Structural Transitions of Chromatin at Low Salt Concentrations: A Flow Linear Dichroism Study†

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Received December 13, 1984

ABSTRACT: We have studied the structure of nuclease-solubilized chromatin from Ehrlich ascites cells by flow linear dichroism (LD) using the anisotropic absorption of the DNA bases and of two intercalated dyes, ethidium bromide and methylene blue. It is confirmed that intercalation occurs preferentially in the linker part of the chromatin fiber, at binding ratios (dye/base) below 0.020. Using this information, we determined the orientation of the linker in relation to the average DNA organization in chromatin. The LD measurements indicate that the conformation of chromatin is considerably changed in the ionic strength interval 0.1–10 mM NaCl: with increasing salt concentration, the LD of the intrinsic DNA base absorption changes signs, from negative to positive, at approximately 2.5 mM NaCl. The LD of the intercalated dyes also changes signs, however, at a somewhat higher salt concentration. The results are analyzed in terms of possible allowed combinations of tilt angles of nucleosomes and pitch or tilt angles of linker DNA sections relative to the fiber axis, at different salt concentrations in the interval 0.1–10 mM NaCl. Two models for the salt-induced structural change of chromatin are discussed.

Today there is fair agreement regarding the basic chromatin structure (Igo-Kemenez et al., 1982). The nucleosome is the generally accepted term for the repeating unit in the lowest order of chromatin organization. It is composed of a nucleosome core and a linker region of variable length (Kornberg, 1974). The core consists of a well-defined length of DNA, 146 base pairs, wrapped one and three-quarter turns around an octamer of two each of the histones H2A, H2B, H3, and H4 (Richmond et at., 1984). Futher, 20 base pairs are associated with a fifth histone, H1, where DNA enters and exits the core. This part of chromatin, containing two full turns of DNA, has been called a *chromatosome* (Simpson, 1978). Together with the linker, it completes the nucleosome (Thoma et al., 1979). The length of the linker varies between 0 and 80 base pairs in different eucaryotic cells (Kornberg, 1977). The resulting chromatin fiber, appearing like "beads on a string" in electron micrographs, is approximately 10 nm in diameter at low ionic strengths (Finch & Klug, 1976). As the ionic strength is increased, the chromatin undergoes further compaction to a fiber ~30 nm in diameter (Renz et al., 1977).

Electric dichroism (Mitra et al., 1984; Mc Ghee et al., 1980, 1983; Yabuki et al., 1982) and flow linear dichroism (Makarov et al., 1983; Tjerneld et al., 1982; Matsuoka et al., 1984) are methods that are particularly useful for obtaining information about the geometry of different chromatin conformations in solution, although there have been apparent discrepancies between both the results and the interpretation of the results (Tjerneld et al., 1982; Matsuoka et al., 1984; Mc Ghee et al., 1983; Yabuki et al., 1982). Flow dichroism (Nordén & Tjerneld, 1976; Nordén & Seth, 1985) has an advantage, to electric dichroism, in that a whole spectrum of very high accuracy is obtained in a single run at steady-state orientation and the risk of perturbing the molecular structure is less with the moderate orienting flow fields. On the other hand, the orientation is not complete, and its determination requires indirect methods. Since chromatin models are necessarily complex, the geometric information available from the anisotropic light absorption of the DNA bases is generally insufficient for a unique assignment of the structure. Great improvement is expected if a specific optical probe can be introduced to selectively monitor the structure of separate parts of chromatin. This approach was recently applied in a study of the 30-nm fiber using psoralen (Mitra et al., 1984).

The cationic dye ethidium binds to DNA by intercalation between the bases with the phenanthridinium plane perpendicular to the DNA helix axis (Sobell et al., 1978; Cantor & Schimmel, 1980) and has also been used in chromatin studies [for a review, see Gabby & Wilson (1978)]. It has been inferred that ethidium binds selectively to the linker region of nucleosomes in chromatin at low binding ratios (Lawrence & Daune, 1976; Paoletti et al., 1977; Angerer & Moudrianakis, 1972; Genest et al., 1981; Angerer et al., 1974). At higher ratios, binding to the nucleosome core takes place accompanied by structural changes of the nucleosomes (Paoletti, 1979).

We have studied flow linear dichroism (LD) of ethidium and another intercalating dye, methylene blue (Nordén & Tjerneld, 1982), upon binding to chromatin. Methylene blue has a considerably higher extinction coefficient than ethidium and can therefore be used to lower binding ratios for optical measurements, which should give less perturbation of the chromatin structure. Second, the optical properties, in terms of transition moments, are for symmetry reasons better definied for methylene blue than for the ethidium chromophore. We find that the reduced linear dichroisms (LD^r) of these dyes when bound to chromatin at low dye/DNA ratios equal each other and infer that the binding sites of ethidium and methylene blue are essentially equivalent. However, their LD^r differs considerably from the LD^r of the DNA bases. This means that the dye molecules are located to certain DNA

† This project is supported by the Swedish Natural Science Research Council. P.E.N. is the recipient of a Niels Bohr Fellowship through the Egmont Foundation.

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0006-2960/85/0424-6336$01.50/0

regions with an orientation differing from the average. This is in agreement with the assumption that these dyes initially bind by intercalation preferentially to the linker region of chromatin. On the basis of this finding, we use the reduced linear dichroism of methylene blue to monitor the orientation of the linker region for elucidating structural details of chromatin.

Furthermore, we confirm that the flow linear dichroism of chromatin is highly dependent on the ionic strength, which may explain some of the diversity among previously reported LD results. We have simultaneously measured the LD of the DNA bases in chromatin and the intercalated methylene blue dye, at low binding ratios and varying salt concentration, and discovered that a structural transition takes place between 0.1 and 10 mM NaCl. These results enable us to encircle two different relatively restricted sets of possible angular coordinates for the nucleosome and linker arrangements at the two ionic strengths. On this basis, we propose a model for the structural change of the chromatin fiber in the 0.1–10 mM ionic strength interval.

Materials and Methods

Chromatin Preparation. Nuclei were prepared from Ehrlich ascites cells essentially according to Lerner & Steitz (1979) as previously described (Matsuoka et al., 1984). Briefly, 10 g of washed cells was lysed by gentle homogenization in 100 mL of 140 mM NaCl, 10 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl), 1.5 mM $MgCl_2$, and 0.5% Nonidet P40, pH 7.4, and the nuclei were isolated by pelleting (3500*g*, 20 min) through a 40-mL 0.8 M sucrose cushion (10 mM Tris-HCl, 5 mM $MgCl_2$, and 0.5% Nonidet P40, pH 7.4). The nuclei were then treated with 250 units of micrococcal nuclease (Boehringer) for 10 min at 37 °C in 20 mL of 0.34 M sucrose, 60 mM KCl, 1.5 mM NaCl, 1 mM $CaCl_2$, 0.5% Nonidet P40, and 10 mM Tris-HCl, pH 7.4. Following reisolation by centrifugation (5000*g*, 5 min), the nuclei were lysed in 20 mL of 1 mM ethylenediaminetetraacetic acid (EDTA), pH 8. The soluble and nonsoluble chromatin fractions were obtained by centrifugation (10000*g*, 10 min) as the supernatant and the pellet (which was resuspended in 20 mL of 1 mM EDTA, pH 8), respectively. Both fractions were dialyzed overnight against 2 L of 0.25 mM EDTA, pH 8. All operations were performed at 0–4 °C. Typically, the DNA isolated from the soluble fraction had a mean length of 4×10^3 base pairs, while the DNA from the pellet fraction had a mean length greater than 2×10^4 base pairs as analyzed by electrophoresis in 1% agarose. A *Hin*dIII restriction enzymatic digest of λ-DNA was used as a size marker. Solutions with different ionic strengths were obtained by dialyzing against 0.25 mM EDTA and 0.1 mM NaCl and diluting to the desired salt concentration.

Flow Linear Dichroism. Linear dichroism, $LD = A_{\parallel} - A_{\perp}$, was measured on a Jasco J-500 spectropolarimeter as described elsewhere (Davidsson & Nordén, 1976; Nordén & Seth, 1985). The chromatin sample was oriented in a flow inner rotating cylinder Couette cell with radial light propagation according to Wada & Kozawa (1964) and Nordén & Tjerneld (1976). An equivalent cell with an outer rotating cylinder was also used in order to eliminate centrifugal instability. Both cells were manufactured of high-quality UV-transparent and birefringence-free fused silica. The LD at different wavelengths was calibrated to be absolutely correct within 3% (absorbance units) according to the "dichrometer formula" (Nordén & Seth, 1985). The flow gradient was normally 1800 s^{-1}. The highest gradient used was 3000 s^{-1}; even at this gradient, the LD signals were completely time stable. It was also confirmed that the magnitude of the LD increased in a continuous and monotonic way as a function of flow gradient, as expected for laminar flow.

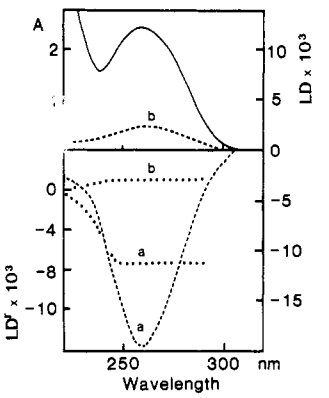

FIGURE 1: Absorbance [A (—)], linear dichroism [LD at a flow gradient $G = 1800\ s^{-1}$ (---)] and reduced linear dichroism [LD^r (···)] spectra of chromatin in 0.1 mM NaCl (a) and in 10 mM NaCl (b). The spectra are normalized to 1-cm optical path length.

The reduced linear dichroism, $LD^r = LD/A_{iso}$, was calculated with A_{iso}, the isotropic absorbance, measured on a Cary 219 spectrophotometer which was wavelength-matched with the Jasco monochromator. All measurements were made at 8 °C. The absence of hyperchromicity effects showed that no DNA denaturation occurs at this temperature even at the present low salt concentrations. Ethidium bromide and methylene blue were of standard reagent p.a. quality.

Results

Figure 1 shows the UV absorbance (A) and flow linear dichroism (LD) spectra and the corresponding reduced linear dichroism (LD^r) spectra of chromatin at two ionic strengths, 0.1 and 10 mM NaCl. The LD of the DNA bases has different signs at the two salt concentrations, being strongly negative at 0.1 mM NaCl and weakly positive at 10 mM NaCl, indicating that a structrual change has occurred in this salt interval. The transition from the negative to the positive form of chromatin could be reversed by decreasing the salt concentration.

The corresponding spectra of chromatin in the presence of the intercalating dyes ethidium bromide and methylene blue, at a binding ratio $r = 0.01$ ($r = n_{\text{bound dye}}/n_{\text{phosphate}}$) in 0.25 mM EDTA, are shown in Figure 2. It can be seen that the LD^r of the dye absorption bands has the same sign as that of the DNA bases but has a significantly larger amplitude, indicating that the binding sites of the dyes are concentrated to parts of DNA with a better orientation than the bases on the average. The LD^r values of the absorption bands of the two dyes are of the same magnitudes as expected if the dyes are bound to equivalent sites of chromatin.

In Figure 3 the LD^r values of the DNA and methylene blue absorption bands are plotted vs. the binding ratio in 10 mM NaCl. The LD^r decreases continuously with increasing r, and an "unperturbed" value is obtained by extrapolation to $r = 0$. With increasing dye concentration, a rapid decrease of the LD^r of the dye is observed at $r \sim 0.02$, suggesting that a structural change takes place in chromatin at this binding ratio. With even higher binding ratios, both of the LD^r values continue to decrease and approach each other. They are expected to coincide at complete saturation if the occupation of binding sites is then homogeneous. Separate measurements on the methylene blue–chromatin complex at binding ratios lower than 0.020, in order to support for a binding located initially to the linker, are presented in Figure 5 and will be discussed below.

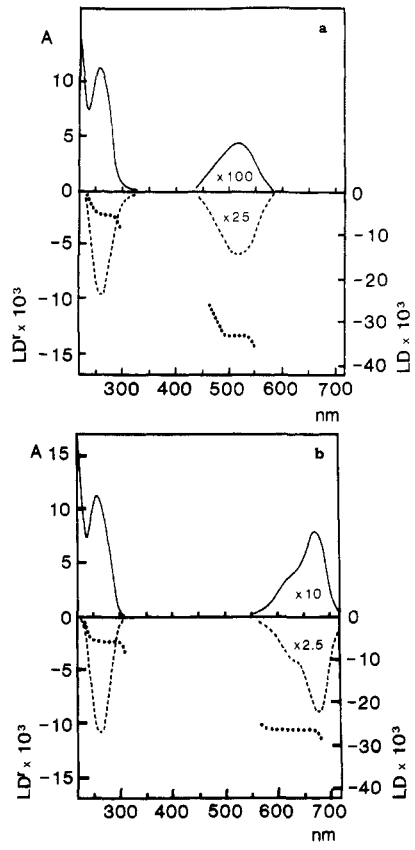

FIGURE 2: Absorbance (A), linear dichroism (LD at G = 3000 s^{-1}), and reduced linear dichroism (LD^r) spectra of the ethidium–chromatin complex (a) and the methylene blue–chromatin complex (b) in 0.25 mM EDTA. The spectra are normalized to 1-cm optical path length, and the notations are the same as in Figure 1. In both cases, r = 0.01.

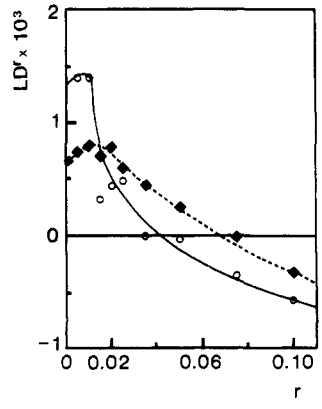

FIGURE 3: Dependence of the reduced linear dichroism (LD^r at 1800 s^{-1}) of the DNA base absorption (♦) and the methylene blue absorption (O) in the methylene blue–chromatin complex as a function of binding ratio (r) in 10 mM NaCl.

Figure 4 shows the LD^r values of DNA and methylene blue, at r = 0.005, as a function of salt concentration. The LD^r of the DNA band changes signs at approximately 2.5 mM NaCl and then reaches a constant level above 5 mM NaCl. The LD^r of the methylene blue band changes signs at a somewhat higher salt concentration.

Discussion

Salt Dependence of LD. There may be several explanations for the observed change in LD upon increasing salt concentration. However, from the following arguments, it is concluded that the change of signs of LD is most probably due to a strongly altered structure within the chromatin fiber: (1)

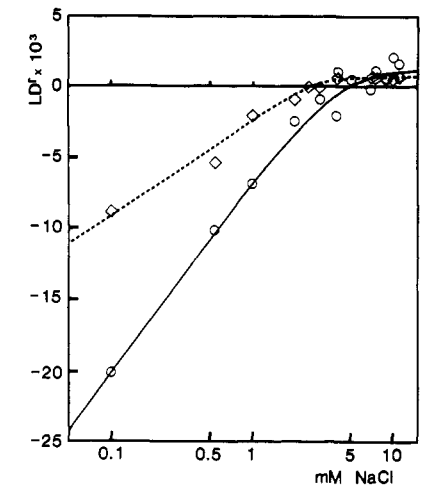

FIGURE 4: Dependence of the reduced linear dichroism (LD^r at 1800 s^{-1}) of the DNA base absorption at 260 nm (◊) and the methylene blue absorption at 674 nm (O) in the methylene blue–chromatin complex at r = 0.005 with increasing salt concentration.

A decrease in |LD| at high salt can arise from a general, unspecific increase in flexibility due to lowered phosphate–phosphate repulsion. Such an effect, however, should not change in *signs* of LD. (2) A distortion of the local DNA structure can affect the LD. However, this is very unlikely since the shape of the LD^r vs. wavelength curve is unchanged at the different salt concentrations. The fact that LD^r is essentially constant between 250 and 290 nm is characteristic for the local base orientation in B-form DNA (Matsuoka & Nordén, 1983). (3) A supercoiling of the fiber into a structure of higher order can cause a sign change (Gårding & Nordén, 1979). However, hydrodynamic (Butler, 1980) and electron microscopy studies (Thoma et al., 1979; Finch & Klug, 1976; Thomas & Butler, 1980) do not provide evidence of any superstructure at ionic strengths below 40 mM NaCl. Furthermore, a uniform transition such as a supercoiling of the entire fiber should change the signs of the LD of the DNA bases and the intercalator at the same salt concentration, in contradiction with the experimental finding.

Alternatively, we propose that the LD change is caused by a change of the organization of DNA *within the fiber*. There are two different internal, structural features which can give a positive LD contribution. The nucleosomes may become tilted at higher salt concentration as suggested by Makarov et al. (1983), or a change in the orientation of the linker region may take place. We propose that both types of changes may occur simultaneously, making the chromatin fiber more heavily packed at 10 mM salt concentration.[1]

Binding of Intercalated Dyes. At very low salt concentrations, where chromatin exhibits the most intense LD (cf. Figure 3), the LD of the ethidium band can be accurately studied at quite low binding ratios. Within experimental errors, the LD^r of ethidium is essentially the same as that of methylene blue (cf. Figure 2). This strongly indicates that ethidium and methylene blue bind to equivalent sites on chromatin.

It has previously been inferred that ethidium binds initially to the linker region of chromatin. We will here review some

[1] The structural change which we discover in chromatin with LD may be related to changes observed before in isolated nucleosome subunits in the same salt interval using various physical methods (Gordon et al., 1978, 1979; Harrington, 1981; Thoma & Koller, 1981). However, since the latter changes seem not to apply to intact chromatin, the discussion of this point will be reserved for a subsequent study on fragmented chromatin.

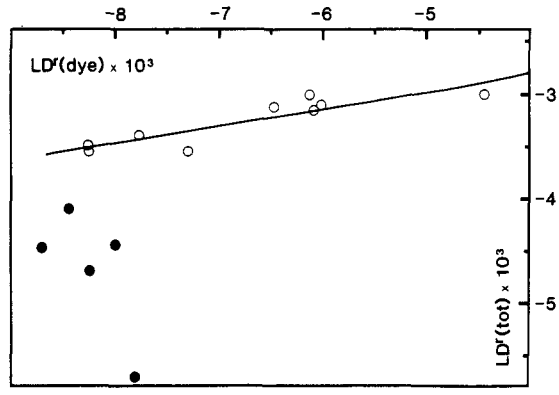

FIGURE 5: Variation of the reduced linear dichroism of the DNA base absorption [LD^r(DNA)] with the reduced linear dichroism of the methylene blue absorption [LD^r(dye)] at $r < 0.01$. Closed circles represent binding ratios above 0.02. The salt concentration is 1 mM NaCl, and $G = 1800\ s^{-1}$.

of the evidence and provide additional support in favor of this hypothesis. (1) In chromatin, two sets of ethidium binding sites, both with spectroscopic characteristics of intercalation, seem to be present, in contrast with pure DNA (Lawrence & Daune, 1976; Paoletti et al., 1977; Genest et al., 1981; Angerer et al., 1974). When the linker region is removed by nuclease digestion, the site with the highest affinity vanishes (Angerer & Moudrianakis, 1972). (2) At $r = 0.010$–0.025, some kind of structural transition takes place (cf. Figure 3). This has been observed by several authors (Paoletti et al., 1977; Paoletti, 1979; Angerer & Maudrianakis, 1972) and has been attributed to a change in the binding of the H1 histone protein. We suggest that this structural change occurs when the intercalation sites of the linker are saturated. In chromatin from Ehrlich ascites cells, the linker DNA is approximately 20 base pairs long, and the total length of a repeating unit is 180 nucleotides (Bakayev et al., 1977; P. E. Nielsen, unpublished results). If the binding density on DNA is 0.13 per base (Nordén & Tjerneld, 1982), the binding ratio consistent with a saturated linker would be $(0.13 \times 20 \times 2)/(180 \times 2) = 0.015$. Both the linker and the nucleosomal DNA will contribute to the total LD^r according to the relation

$$LD^r(\text{tot}) = \frac{a}{a+b} LD^r(\text{linker}) + \frac{b}{a+b} LD^r(\text{chrom}) \qquad (1)$$

where a and b are the base pair contents in the linker and the chromatosomal DNA, respectively. If LD^r(linker) is plotted vs. LD^r(tot), a straight line with the slope $a/(a + b)$ would be obtained provided the dye binds exclusively to the linker region (low binding ratios). In Figure 5, the observed LD^r(dye) values are plotted against LD^r(DNA) for chromatin + methylene blue in 1 mM NaCl. If LD^r(dye) = LD^r(linker) and LD^r(DNA) = LD^r(tot), the slope of the line for $r < 0.02$ should equal $20/(20 + 160) = 0.11$ for Ehrlich cell chromatin. The slope in Figure 5 is in fact 0.15 which is thus in fair agreement with the calculation.

The arguments above strongly indicate that the intercalating dyes ethidium and methylene bind initially only to the linker region of the chromatin fiber. The implication of this conclusion is clear: by adding a small amount ($r < 0.005$) of an intercalating dye, which binds perpendicularly to the DNA axis, the orientation of the linker DNA can be evaluated separately through the LD^r of the dye absorption band. As seen in Figure 3, $|LD^r|$ of DNA increases initially (below $r = 0.01$) by some 20% with increasing amount of bound methylene blue. This effect can most likely be ascribed to an elongation and stiffening of the DNA helix (Hogan et al., 1979) providing a more efficient DNA orientation.

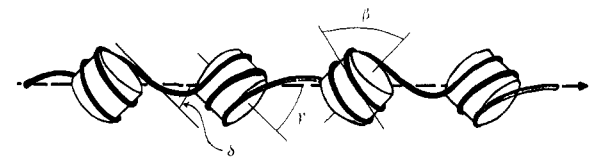

FIGURE 6: Definition of the structural parameters β, γ, and δ of chromatin. β is the pitch angle of the DNA wrapped around the chromatosome, γ is the tilt angle of the chromatosome with respect to the fiber axis, and δ is the pitch angle of a supercoiled linker around the fiber axis.

One is motivated to question whether the dye really probes the orientation of the linker at low binding ratios or if the binding introduces any perturbation of the structure. For the DNA–methylene blue complex, when studied on pure DNA, the LD^r values of the dye and base chromophores equal each other within experimental error (Nordén & Tjerneld, 1982). This indicates that the dye in a proper way reflects the local orientation of the unperturbed DNA bases. In chromatin, there is also a risk of distorting the higher order structure. Regarding the measuring uncertainty at low r values, such a distortion cannot be completely eliminated. However, the essentially linear behavior of the plot in Figure 5, at low r values, is consistent with a constant LD^r(chrom), i.e., with an unperturbed chromatosome structure. If the DNA orientation within the chromatosome had changed considerably upon binding, the second term in eq 1, and not only the first one, would have varied with r, and a nonlinear behavior or a slope differing from 0.1 would have been obtained.

Geometry of Low-Salt Fiber. For the basic structure of the chromatin fiber, referred to in the introduction, we shall now introduce structural parameters of importance for evaluating the LD of DNA.

In Figure 6, three angles are defined. β is the pitch angle of DNA wrapped as a helix around the histone core in the nucleosome. The geometry of the nuclesome has been studied by X-ray and neutron scattering and, the angle β can be estimated to approximately 85° (Finch et al., 1977, 1981). If upon averaging a cylindrical symmetry of the fiber can be assumed, which is generally justified for hydrodynamic orientation of very elongated species (Nordén, 1978), the orientation of the chromatosome axis with respect to the fiber axis is satisfactorily defined through a single tilt angle γ.

The linker DNA can be supercoiled or have some other less regular orientation with respect to the fiber axis. In a general case, a large number of structural parameters (two for each base) are needed for describing the orientation of the linker within the fiber. Regarding the limited information that is available from LD and the effect of averaging upon rotation around the fiber axis, it is motivated to describe the basic geometry of the linker in terms of a superhelix (or a part of a superhelix) with a pitch angle δ.

Linear Dichroism of Chromatin. An expression for the reduced linear dichroism of chromatin will be derived according to the ensemble principle developed by Gårding & Nordén (1979). According to the previous discussion, LD^r consists of contributions from the linker and the nucleosomal DNA. LD^r of a perfectly oriented DNA segment is equal to $^3/_2(3\langle\cos^2\alpha\rangle - 1)$ where $\langle\cos^2\alpha\rangle$ denotes the ensemble average orientation of an effective transition moment (Arnott et al., 1969; Matsuoka & Nordén, 1982, 1983) in the DNA bases with respect to the helix axis; an effective value of $\alpha = 86°$ has been derived for B-form DNA (Matsuoka & Nordén, 1982). For each further supercoiling, $i = 1, 2, \ldots$ with pitch angle θ_i, LD^r is multiplied by a factor $^1/_2(3\ \langle\cos^2\theta_i\rangle - 1)$. Considering the linker and nucleosomal DNA and the geo-

metrical parameters defined above, LD^r turns out as

$$LD^r(\text{linker}) = 3S\frac{1}{2}(3\langle\cos^2\alpha\rangle - 1)\frac{1}{2}(3\langle\cos^2\delta\rangle - 1) \quad (2)$$

and

$$LD^r(\text{chrom}) = 3S\frac{1}{2}(3\langle\cos^2\alpha\rangle - 1)\frac{1}{2}(3\langle\cos^2\beta\rangle - 1)\frac{1}{2}(3\langle\cos^2\gamma\rangle - 1) \quad (3)$$

where S is an orientation factor for the common orientation direction (fiber axis) defined by the arrow in Figure 6. The orientation of the fiber depends on the flow gradient, S being equal to unity in a perfectly oriented sample. Equation 2 can now be used to evaluate the orientation of the linker from the LD^r of an intercalating dye at low binding ratios using $LD^r(\text{dye}) = LD^r(\text{linker})$. The LD^r of the DNA bases consists of contributions from LD^r(linker) and LD^r(chrom) weighted with their respective amounts of base pairs. Substitution into eq 1 gives for $LD^r(\text{tot}) = LD^r(\text{DNA})$

$$LD^r(\text{DNA}) = 3S\left\{\frac{a}{a+b}\frac{1}{4}(3\langle\cos^2\alpha\rangle - 1)(3\langle\cos^2\delta\rangle - 1) + \frac{b}{a+b}\frac{1}{8}(3\langle\cos^2\alpha\rangle - 1)(3\langle\cos^2\beta\rangle - 1)(3\langle\cos^2\gamma\rangle - 1)\right\} \quad (4)$$

where $a = 20$ and $b = 160$ in chromatin from Ehrlich cells.

It should be noted that eq 2–4 are valid for cylindrical distributions of the angles α, β, γ, and δ around their respective reference axes but do not require uniaxial orientation of the chromatin fiber. This assumption is justified for structural and dynamic reasons, for example, due to rotation of the fiber around its long axis. The orientation factor S is a more complicated average, involving two angular parameters for the orientation of the fiber in the flow field which generally does not have cylindrical symmetry (Nordén, 1978; Wada & Kosawa, 1964; Wada, 1972).

Calculation of Structure. In eq 4, there are three unknown parameters: S, γ, and δ ($\alpha = 86°$ and $\beta = 85°$). They cannot be determined separately since only two experimental values, the LD^r(link) and the LD^r(tot), are available. By dividing eq 4 by eq 2, it is possible to eliminate the unknown orientation parameter S (and also α) and end up with a continuous equation in γ and δ:

$$\frac{2[K(a+b)-a]}{b(3\langle\cos^2\beta\rangle - 1)}(3\langle\cos^2\delta\rangle - 1) = 3\langle\cos^2\gamma\rangle - 1 \quad (5)$$

where K is the ratio $LD^r(\text{tot})/LD^r(\text{link})$. Using this relation, it is possible to construct a diagram representing all combinations of the γ and δ angles. In Figure 7, the possible ranges of these structural parameters are obtained by correlation with the experimentally observed LD^r values from all DNA bases and from the linker probe. The area of each circle in the diagram is proportional to $|LD^r|$. Filled and unfilled circles represent positive and negative LD^r, respectively. The circles in the left column and the upper row correspond, respectively, to the LD^r(link) and the LD^r(chrom). The origin in this "coordinate system" (marked with a star) represents the angles $\gamma = \delta = 54.7°$ where both LD^r(link) and LD^r(DNA) vanish. To the right of the vertical nodal curve through the origin, $LD^r(\text{tot}) < 0$. Above the horizontal line through the origin, $LD^r(\text{link}) < 0$. From the signs of the observed dichroisms, we can identify the relevant quadrants. Thus, at 0.1 mM NaCl where both LD^r(DNA) and LD^r(dye) are negative, the angles should be found in the upper right quadrant. The ratio K was determined to 0.45, with the estimated experimental uncertainities $0.39 < K < 0.52$. The corresponding solutions of eq 5 are indicated in Figure 7. In 10 mM NaCl, K is equal to 1.25 (experimental uncertainty $0.85 < K < 2.00$); both LD^r(dye) and LD^r(DNA) are positive, corresponding to an area in the lower left quadrant.

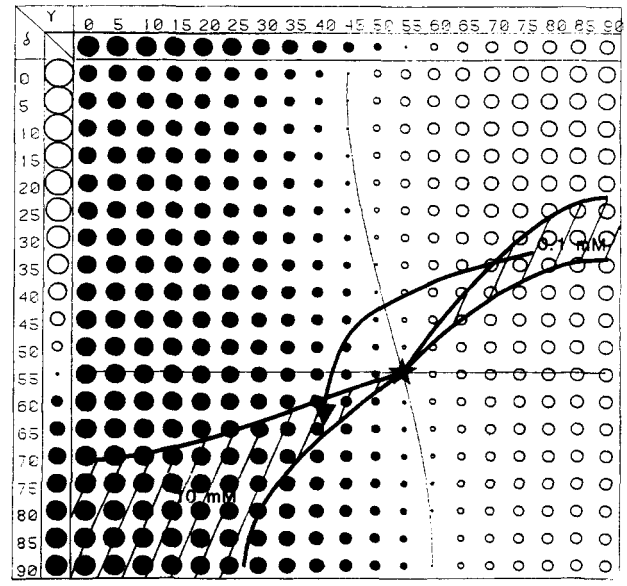

FIGURE 7: Possible linker and chromatosome arrangements in 0.1 mM NaCl and in 10 mM NaCl. Angle combinations are represented in a coordinate system with γ as the abscissa and δ as the ordinate. Areas of circles represents $|LD^r|$, closed circles indicating positive values and open circles representing negative values. The upper row and the left column denote LD^r(chrom) and LD^r(link), respectively. Circles in the coordinate system represent LD^r(tot), the weighted sum of the contributions from the linker and chromatosome. Two lines, one vertical corresponding to $LD^r(\text{tot}) = 0$ and one horizontal corresponding to $LD^r(\text{link}) = 0$, divide the coordinate system into four quadrants. The intersection of these lines, marked with a star, is the origin of the coordinate system, where both $LD^r(tot)$ and $LD^r(link)$ vanish. The experimentally determined angle combinations of β and γ in 0.1 mM NaCl (upper right quadrant) and in 10 mM NaCl (lower left quadrant) are represented by dashed areas. The proposed path for the transition upon salt addition is indicated with an arrow.

We now know that the direction of the salt-induced transition goes from the upper right to the lower left quadrant. Futher, since LD^r(DNA) changes signs at a lower concentration than LD^r(dye), the path of the transition should go through the quadrant where $LD^r(\text{tot})$ is positive and LD^r(link) is negative as indicated by the arrow in Figure 7.

Salt-Induced Condensation of Chromatin. On the basis of the discussion above, we will try to give a physical interpretation of the structural transition that we observe in the chromatin fiber at low salt concentration.

At very low ionic strength, the fiber is, as a result of electrostatic repulsion, in an extended conformation with the flat faces of the nucleosomes more or less parallel to the fiber axis (Figure 8a). As the ionic strength is increased, the negatively charged phosphate groups of the linker DNA become gradually more shielded, allowing an increasing degree of folding or supercoiling ["electrostatically induced" compaction is also observed in pure DNA (Gosule & Schellman, 1978)]. Simultaneously, the chromatosomes tilt, and internucleosomal contacts could further stabilize the structure (Felsenfeld et al., 1983).

Two basically different structures may be envisaged, producing the observed LD^r values and the correspondingly calculated angles γ and δ at 10 mM NaCl. Both structures have a face to face nucleosomal arrangement. However, one

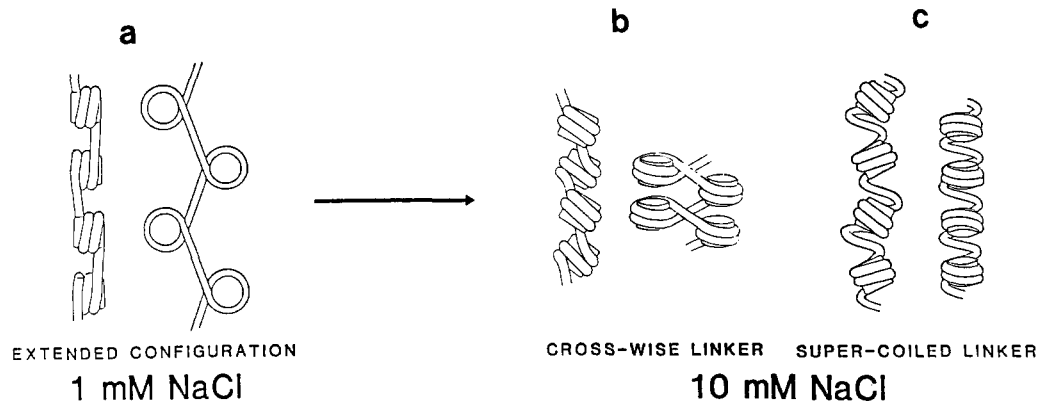

FIGURE 8: Models of the structures of chromatin in 1 and 10 mM NaCl, $\gamma = 90°$, $\delta = 30°$. (b and c) Condensed chromatin conformation in 10 mM NaCl, $\gamma = 10°$, $\delta = 80°$. (b) Zig-zag, cross-wise linker model; (c) supercoiled linker model. The histone H1 has been omitted for clarity.

is a "two-row" model retaining the zig-zag conformation with a "*cross-wise linker*" (Figure 8b) while the other is a "one-row" model having a *supercoiled linker* (Figure 8c). The linking number of the cross-wise linker model is −2 per nucleosome, if it is assumed that there are two full turns of DNA around each chromatosome [for definition and sign convention of linking number, see Crick (1976)]. The linking number for the supercoiled linker model will be the sum of the contributions from the chromatosome and the linker (Fuller, 1978). Since the linker is proposed to adopt the same handedness as the DNA around the chromatosome, the linking number must be more negative than −2 per nucleosome and depend on the length of the linker. Work on Simian virus 40 (Germond et al., 1975), however, suggests that the linking number is approximately −1 per nucleosome. This lower value can be explained by an overwinding of DNA upon binding to the histone core (Klug & Lutter, 1981; Klug, 1983). If there are 160 base pairs associated with the histone core, a decrease of the pitch angle between adjacent base pairs by 360°/160° = 2.5° is enough to increase the twisting number of DNA with 1 per nucleosome, resulting in an overall linking number of −1 per nucleosome.

CD measurements on chromatin suggest that the CD of the DNA in the core domain in fact differs from that of B-form DNA in solution (Kreuger, 1984). This might be due to perturbations from the histones or to interactions between the bases in adjacent DNA chains; however, a contribution to the changed CD from an overwinding of DNA is quite plausible. The same studies indicate that the linker region consists of B-form DNA. This should imply that any supercoiling of the linker is not compensated for by overwinding the DNA. To maintain the linking number constant in the supercoiled linker model, during the observed transition, the chromatin fiber would have to be smoothly coiled already in its extended form (low salt concentration). When the salt concentration is increased, this coiling is localized to the linker region. The necessity to assume such a smooth coiling of the extended form, in case of the supercoiled linker model, clearly makes this model less attractive.

Finally, chromatin in approximately 10 mM NaCl has been observed in electron micrographs to appear as zig-zag-shaped fibers with a diameter of about two nucleosomes (Thoma & Koller, 1977), a structure closely resembling the cross-wise linker model. A structure related to the cross-wise linker model has been suggested by Worcel et al. (1981). The difference is that in their model every linker crosses the preceding one. This gives an overall linking number of −1 per nucleosome, but for steric reasons, the crossing requires a rather long linker.

It is also motivated to see how the models compare with the chromatin structure at higher salt concentrations. When the salt concentration is increased to above approximately 50 mM, chromatin is observed in electron micrographs to condense into a fiber of approximately 30 nm in diameter (Thoma et al., 1979). This fiber has been proposed to be a "solenoid" with a pitch of ~11 nm, a diameter of ~30 nm, and with ~6 nucleosomes per solenoidal turn (Finch & Klug, 1976; Lee et al., 1981; Suau et al., 1979). A solenoid is easy to construct from the supercoiled linker model but appears more difficult to obtain from the cross-wise linker model [however, cf. Worcel et al. (1981)]. This could be taken as an argument for the supercoiled linker model; on the other hand, the solenoid structure has not yet been unequivocally proven, and other structures have been proposed as well (Hozier et al., 1977). A solenoid has a linear dichroism of the linear form multiplied by a factor $(3\cos^2\theta - 1)/2$ (Gårding & Nordén, 1979) where θ is the pitch angle of the solenoid. This means that upon coiling chromatin into a solenoid, the LD should *reverse* signs when the pitch angle exceeds 54.7°. A solenoid with the parameters above has a pitch angle of 83° and should therefore have a sign opposite to that of the extended form (in this case, a negative sign since the sign is positive in 10 mM NaCl). Such a sign change has not been observed in flow LD measurements (Tjerneld et al., 1980; Makarov et al., 1983; Matsuoka et al., 1984). With electric dichroism, a negative LD has been observed for the condensed structure obtained with Mg^{2+} but not any intermediate form with positive LD (Felsenfeld et al., 1983). Furthermore, when the 30-nm fiber was induced with NaCl, a positive LD was observed (Lee & Crothers 1982).

On the basis of these arguments, we propose that the condensation of chromatin at 10 mM NaCl results in a structure with the nucleosomes arranged in a cross-wise manner, each nucleosome facing its second neighbors. This "intermediate" form will undergo further folding or supercoiling at physiological salt concentration.

CONCLUSIONS

The LD measurements indicate that the conformation of chromatin is considerably changed when the ionic strength is increased from 0.1 to 10 mM NaCl. The LD results can be rationalized in terms of a two-dimensional map of possible orientations of the nucleosomes within the chromatin fiber. Two principally different models of chromatin structure are discussed. On the basis of the experimental results in combination with other arguments, it is proposed that the structure obtained in 10 mM NaCl can be described by a *cross-wise*

linker model according to Figure 8b.

ACKNOWLEDGMENTS

We acknowledge Peder Öhman for valuable help in designing the figure on the chromatin models.

Registry No. NaCl, 7647-14-5; ethidium bromide, 1239-45-8; methylene blue, 61-73-4.

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