Volume 262, number 1, 131–134 FEBS 08236 March 1990

Changes in chromatin chain flexibility during condensation induced by sodium chloride, as evidenced by electric dichroism

Roland Marquet, Marisa Favazza, Michel H.J. Koch* and Claude Houssier

Laboratoire de Chimie Macromoléculaire et Chimie Physique, Université de Liège, Sart-Tilman (B6), B-4000 Liège, Belgium and **European Molecular Biology Laboratory, c/o DESY, Notkestrasse 85, D-2000 Hamburg 52, FRG*

Received 10 January 1990

The electric linear dichroism of chicken erythrocyte chromatin has been measured as a function of NaCl concentration in the 1–28 mM ionic strength range, using a specially designed Kerr cell with reduced pathlength, and thus, smaller electrode surface. This allowed the determination of the dichroism of compact chromatin in conditions where artifacts due to possible contribution from turbidity are avoided, which was not the case for previous studies in the presence of di- or multivalent cations. The linear dichroism of compact chromatin was found to be positive, as expected from models of the 30-nm fibre in which the linker DNA runs perpendicular to the fibre axis. The dependence of the relaxation times on ionic strength reveals that the process of compaction is first accompanied by an increase in flexibility of the chain followed by a decrease, in the range of 5–10 mM NaCl, and a further decrease above 10 mM NaCl, corresponding to the compaction of the 30 nm fibre.

Chromatin; Compaction; Flexibility; Electric dichroism

1. INTRODUCTION

Linear dichroism is one of the most powerful methods, together with X-ray and neutron scattering, to obtain characteristic parameters of the solution conformation of compact chromatin fibres. Despite numerous flow and electric dichroism studies, there is however no full agreement yet about a definitive structural model ([1,2] and references therein). Flow linear dichroism (FLD) of chromatin condensed by monovalent or multivalent cations was found to be slightly positive [1]. In contrast, Crothers and coworkers [3–8] and McGhee and coworkers [9,10] measured negative electric linear dichroism (ELD) for chromatin fibers condensed by Mg^{2+} or multivalent cations.

Reconciliation of these conflicting results was thought to be attained with our observations of a positive ELD for chromatin above 400 μM $MgCl_2$ [1,11], indicating that the maximum compaction was probably not reached in the experiments of Crothers' and McGhee's groups. Sen and Crothers [4,5] deduced the point of maximum compaction from mean relaxation time measurements. Upon addition of di- and multivalent cations, a four- to fivefold decrease of $\bar{\tau}$ was observed at about 200 μM $MgCl_2$, followed by a two- to eightfold increase at higher concentrations. The authors postulated that the point of minimal relaxation time (or maximal relaxation rate) corresponded to the optimal compaction, and that further increase in $\bar{\tau}$ reflected aggregation. On the contrary, we measured the ELD in the whole range of chromatin solubility, up to 800–900 μM $MgCl_2$ [1].

Both approaches are subject to criticism: (i) the relaxation times depend not only on particle size (i.e. on the compaction factor of the chromatin fibre), but also on fibre flexibility which may be affected by cation addition, regardless of the compaction process; (ii) when measuring electric dichroism at high multivalent cation concentration, some turbidity was always present, which could affect the electro-optical signal (a small dichroism signal was also observed at 350 nm, outside the absorption region of the heterocyclic bases). However, the turbidity measured in 800 μM $MgCl_2$, was found to be only 2% of the absorbance at 260 nm [11].

In order to circumvent these two problems, we undertook electric dichroism measurements on chromatin samples as a function of sodium chloride concentration, up to the range where this monovalent salt can produce compaction, using a Kerr cell with small pathlength, and thus reduced electrode surface. Inducing chromatin compaction by monovalent cations excludes artifacts due to turbidity and sample aggregation, since no turbidity nor aggregation could be observed up to 80 mM NaCl. In parallel, we measured the salt concentration dependence of the dichroism relaxation times. This allowed us to determine if the

Correspondence address: C. Houssier, Laboratoire de Chimie Macromoléculaire et Chimie Physique, Université de Liège, Sart-Tilman (B6), B-4000 Liège, Belgium

Abbreviations: FLD, flow linear dichroism; ELD, electric linear dichroism

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/90/$3.50

relaxation times measurements could serve to determine the maximum compaction.

2. MATERIALS AND METHODS

Chicken erythrocyte chromatin was prepared as previously described [1,11]. ELD was determined with the previously described instrumentation [12,13]. Measurements at ionic strengths higher than 2–3 mM NaCl were made in a Kerr cell with a 1 mm optical pathlength and 3 mm separation between the Pt electrodes. This cell is identical to the cell used in the first electric-field X-ray scattering experiments [14,15], except that the windows were made of quartz SUPRASIL (Hellma, Germany). This cell allows us to study the Na^+-induced compaction of chicken erythrocyte chromatin up to 30 mM sodium chloride, in the 0–7 kV/cm electric field range. In order to increase the sensitivity of the detection, we used a 2 mm UV light beam width (instead of 1 mm with our usual cells).

Identical results were obtained when the final ionic strength was reached by addition of aliquots of either a 50 mM NaCl or a 200 mM NaCl stock solution in 1 mM sodium cacodylate pH 6.5. Thus, the addition of small volumes of the 200 mM sodium chloride solution produced no irreversible local aggregation.

3. RESULTS AND DISCUSSION

The field strength dependence of the ELD at increasing sodium chloride concentrations is shown in fig.1. At low ionic strength, a very strong dependence of the ELD on salt concentration was found (figs 1 and 2). This supports our previous observations [1] and suggests an explanation for some of the important variations in the negative ELD amplitudes reported for uncondensed chromatin by different groups, using slightly different low ionic strength buffers [4,9–11]. A sign reversal of the ELD was observed when 4 mM sodium chloride were added to the initial 1 mM cacodylate pH 6.5 buffer (figs 1 and 2): this is a substantially higher NaCl concentration than that required to produce a sign-change in FLD (2 mM NaCl, see [1]). The slightly positive dichroism (about +0.005) was constant between 7.7 and 28 mM NaCl. There was no modification in the shape of the field strength dependence of the ELD above 7.7 mM NaCl (data not shown). This indicates that addition of Na^+ cations, in the 8–28 mM range does not modify the mechanism of orientation of the chromatin fibres in the electric field. Thus, changes evidenced in this ionic strength range by FLD [1,16] are mainly due to a decrease in the hydrodynamic orientation function, at high ionic strength. This technique indeed uses very low orientation degrees and is therefore much more sensitive to changes in the orientation function and to polydispersity.

In the case of Na^+-induced chromatin compaction, we can be confident that the positive dichroism was not produced by turbidity anisotropy, since our chromatin samples displayed no turbidity up to 80–100 mM NaCl. No abrupt change in sedimentation coefficient [17] nor in mass per unit length [18], has been either observed in this ionic strength range. Our results are

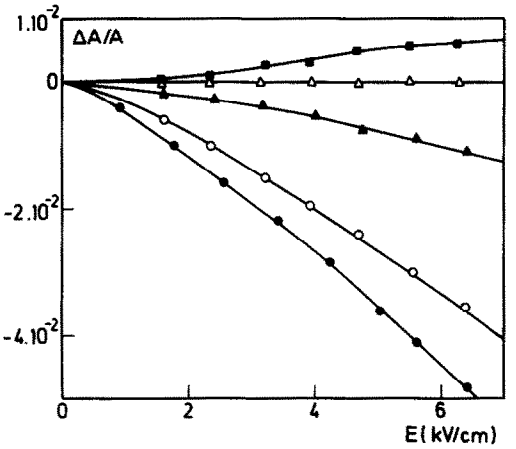

Fig.1. Field strength dependence of the ELD of chicken erythrocyte chromatin in the presence of Na^+: 0 (●), 0.49 (○), 1.92 (▲), 3.92 (△) and 7.7 (■) mM.

compatible with the observations of Yabuki et al. [8] and Lee et al. [6,7] who measured, at low ionic strength, the ELD of samples previously cross-linked at 80 mM NaCl.

The mean relaxation time ($\bar{\tau}$) of chromatin fibres, measured after the end of the electric pulse, as a function of NaCl concentration, displayed a more complex behaviour than the dichroism amplitude. The relaxation time first decreased from 480 μs to 25 μs when the Na^+ concentration was increased from 0 to 2 mM, and then rose to a maximum of 360 μs at 11.5 mM. Finally, $\bar{\tau}$ decreased monotoneously between 12 and 28 mM NaCl (fig.2). Comparison with other methods, such as electron microscopy and X-ray scattering [2,19,20] indicates that only the salt concentration dependence of $\bar{\tau}$ at Na^+ higher than 12 mM can reflect the change of fibre length upon compaction, while the measurements at lower ionic strength mainly reflect changes in the fibre flexibility. X-ray scattering measurements of Koch et al. [2] performed in the same buffer, at the same ionic strength, and with similar chromatin samples, indicated that the mass per unit length (m/l) of the chromatin fibres gradually increased when NaCl was added. The relative change of m/l with respect to the most compact state at 28 mM NaCl has been redrawn in fig.2 on the basis of these X-ray scattering data. The differences with our experimental data clearly indicate the predominance of flexibility effects in the relaxation behaviour of chromatin fibres.

Although our results were obtained with chicken erythrocyte chromatin, they can probably be extrapolated to chromatin from other sources. Fujiwara et al. [21] observed, with rat thymus chromatin, qualitatively the same behaviour as Koch et al. [2] with chicken erythrocyte chromatin. At ionic strengths below 5 mM NaCl, there is an expansion of the fibre characterized by a marked decrease in mass per unit length and in radius of gyration of the cross-section.

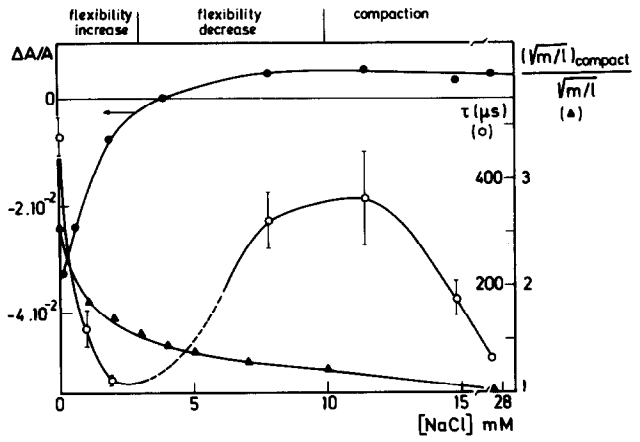

Fig.2. Influence of the Na^+ concentration on the ELD (•) and the mean relaxation time $\bar{\tau}$ (○) of CE chromatin at 4.7 kV/cm. The error bars correspond to $2d$. The symbols (▲) represent the compaction factor calculated on the basis of the $(I(O)_x)^{1/2} \div (m/l)^{1/2}$ values taken from Koch et al. [2]. The various stages of compaction depicted in fig.3 are indicated on top of this figure.

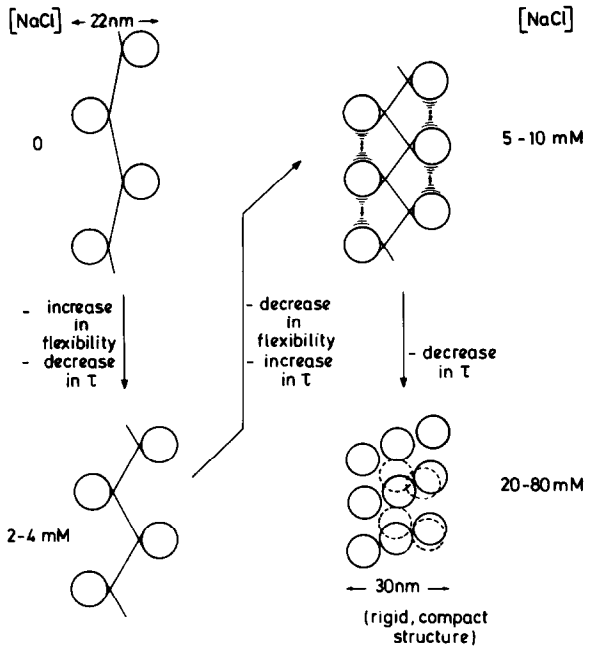

Fig.3. Schematic representation (not to scale) of the various compaction stages of chromatin as a function of ionic strength, and their relaxation times characteristics.

In the initial buffer, the electrostatic repulsion between phosphate groups, mainly in the linker region, is responsible for the fibre stiffness, since the charge on the core DNA should be largely neutralized by the histones. When sodium chloride is added in the 0–4 mM range, the electrostatic repulsion decreases. The chromatin fibre thus becomes less extended and the flexibility increases. This is a typical polyelectrolyte effect [22,23]. As the fibre starts to condense, the distance between nucleosomes decreases and, at N^+ concentrations higher than 5 mM, attractive interactions and/or steric hindrances develop, and the fibre flexibility consequently decreases. This process is specific of chromatin and could not be observed with DNA since it would require a charge neutralization only attainable at higher ionic strengths or in the presence of multivalent cations. At 4 mM sodium chloride, the relaxation signals were complex and displayed negative and positive contributions with fast and slow relaxation components respectively, indicating sample heterogeneity. In the electric field, both contributions compensate, and in the steady-state region, the ELD is zero. On the other hand, the stiffer component is the easiest to orient in the hydrodynamic fields, and positive FLD is therefore measured at this ionic strength [1,16,24].

Using the approach of Sen and Crothers [4,5], one would deduce that chromatin is fully condensed in 5 mM monovalent cations, in contradiction with all other physicochemical measurements. Thus, it seems likely that Crothers and coworkers stopped their titration curves at the beginning of the compaction step; the maximal compaction of chromatin thus cannot be detected in this way. First, as the relaxation time of stiff particles depends on the third power of their length, aggregation should produce an increase of $\bar{\tau}$ much larger than a two- to eightfold factor. By comparison, the aggregation of calf thymus chromatin that we previously evidenced [25] produced more than a hundredfold increase of $\bar{\tau}$. Furthermore, working at a chromatin concentration of about 40 μg/ml, we previously observed [11] the onset of chromatin precipitation at about 1 mM $MgCl_2$ in agreement with Widom [26] who was working with chicken erythrocyte chromatin at 10 μg/ml. He observed aggregation of the samples at 1 mM $MgCl_2$ in 0.2 mM Na^+, or at 2 mM $MgCl_2$ in 5 mM Na^+. In addition, our measurements of the relaxation time as a function of the NaCl concentration clearly indicate that an increase of $\bar{\tau}$ due to stiffening of the chromatin fibers (and not to aggregation) takes place at the beginning of the compaction process (and not at the optimal compaction). Moreover, the fact that we observed positive dichroism for chromatin at 28 mM sodium chloride, in the absence of any possible turbidity and/or aggregation, definitely proves that we measured a true optical dichroism rather than artifacts due to turbidity.

In conclusion, the linear dichroism of compact chromatin is positive as expected from models of the 30 nm fibre in which the linker DNA runs perpendicular to the fibre axis. The change of linear dichroism sign occurs in the first stage of compaction (2–4 mM NaCl; 100–400 μM $MgCl_2$). The relaxation times are much influenced by the flexibility, and the minimum in the dependence of $\bar{\tau}$ on NaCl concentration does not correspond to maximum compaction. Fig.3 summarizes various stages in the compaction process which are detected by ELD. They do not represent distinct

compact states but occur in a continuous manner as the mass per unit length measured by X-ray scattering increases monotonously with ionic strength [2].

Acknowledgements: This work has received the financial support of the Services de Programmation de la Politique Scientifique (ARC contract 80/85-90), and of the Fonds National de la Recherche Scientifique (IISN contract no. 4.9004.88). Thanks are also due to Professor E. Fredericq and Dr P. Colson, for constant interest in this work and fruitful discussions, and to Mr R. Kläring for building the Kerr cell. The skillful assistance of G. Bertin has also been greatly appreciated.

REFERENCES

[1] Hagmar, P., Marquet, R., Colson, P., Kubista, M., Nielsen, P., Norden, B. and Houssier, C. (1989) J. Biomol. Struct. Dyn. 7, 19–33.
[2] Koch, M.H.J., Sayers, Z., Michon, A.M., Sicre, P., Marquet, R. and Houssier, C. (1989) Eur. Biophys. J. 17, in press.
[3] Crothers, D.M., Dattagupta, M., Hogan, L. and Lee, C.S. (1978) Biochemistry 17, 4525–4533.
[4] Sen, D. and Crothers, D.M. (1986) Biochemistry 25, 1495–1503.
[5] Sen, D., Mitra, S. and Crothers, D.M. (1986) Biochemistry 25, 3441–3447.
[6] Lee, K.S., Mandelkern, M. and Crothers, D.M. (1981) Biochemistry 20, 1438–1445.
[7] Lee, K.S. and Crothers, D.M. (1982) Biopolymers 21, 101–116.
[8] Yabuki, H., Dattagupta, N. and Crothers, D.M. (1982) Biochemistry 21, 5015–5020.
[9] McGhee, J.D., Rau, D.C., Charney, E. and Felsenfeld, G. (1980) Cell 22, 87–96.
[10] McGhee, J.D., Nickol, J.M., Felsenfeld, G. and Rau, D.C. (1983) Cell 33, 831–841.
[11] Marquet, R., Colson, P., Matton, A.M., Houssier, C., Thiry, M. and Goessens, G. (1988) J. Biomol. Struct. Dyn. 5, 839–857.
[12] Fredericq, E. and Houssier, C. (1973) Electric Dichroism and Electric Birefringence, Clarendon Press, Oxford.
[13] Houssier, C. and O'Konski, C.T. (1981) in: Molecular Electrooptics (Krause, S. ed.) Ser. B, vol.64, pp. 309–339, NASO ASI Series.
[14] Koch, M.H.J., Dorrington, E., Klaring, R., Michon, A.M., Sayers, Z., Marquet, R. and Houssier, C. (1988) Science 240, 194–196.
[15] Marquet, R., Koch, M.H.J. and Houssier, C. (1989) in: Dynamic Behavior of Macromolecules, Colloids, Liquid Crystals and Biological Systems by Optical and Electro-optical Methods (Watanabe, H. ed.) pp. 105–110, Hirokawa Publ. Co., Tokyo.
[16] Kubista, M., Hard, T., Nielsen, P.E. and Norden, B. (1985) Biochemistry 24, 6336–6342.
[17] Bates, D.L., Butler, P.J.G., Pearson, E.C. and Thomas, J.O. (1981) Eur. J. Biochem. 119, 469–476.
[18] Koch, M.H.J., Sayers, Z., Michon, A.M., Marquet, R., Houssier, C. and Willführ, J. (1988) Eur. Biophys. J. 16, 177–185.
[19] Thoma, F., Koller, T. and Klug, A. (1979) J. Cell Biol. 83, 403–427.
[20] Labhart, P. and Koller, T. (1981) Eur. J. Cell Biol. 24, 309–316.
[21] Fujiwara, S., Inoko, Y. and Ueki, T. (1989) J. Biochem. 106, 119–125.
[22] Tanford, C. (1961) Physical Chemistry of Macromolecules, Wiley, New York.
[23] Oosawa, F. (1971) Polyelectrolytes, Dekker, New York.
[24] Smirnov, I.V., Dimitrov, S.I. and Makarov, V.L. (1988) J. Biomol. Struct. Dyn. 5, 1149–1161.
[25] Marquet, R., Colson, P. and Houssier, C. (1986) J. Biomol. Struct. Dyn. 4, 205–218.
[26] Widom, J. (1986) J. Mol. Biol. 190, 411–424.