reflects the beginning of condensation of the chromatin structure. At $0.3 < I/P < 0.8$ and above 0.5 for Mn^{2+} and Mg^{2+} respectively (composite signals), the optical anisotropy of the positive birefringence component was constant at all *I/P* (table 1). Consequently, the molecular entity responsible for this component is strongly condensed (positive birefringence) and the condensation which is more important in the presence of Mn^{2+}, has reached a maximum value (constancy of the anisotropy with *I/P*). On the other hand, the molecular entity responsible for the negative component of the birefringence is more progressively folded when *I/P* increases (fig. 4, part B); this folding is restricted in the presence of Mg^{2+} cations since the anisotropy reaches a constant negative value at very high *I/P*. In the presence of Mn^{2+} cations, the folding becomes gradually more pronounced until a positive optical anisotropy is reached for the overall chromatin structure (fig. 4, part C). These results also show that, in opposition to the case of DNA, Mg^{2+} ions are able to induce a specific folding of the chromatin structure as well as Mn^{2+} ions. Consequently, this folding primarily depends on the binding of divalent cations to phosphate residues. However, it can only become important in the presence of Mn^{2+} which also indicates the requirement for an interaction of the cation with the DNA bases.

Important variations of the relaxation times have been evidenced (fig. 6, table 1). At low *I/P*, a very small decrease of the mean relaxation time $\bar{\tau}$ was observed in the presence of Mg^{2+}, while Mn^{2+} yielded an extension phase similar to that observed for DNA, as shown by the increase of $\bar{\tau}$ in the range of $I/P \approx 0.2$ (fig. 6, part A). At *I/P* above 0.3 and 0.5 for Mn^{2+} and Mg^{2+} respectively (composite signals) the mean relaxation time of the negative birefringence component remained very close to the value observed in the presence of Na^+ ions (table 1) and was not appreciably affected by the ratio *I/P*. The positive birefringence

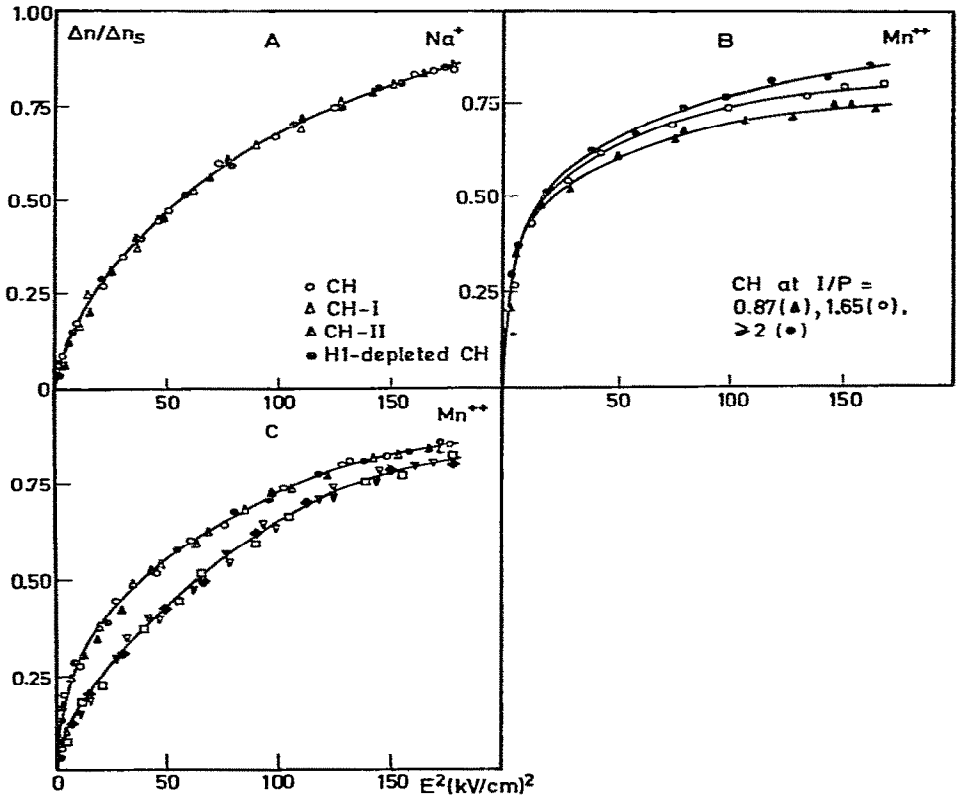

Fig. 5. Field strength dependence of the electric birefringence of whole chromatin (CH), of its chromatographic fractions (CH-I and CH-II) and of H1-depleted chromatin in the presence of Na^+ (part A) and Mn^{2+} (parts B and C). Part C: ○, △: CH at $I/P = 0.4$ and 0.65 respectively, for positive birefringence component. ◆, □: CH at I/P below and above 0.3 respectively, for negative birefringence component. ●, ▲: CH-I at $I/P = 0.5$ and 2 respectively. ▼, ▽: CH-II and H1-depleted chromatin, respectively for $0 < I/P < 5$.

component showed much larger $\bar{\tau}$ values independent of the nature of the divalent cation and of I/P (table 1). The reported values of $\bar{\tau}$ in the case of composite birefringence signals should be considered only as rough estimations since their determination was made difficult by the decomposition of the signals. When whole chromatin displayed a pure positive birefringence, i.e. in the presence of Mn^{2+} at I/P above 0.8, $\bar{\tau}$ was greater than the values determined in the presence of Na^+ ions and monotonously decreased with increasing I/P (fig. 6, part A).

(ii) H1-depleted chromatin. The interaction of Mn^{2+} ions with H1-depleted chromatin produced a decrease of the intrinsic optical anisotropy (fig. 4, part D) without change of the birefringence sign. Thus, the absence of histone H1 does not appear to prevent a restricted folding of the chromatin structure but its presence seems to be required for the formation or stabilization of the folded structure responsible for the positive birefringence. A phase of extension of the chain was also evidenced by the increase of the mean relaxation times in the range of $I/P \approx 0.4$, followed by a continuous decrease of $\bar{\tau}$ at higher I/P (fig. 6, part A).

(iii) CH-I and CH-II fractions. More distinctive features for the behaviour of the CH-I and CH-II fractions were observed in the presence of Mg^{2+} and Mn^{2+} than in 1 mM NaCl solution. The electric birefringence of CH-II remained negative at all I/P either in the presence of Mg^{2+} or Mn^{2+} cations, while CH-I displayed

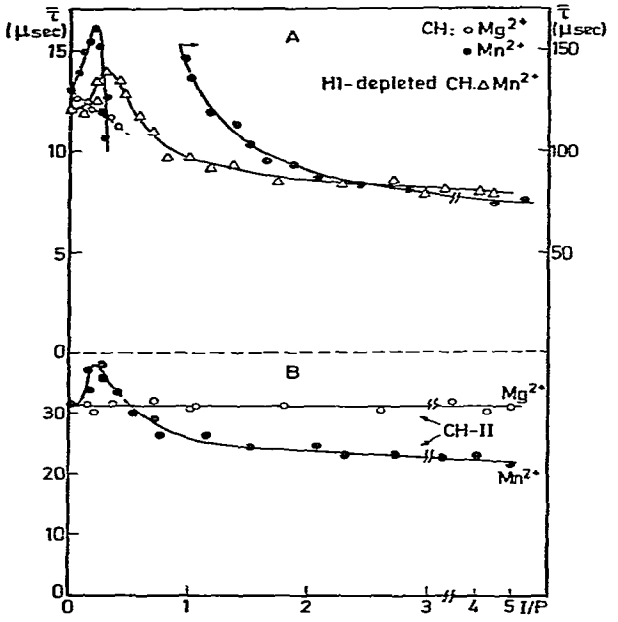

Fig. 6. Dependence of the mean relaxation times $\bar{\tau}$ on the ratio I/P in the presence of Mn^{2+} and Mg^{2+}, for whole chromatin (CH; negative birefringence component (left scale) and positive birefringence component (right scale)), for H1-depleted chromatin, and for the CH-II chromatin fraction. All the relaxation times are determined at $E = 13$ kV/cm.

a positive birefringence at high I/P (fig. 4, table 1). In the presence of Mn^{2+}, the phase of extension of the chain evidenced by the variation of $\bar{\tau}$ at low I/P in the case of whole chromatin, H1-depleted chromatin, and of DNA, was also observed with the CH-II fraction (fig. 6, part B) but not with the CH-I fraction. The mean relaxation times of the latter fraction (in the presence of Mg^{2+} or Mn^{2+} and independently of the I/P ratio) were bigger than those determined for the former and were of the same order of magnitude as the values determined for the positive component of the composite signals of whole chromatin in the range of intermediate I/P values (table 1).

Composite electro-optical signals as observed with whole chromatin have never been detected in the case of the CH-I and CH-II fractions.

All these findings are indications of a possible correlation between the CH-I fraction and the molecular entity responsible for the positive contribution to the electric birefringence of whole chromatin in the presence of divalent cations.

4. General conclusions

The following conclusions can be drawn from the present study.

4.1. DNA

Binding of Mg^{2+} cations, which interact only with the phosphate groups and reduce the electrostatic repulsions between them, brings about a small and non-specific coiling of DNA by an increase of molecular flexibility which is evidenced by the decrease of relaxation times and, partially, by the modification of electric polarizability. This kind of conformational change does not produce any alteration of the intrinsic optical anisotropy of DNA since the macromolecular orientation in the electric field proceeds essentially by an orientation of smaller segments in which the arrangement of base planes is almost unaltered, as shown by circular dichroism studies [5,7].

On the contrary, coordination of Mn^{2+} and Cu^{2+} to DNA brings about a specific organization of this macromolecule into a more compact structure as shown by the drastic decrease of intrinsic optical anisotropy and relaxation times. This compact structure seems also to be more rigid as is qualitatively indicated by the electric polarizability determinations. Another possible explanation for the drastic decrease of optical anisotropy would be a modification of the intrinsic optical properties of the bases chromophores upon interaction with Cu^{2+} and Mn^{2+}, resulting in the appearance of a component of transition dipole moment parallel to the double-helical axis, without conformational modification. We do not favour this latter interpretation because of the important variations of the relaxation times, which are strong evidence for alterations in the DNA structure.

The difference in the amplitude of the structural changes observed and in the range of I/P where they occur can be accounted for by the different electronic structures of the Mn^{2+} and Cu^{2+} cations which can introduce slight differences in the geometry of the complexes formed, or by the fact that Cu^{2+} can form various kinds of complexes with DNA bases [7,8, 13–15] so that a competition between the modes of binding is always present.

The mechanism of DNA condensation in the presence of Mn^{2+} and Cu^{2+} seems to be very complex as dis-

played by the biphasic variation of relaxation times with I/P (fig. 2). The extension of the macromolecule at intermediate I/P could be interpreted by an unstacking of some bases which then permits a further condensation. Such an extension phase of the macromolecule prior to the condensation has been theoretically considered in the folding of DNA, by Crick and Klug [50]. In this context, the mode of binding of Mn^{2+} and Cu^{2+} by chelation to the phosphate group and the N_7 of guanine [1–4,6–10] seems to us to be particularly important since it can simultaneously induce perturbations in sugar-phosphate linkage and in base stacking.

As already stated in the discussion of the results, the interpretation of the changes in relaxation times cannot be made unambiguously because of the concomitant influence of flexibility and overall dimensions on this parameter. A more detailed examination of the influence of molecular weight on the observations described in this paper should be performed using monodispersed short DNA fragments such as those obtained with the aid of restriction enzymes.

Thus, our electro-optical study clearly shows that the change of DNA conformation in the presence of Mn^{2+} and Cu^{2+} is not only restricted to a small variation of secondary structure as previously reported in circular dichroism studies [4–7,9] but implies a great modification of the overall spatial structure of DNA.

4.2. Chromatin

Chromatin can also be condensed into a compact structure when interacting with divalent cations. At low ionic strength and in the absence of divalent metal cations, chromatin fibers of about 100 Å diameter consist of linear and flexible arrays of nucleosomes, while in the condensed state, a superhelical organization (quaternary structure) would be formed, yielding a chromatin fiber of 200–300 Å diameter in which each nucleosome is in close contact with four or six others [51,52]. If native chromatin corresponds to this model, then shearing and ultrasonic degradation which are statistical processes, would produce in solution a mixture of: (i) fragments with closely spaced nucleosomes; (ii) fragments with relatively long internucleosome segments resulting either from an unfolding of the original internucleosome segments or from a partial disruption of nucleosomes; (iii) perhaps some free nucleosomes with or without tail; (iv) variable amounts of fragments with many structural intermediates containing regions of closely spaced nucleosomes and other regions of more extended conformation.

Even though there is an important supercoiling of DNA helix in the nucleosomes, the intrinsic optical anisotropy of whole chromatin (and of the chromatographic fractions CH-I and CH-II) remains negative in the presence of Na^+ ions (table 1) probably because of a sufficient amount of unfolded internucleosome segments and of the absence of closely packed superhelical arrangement of nucleosomes. It should be noted that the electric dichroism of nuclease-resistant fragments of chromatin was also found to be negative [53]; these fragments are probably not isolated nucleosomes but linear arrays of nucleosomes or nucleosomes with tails, in which a sufficient amount of DNA is in an extended conformation.

The action of divalent cations can then be understood as a folding of the linear arrangement of nucleosome strings into a superhelical arrangement, in the presence of the histone H1. On the basis of the structural parameters presently reported for the nucleosome particle and for the condensed superhelical array of nucleosomes, we previously estimated [54] that the superhelicoidal axis of the nucleosome DNA should be perpendicular to the main axis of this quaternary structure in order for the resulting optical anisotropy to be positive, as observed in the presence of Mg^{2+} and Mn^{2+} at high I/P. This interpretation rests on the assumption that the form contribution to the measured anisotropy is not the dominant effect; the estimation of this contribution is still subject to such uncertainty that a satisfactory appreciation of its importance cannot be made in such cases. We cannot completely exclude the possibility that the changes in relaxation times and in the sign of the anisotropy could be partially due to some aggregation of the samples. Some turbidity was present for the highest I/P in the case of the chromatin-Mn^{2+} interaction but not in the case of DNA. It should however be emphasized that the most important alterations of the electro-optical parameters occurred for the highest I/P values where the polymer concentration is the lowest. The effect of the divalent cations would result from a decrease of electrostatic repulsion between the phosphate groups. Mn^{2+} ions, however, appear more effec-

tive for the formation of the quaternary structure owing to its specific interaction with the DNA bases. Indeed, the extension phase evidenced by the relaxation behaviour at low I/P during the condensation of the DNA chain, was also observed with chromatin (fig. 6). This would also occur in the internucleosome segments. The observation of larger relaxation times for the positive birefringence components is understandable on the basis of the much more rigid character of the compact superhelicoidal structures in comparison to the flexibility of the unfolded structures.

These interpretations are in line with the observations that the addition of divalent cations (Mg^{2+}, Ca^{2+}, Mn^{2+}) produces an important condensation of chromatin at ionic strengths smaller than in the presence of monovalent cations and that it is intimately dependent on the presence of histone H1 [27–30]. Electron microscopic observations of Finch and Klug [23] also indicated a helical arrangement of nucleosomes in the simultaneous presence of histone H1 and Mg^{2+} ions at a concentration of 2×10^{-4} M (our divalent cation concentration was 3.3×10^{-4} M). Rosenberg [55] also suggested that the removal of histone H1 produces an "opening" of the chromatin structure resulting from the unfolding of the internucleosome segments.

Although the ECTHAM chromatography of sonicated chromatin cannot be expected to allow a clear separation of two distinct entities, it can however yield two fractions CH-I and CH-II which display some distinctive features characteristic of more condensed and more extended regions of whole chromatin, respectively. The CH-I fraction would contain more closely spaced nucleosomes allowing the formation of compact superhelical arrangements with positive birefringence, while the presence of relatively long internucleosome segments in CH-II fraction inhibits this condensation. It would be now useful to study the electro-optical behaviour of nucleosome strings of various sizes obtained by mild micrococcal digestion.

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