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# Orientation of Nucleosomes in the Thirty-Nanometer Chromatin Fiber†

Hiroko Yabuki,‡ Nanibhushan Dattagupta, and Donald M. Crothers*

ABSTRACT: We have measured the linear dichroism of $Mg^{2+}$-stabilized and dimethylsuberimidate cross-linked 30-nm chromatin fibers, using electric fields to produce orientation. The limiting dichroism of $Mg^{2+}$-stabilized fibers at low monovalent ion concentration is −0.09, with indistinguishable results for avian erythrocyte and calf thymus chromatin. Correction of the apparent dichroism signal for an absorbance change that becomes significant at high electric field yields the dichroism of −0.09 and reveals that the fibers reach saturation of their orientation by about 15 kV/cm. Calf thymus chromatin fibers cross-linked at 100 mM NaCl concentration have a dichroism of +0.05, with a dependence of orientation on field similar to that observed for $Mg^{2+}$-stabilized fibers. Comparative sedimentation studies of $Mg^{2+}$-stabilized fibers and fibers in 100 mM cross-linking buffer revealed a 25% sedimentation coefficient increase accompanying the dichroism increase from −0.09 to +0.05. We interpret the results to mean that the nucleosomal disk diameters form an angle of about 30° to the chromatin fiber axis in $Mg^{2+}$-stabilized fibers at low monovalent ion concentration. When 100 mM NaCl is added, the fiber becomes more compact, and the disk diameter angles increase by 8°, to about 38°. Cross-linking itself may also contribute slightly to fiber compaction. The results are consistent with a large longitudinal compressibility of the 30-nm fiber, a feature required for the bendability necessary when the fiber is further coiled to form structures such as chromosomes. Our results indicate that compression is accommodated by small changes in the angular orientation of the nucleosomal disks.

The detailed structure of the 30-nm chromatin fiber, which is readily visualized in the electron microscope (Ris & Kubai, 1970), remains unknown. The organization of nucleosomal disks in chromatin fibers has been studied by electron microscopy (Finch & Klug, 1976; Rattner & Hamkalo, 1978; Thoma et al., 1979), neutron scattering (Suau et al., 1979), X-ray diffraction (Sperling & Klug, 1977), light scattering (Campbell et al., 1978), electric dichroism (Rill & Van Holde, 1974; Houssier et al., 1977; McGhee et al., 1980; Lee et al., 1981; Lee & Crothers, 1982), and flow dichroism (Tjerneld & Norden, 1982). It has been proposed that in the 10-nm fiber observed at low salt concentration, the nucleosomal disks are arranged with their diameters nearly parallel to the fiber axis. However, this conclusion is not supported by the flow dichroism studies of Tjerneld & Norden (1982). The 30-nm fiber results from further coiling or folding of the 10-nm fiber, which is induced by higher salt concentration or addition of multivalent cations, and occurs over a range of salt concentrations (Thoma et al., 1970; Butler & Thomas, 1980; Thomas & Butler, 1980; Bates et al., 1981).

The linear dichroism of oriented 30-nm chromatin fibers is expected to be a sensitive function of the orientation of nucleosomal disks relative to the fiber axis. Recent experiments by McGhee et al. (1980) and in our laboratory (Lee et al., 1981; Lee & Crothers, 1982) have used electric field orientation to obtain the dichroism of the fiber. The results of those studies left some unresolved discrepancies, which we seek to clear up in this paper. Specifically, McGhee et al. (1980), working with unfixed erythrocyte chromatin at low ionic strength in the presence of $Mg^{2+}$, found a limiting dichroism of about −0.2 and did not observe saturation of the orientation at increasing electric field. Our experiments (Lee

† From the Department of Chemistry, Yale University, New Haven, Connecticut 06511. *Received April 5, 1982.* Supported by Grant GM 21966 from the National Institutes of Health.
‡ Present address: Institute of Endocrinology, Gunma University, Maebashi, Japan.

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et al., 1981) characterized calf thymus chromatin fixed by dimethylsuberimidate cross-linking in 100 mM NaCl and showed a limiting dichroism of +0.06, with saturation of orientation at voltages above 15 kV/cm.

In the present paper we report that unfixed erythrocyte and calf thymus chromatin samples in $Mg^{2+}$ and low ionic strength show identical limiting dichroism values of −0.09 if correction is made for a nondichroism signal that becomes significant above 15 kV/cm. Furthermore, after this correction one finds saturation of orientation at about 15 kV/cm, as observed for cross-linked samples. However, even in the presence of $Mg^{2+}$, chromatin samples at low ionic strength are not as compact as samples at more nearly physiological salt concentration, as demonstrated by a 25% increase in the *s* value upon increasing the NaCl concentration to the 100 mM level used for cross-linking. Accompanying this compaction is a change of the dichroism from −0.09 to +0.05, the latter measured after cross-linking.

We interpret the dichroism changes as implying an increase of the tilt of the nucleosome diameters relative to the fiber axis, from about 30° in the presence of $Mg^{2+}$ at low salt to about 38° in the compact cross-linked form. Much larger angular alterations of the linker DNA would be required to produce the observed dichroism increase upon compaction. Taken together, the results argue strongly for angular placement of nucleosomes relative to the fiber axis. Furthermore, the structure appears to be rather flexible, without a single strongly preferred disk orientation angle. This elasticity is probably required for further coiling of the 30-nm fiber into structures with even greater degrees of DNA compaction.

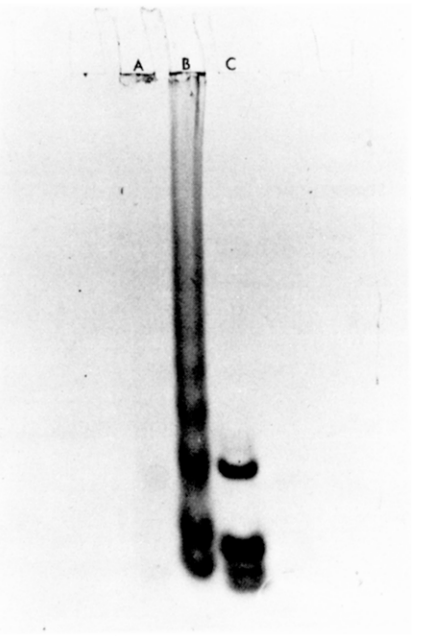


FIGURE 1: 5% polyacylamide–$NaDodSO_4$ gel of native and cross-linked histone fractions. (A) Histones from the cross-linked calf thymus polysomal fraction, showing no material entering the gel. (B) Histones from polysomal chicken erythrocyte chromatin reacted extensively with dimethylsuberimidate, showing that cross-linking is incomplete by comparison with (A). (C) Un-cross-linked histones from polysomal chicken erythrocyte chromatin. All histones were extracted from the polysomal fraction with 0.25 N HCl and electrophoresed as described by Lee et al. (1981).

## Materials and Methods

*Calf thymus chromatin* fragments were prepared and cross-linked as described earlier (Lee et al., 1981; Lee & Crothers, 1982). Calf thymus nuclei were briefly digested with micrococcal nuclease to yield 20–30% solubilized chromatin. For un-cross-linked preparations, the solubilized material was immediately fractionated on a 10–30% sucrose gradient in cross-linking buffer (10 mM Hepes–0.1 mM EDTA–1 mM PMSF–80–100 mM NaCl, pH 8.05) and centrifuged for 4 h at 27 krpm in an SW 27 rotor. For cross-linked samples the solubilized fraction was dialyzed against cross-linking buffer and reacted as described earlier (Lee, 1980; Lee et al., 1981; Lee & Crothers, 1982) with freshly dissolved dimethylsuberimidate. The cross-linked chromatin fragments were fractionated on a 10–30% sucrose gradient in TE buffer (10 mM Tris–1 mM EDTA, pH 7.3). In both cases, fractions containing 50–75 nucleosomes were collected.

*Chicken erythrocyte chromatin* was prepared by using the same procedure as adopted for calf thymus, except that 0.3% saponin solution was added to lyse chicken erythrocytes rather than blending in a Waring Blendor. The yield of solubilized chromatin after nuclear digestion was about 10–15%.

*DNA sizes* were estimated by 0.5% agarose gel electrophoresis in 2 mM EDTA–20 mM sodium acetate buffer, pH 7.8, by using *Hin*dIII fragments of λ DNA as size standards.

*Protein cross-linking* was judged by $NaDodSO_4$ slab gel [5% acrylamide–0.07% *N,N'*-methylenebis(acrylamide)] electrophoresis as described by Weber & Osborn (1969) [see also Lee et al. (1981)].

*Electric dichroism and sedimentation* measurements were performed as described earlier (Lee et al., 1981).

## Results

*Incomplete Cross-Linking of Erythrocyte Chromatin.* One of our objectives upon initiating this study was to compare the dichroism properties of calf thymus and avian erythrocyte chromatin, both with cross-linking and without cross-linking. We found, however, that maximal reaction of erythrocyte chromatin with dimethylsuberimidate did not produce exclusively high molecular weight cross-linked protein aggregates, in contrast to the results we reported earlier for calf thymus chromatin. Figure 1 shows comparative gel electrophoresis results for protein samples from the two cross-linked chromatin preparations. Cross-linking conditions that prevent thymus histone proteins from even entering the gel leave large amounts of smaller aggregates in erythrocyte chromatin. Presumably this contrast reflects the replacement of histone H1 in thymus chromatin by H5 in erythrocytes. Given the similar dichroism properties of the two un-cross-linked chromatin samples that our subsequent measurements revealed, we believe that the cross-linking difference originates simply in the relative availability of suitably placed imidate-reactive sites in the two materials and does not reflect a substantial structural difference between them. Partially cross-linked erythrocyte chromatin samples gave large field-induced nondichroism optical signals of the kind reported earlier (Lee & Crothers, 1982) and were not studied further.

*Compaction of Chromatin by $Mg^{2+}$ at Low Salt.* McGhee et al. (1980) showed that chromatin fibers are strongly compacted by small amounts of $Mg^{2+}$, even at very low monovalent ion concentration. We confirm their qualitative conclusion, although the increase in the *s* value we observed is somewhat smaller than they reported. Figure 2 shows the sedimentation coefficient of 12–15 kilobase calf thymus chromatin samples in the presence of $r_{Mg^{2+}}$ moles of $Mg^{2+}$ per mole of DNA phosphate. The results are expressed as the ratio of *s* at $r_{Mg^{2+}}$ to the *s* value with $r_{Mg^{2+}} = 0$ (0.1 mM Tris–0.01 mM EDTA, pH 7.3). With three $Mg^{2+}$ per DNA phosphate, *s* is increased nearly 3-fold over the value in the absence of $Mg^{2+}$, confirming the expected strong compaction of the fiber.

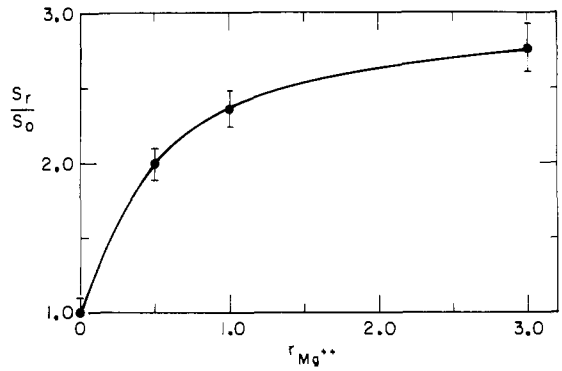


FIGURE 2: Effect of $Mg^{2+}$ on the sedimentation coefficient of calf thymus chromatin. $s_r/s_0$ is the ratio of sedimentation coefficient in the presence of $r_{Mg^{2+}}$ per DNA phosphate to no added $Mg^{2+}$. Buffer 0.1 mM Tris–0.01 mM EDTA, pH 7.3; temperature 22 °C; speed 18 krpm. Chromatin size = 75 nucleosomes.

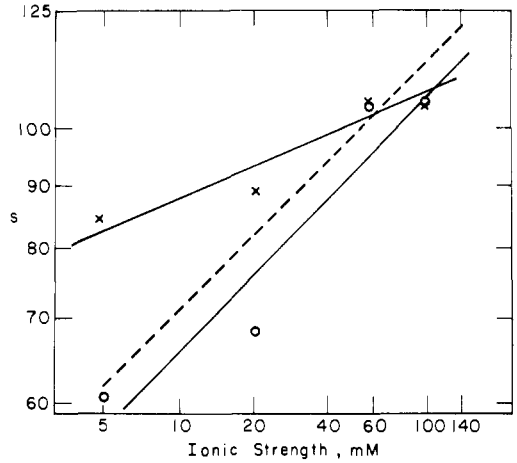


FIGURE 3: Variation of the sedimentation coefficient of chicken erythrocyte chromatin under different ionic conditions: (×) in the presence of $Mg^{2+}$ ($r_{Mg^{2+}}$ = 3.0), (○) in the absence of $Mg^{2+}$; (---) results obtained for the 44 nucleosome fraction by Bates et al. (1981). Chromatin size = 50 nucleosomes; other conditions were as in Figure 3.

*Further Compaction by NaCl.* Measurement of the dichroism of low-salt chromatin samples in the presence of $Mg^{2+}$ raises the question of the relationship of the fiber compaction under these conditions to its state under a more nearly physiological concentration of monovalent ion. Figure 3 shows a log–log plot of $s$ value against ionic strength, with and without added $Mg^{2+}$ ($r_{Mg^{2+}}$ = 0 and 3.0). It is evident that $Mg^{2+}$ causes compaction of the fiber but not to the extent caused by 100 mM NaCl, with or without $Mg^{2+}$. From the starting point of 83 S at 0.013 mM Tris–EDTA buffer, with $r_{Mg^{2+}}$ = 3.0, $s$ increases by over 25% of its value of 103 S when 100 mM NaCl is added. Hence it is clear that chromatin with only $Mg^{2+}$ added is not as compact as it is at 100 mM salt concentration. The dichroism results that follow are consistent with a modest structural change of the $Mg^{2+}$-containing fiber when NaCl is added.

*Dichroism of Cross-Linked Calf Thymus Chromatin.* We repeated our earlier (Lee et al., 1981) measurement of the dichroism of calf thymus chromatin, stabilized against unfolding in the low-salt dichroism measurement buffer by previous dimethylsuberimidate cross-linking in 80 mM NaCl, with nearly identical results. Figure 4 shows the field dependence of the reduced dichroism $\rho$. The signal is positive and reaches a saturating value of 0.05 by about 10 kV/cm. Saturation of the signal means that orientation is essentially complete at voltages above 10 kV/cm. This result is expected

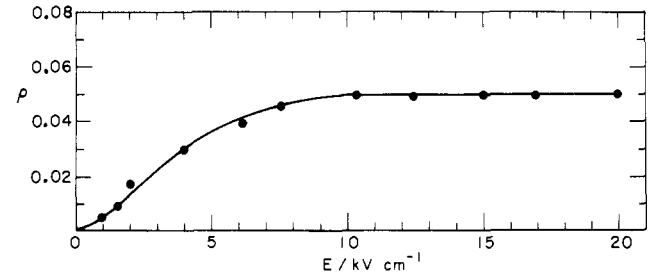


FIGURE 4: Dependence of the reduced dichroism ($\rho$) of cross-linked calf thymus chromatin on field strength. Temperature 7 °C; $r_{Mg^{2+}}$ = 0; buffer 0.1 mM Tris–0.01 mM EDTA, pH 7.3. The chromatin sample (~75 nucleosomes in length) had been cross-linked in 80 mM NaCl.

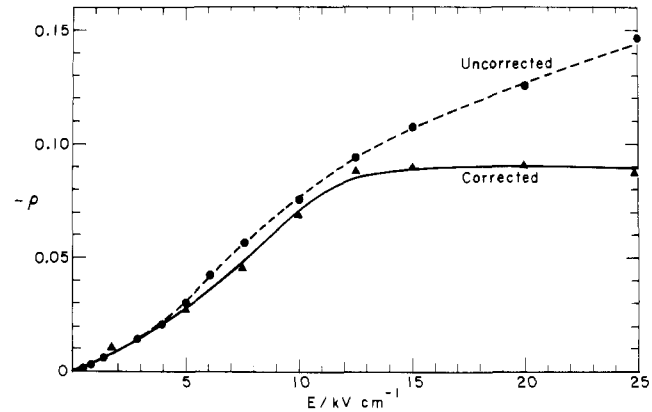


FIGURE 5: Variation of the reduced dichroism ($\rho$) of un-cross-linked chicken erythrocyte chromatin with field strength. Temperature 7 °C; buffer 0.10 mM Tris–0.01 mM EDTA, pH 7.3; $[Mg^{2+}]$ = 0.5 mM ($r$ = 3.0). (●) Uncorrected values; (△) corrected for the nondichroism signal measured at a 54° polarizer angle.

because of the very large ionic polarizability of a highly charged chromatin fiber roughly 1000 Å in length (Lee et al., 1981; Lee & Crothers, 1982). When the polarizer was set at the "magic angle" of 54° to the field direction for the sample in Figure 4, no signal was observed, confirming that the optical change found with a 0° polarizer angle is indeed due to dichroism.

*The Dichroism of Unfixed Samples Must Be Corrected for a High-Field Nondichroism Signal.* Figure 5 shows a crucial result of this study. When the apparent dichroism is measured with a 0° polarizer angle, the dichroism amplitude continues to increase at high field. Extrapolation of these values to infinite field yields a dichroism of about –0.2, as reported by McGhee et al. (1980). However, at high fields there is a significant optical change with the polarizer set at 54°, a result we reported earlier for chromatin cross-linked at intermediate ionic strength (Lee & Crothers, 1982). Subtraction of the nondichroism signal measured at polarizer angle 54° yields the saturation dichroism of –0.09 shown for the corrected curve in Figure 5.

The observed saturation of the dichroism at about 13 kV/cm greatly increases confidence in assigning the signal to overall orientation of the particle. The slightly higher field required for saturation of orientation of the sample in Figure 5 compared to that in Figure 4 probably results from the higher ionic strength, which reduces the polarizability (Lee & Crothers, 1982). The nondichroism signal is an absorbance decrease of the sample, which we have earlier tentatively ascribed to a field-induced local distortion of DNA structure (Lee & Crothers, 1982).

Parallel studies on unfixed erythrocyte and thymus chromatin in the presence of $Mg^{2+}$ gave indistinguishable results

for the two samples. Hence, the difference between our earlier results for cross-linked thymus chromatin (Lee et al., 1981) and those reported by McGhee et al. (1980) for unfixed erythrocyte chromatin is not due to the different biological source of the samples.

## Discussion

*Qualitative Conclusions.* Our results show that chromatin samples in $Mg^{2+}$-containing low-salt buffer and in 100 mM salt cross-linking buffer differ by about 25% in sedimentation coefficient, implying further compaction by addition of NaCl to the $Mg^{2+}$-stabilized fiber. Once correction is made for the significant nondichroism signal observed at high fields for non-cross-linked samples, the orientation vs. field curves are very similar for cross-linked and un-cross-linked samples. However, the limiting dichroism values are clearly different, −0.09 for $Mg^{2+}$-stabilized fibers at low salt and +0.05 for cross-linked fibers. Our earlier results (Lee, 1980; Lee et al., 1981) showed no significant effect on the limiting dichroism of adding $Mg^{2+}$ to the cross-linking buffer or to the low-salt measurement buffer for fully cross-linked samples.

When the DNA helix lies parallel to the field direction, dichroism is negative, with a theoretical limit of −1.5 if the bases are perpendicular to the DNA helix axis. DNA oriented with its helix axis perpendicular to the field has a theoretical limiting dichroism of +0.75. Hence, the shift of dichroism from −0.09 to +0.05 in un-cross-linked and cross-linked samples, respectively, implies that the average DNA helix axis direction becomes more tilted relative to the fiber axis after cross-linking in the higher salt condition. This result is consistent with compaction of the fiber: the projection of the DNA axis perpendicular to the 30-nm fiber axis should increase relative to the projection along the fiber axis, if it is assumed the compaction occurs by small changes in angular orientation of nucleosomes rather than by drastic structural reorganization.

*Influence of Cross-Linking.* Our comparison of the dichroism properties of low-salt plus $Mg^{2+}$ and high-salt-treated chromatin fibers is complicated by inclusion of a cross-linking step in the latter case. The recent results of Fulmer & Bloomfield (1982) on the dimensions of un-cross-linked chromatin fibers in high salt allow further evaluation of the influence of cross-linking itself on compaction. The sample sets of both Lee et al. (1981) and Fulmer and Bloomfield include material estimated by sedimentation–diffusion to be 45–46 nucleosomes in average length. Lee et al. (1981) found a sedimentation coefficient of 125 S for this material after cross-linking, whereas the results of Fulmer & Bloomfield (1982) show 110–115 S for their samples in 85 mM salt concentration. The near agreement of these values is consistent with our earlier observations indicating little change in the $s$ value upon cross-linking (Lee et al., 1981). However, since the cross-linked material sediments slightly faster, it is possible that cross-linking produces a 10–15% compaction.

The hydrodynamic diameter of the chromatin fiber in high salt was estimated by Lee et al. (1981) to be 33 nm (after cross-linking) and by Fulmer and Bloomfield to be 40 nm. Given the uncertainty of the measurements, the insensitivity of the measured parameters to fiber diameter, and the difference in hydrodynamic models, the two numbers are probably not significantly different. However, the values obtained for the rise per nucleosome, 15 nm (Lee et al., 1981) and 22 nm (Fulmer & Bloomfield, 1982), differ more dramatically than do the sedimentation coefficients. Possibly the discrepancy arises from the use of different techniques in the two studies. Both groups measured translational diffusion coefficients, combined in the case of Lee et al. (1981) with rotational relaxation and in the case of Fulmer & Bloomfield (1982) with the angular dependence of light scattering, to yield fiber length and diameter. Lee et al. (1981) reported that the light scattering radius of gyration of cross-linked fibers (measured in low-salt solution) was clearly larger than either electron microscopic or hydrodynamic dimensions and chose to ignore the light scattering results. In addition, the gel filtration step included in the preparation of Fulmer & Bloomfield (1982) could lead to a loss of nonhistone proteins that remain bound in our cross-linked preparation. Thus, on balance, while the significant disagreement over the calculated rise per nucleosome of cross-linked and un-cross-linked fibers leaves some residual grounds for disquiet, the small change in directly measured sedimentation behavior upon cross-linking supports the view that cross-linking produces at most only modest (10–15%) further compaction beyond that induced by increased salt concentration.

*Comparison with Other Work.* Our results show a limiting negative dichroism for $Mg^{2+}$-stabilized chromatin that is less than half as large as that reported by McGhee et al. (1980). If we ignore the high-field absorbance change revealed by the signal observed when the polarizer angle was set at 54°, our extrapolated dichroism is consistent with theirs. Hence, it is plausible that their results are in error because of neglect of this factor.

As a more general principle, it is dangerous to assume that an electric field induced optical signal corresponds to the overall orientation of a macromolecule. As we stressed in an earlier paper (Lee & Crothers, 1982), segmental orientation and local distortion can give rise respectively to dichroism and absorbance changes that are unrelated to the optical anisotropy of the unperturbed particle. An instructive example is provided by the dichroism of chromatin in low-salt conditions, without added $Mg^{2+}$. McGhee et al. (1980), using electric field orientation, reported a substantial negative dichroism, from which they concluded that the nucleosomal disk diameters in the 10-nm fiber are roughly parallel to the fiber axis. On the other hand, Tjerneld & Norden (1982), using flow orientation, found positive dichroism for the 10-nm fiber, supporting models in which the disks stack face to face with diameters perpendicular to the low-salt fiber axis. No definitive resolution of this discrepancy is yet possible, but the large dipole moment of mononucleosomes (Crothers et al., 1978) and relative flexibility of the 10-nm fiber make it plausible that electric fields cause segmental orientation of nucleosomes, yielding an incorrect picture of their disposition relative to the fiber axis in an unperturbed molecule.

Adequate experimental criteria are clearly needed to assess the contribution of overall orientation to observed electric dichroism signals. We believe that a minimum requirement for a large DNA-containing chromatin fragments is that overall orientation should saturate at low values of the field (10–15 kV/cm), as shown in Figures 4 and 5 (corrected curve). Additional evidence can be obtained by comparing the apparent dimensions deduced from the rotational and translational diffusion constants of the particle with dimensions determined by electron microscopy (Lee et al., 1981). Further support for our conclusion that the dichroism of the 30-nm fiber is very small (−0.09 to +0.06) is provided by the observation by Tjerneld & Norden (1982) of a nearly zero dichroism signal for $Mg^{2+}$-stabilized chromatin fibers oriented by flow.

*Quantitative Interpretation.* As discussed by McGhee et al. (1980), the unknown path of the linker DNA between

nucleosomes leads to potential ambiguity in quantitative interpretation of the dichroism data. We take here two limiting views of the problem to illustrate the possibilities. First, we consider the case in which the entire dichroism change (−0.09 to +0.05 from low-salt plus $Mg^{2+}$ to cross-linking conditions) is ascribed to change in the angular orientation of the linker DNA, which we take to be 32 base pairs out of the 200 total base pairs in the repeat (Finch et al., 1981). The dichroism $\rho_{linker}$ of an ideal DNA rod, with bases perpendicular to the DNA axis and with the linker DNA axis at an angle $\beta_{linker}$ to the field, is [see Crothers et al. (1978)]

$$\rho_{linker} = -\frac{3}{4}(3 \cos^2 \beta_{linker} - 1) \quad (1)$$

Since the linker DNA constitutes only 32/200 of the total DNA, its dichroism would have to change by (200/32) × 0.14 = 0.88 to account for the observed dichroism increase of 0.14. The minimum angular change that could account for this dichroism change occurs in the neighborhood of the magic angle $\beta = 54°$; a change of at least 24° would be required. For example, changing $\beta_{linker}$ from 43° to 68° changes $\rho_{linker}$ from −0.44 to +0.44.

Change of the linker DNA angle $\beta_{linker}$ from 43° to 68° would have a large effect on fiber length if there were no compensating structural changes. The projection of a 32 base pair (~100-Å) linker DNA segment along the fiber axis is 100 cos $\beta_{linker}$ (Å). The change from $\beta_{linker} = 43°$ to 68° would change the projection by 36 Å from 73 to 37 Å/nucleosome. Since the average fiber length per nucleosome in our cross-linked fibers is only about 15 Å (Lee et al., 1981), the change of 36 Å is unacceptably large in view of the small change in length and apparent morphology on cross-linking.

The alternate limiting model we can take assumes, as we did earlier (Lee et al., 1981), that the linker DNA continues on the same local superhelical path as the core DNA and that the total nucleosome contains an integral number of half turns of DNA. The dichroism is then closely approximated by (Crothers et al., 1978; Lee et al., 1981)

$$\rho = \frac{3}{8}(3 \cos^2 \gamma - 1) \quad (2)$$

in which $\gamma$ is the angle between the fiber axis and the local superhelix axis of the nucleosomal DNA. When $\rho = -0.09$, eq 2 yields $\gamma = 60°$; with $\rho = +0.05$ we get $\gamma = 52°$. Hence, the angular orientation of the nucleosomal disks need change by only 8° to explain the dichroism difference between our unfixed and cross-linked chromatin samples.

Corresponding to the $\gamma$ angles of 60° and 52°, the nucleosomal disk diameters form angles of 30° and 38°, respectively, to the chromatin fiber axis. It should be noted that it is because the disks are at an angle to the fiber axis that the dichroism is very sensitive to small angular changes. If the disk diameters were parallel to the fiber axis in the $Mg^{2+}$-containing un-cross-linked fiber at low salt, $\gamma$ would have to change by 20°, from 90° to 70°, to explain the dichroism increase of 0.14. Hence, not only is the dichroism itself not consistent with disk diameters arranged parallel to the helix axis but it is also more difficult with this model to explain the significant dichroism changes that accompany modest compaction under the cross-linking condition. Hence, the results, taken together, support general models in which the nucleosomal disks lie at an angle relative to the chromatin fiber axis.

*Possible Packing Models.* There is not at present sufficient information to construct a definitive structural model for the organization of nucleosomes in the 30-nm chromatin fiber. However, it is appropriate at this time to ask whether plausible models can be built. Figure 6 shows two possibilities, differing in the postulation of either a mononucleosome or dinucleosome repeat in a solenoidal superhelix (Lee, 1980). The figure shows a view normal at each point to the cylindrical fiber surface. The repeat along the fiber axis is taken to be 110 Å, with a 110-Å diameter for the nucleosomal disks. In exploring crude three-dimensional models, we found that there is no apparent stereochemical reason why nucleosomal disks 55 Å × 110 Å × 110 Å cannot be packed in a helical array with their diameters at angles of 30–40° to the solenoidal axis.

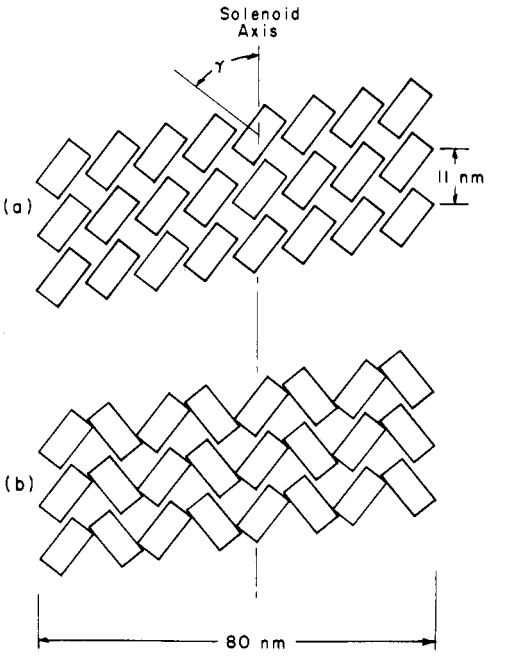


FIGURE 6: Possible arrangements of nucleosomes in the compact chromatin fiber. Nucleosomes are veiwed in projection along the normal to the surface of the cylinder. The following structural parameters are used: pitch = 11 nm, circumference 80 nm, $\gamma = 52°$, and the number of nucleosomes per turn = 8. Similar structures are possible with six to eight nucleosomes per turn. According to our results, the angle $\gamma$ depends on the compaction of the fiber, varying by 8° from about 60° in low-salt, $Mg^{2+}$-stabilized fibers to about 52° in cross-linked fibers. The number of nucleosomes per turn may also vary with compaction [see Thoma et al. (1979)]. (a) Mononucleosome repeat. (b) Dinucleosome repeat.

*Bendability and Longitudinal Compressibility.* The ease of bending a rod is proportional to its longitudinal compressibility, as one can understand by recognizing that the rod is compressed along the inner edge of a curve and elongated at the outer edge. Our sedimentation results, along with the much more extensive studies of Thomas and collaborators (Butler & Thomas, 1980; Thomas & Butler, 1980; Bates et al., 1981), indicate a progressive compaction of the chromatin fiber when ionic concentration is increased, even when $Mg^{2+}$ is present. Such a response, not found for rodlike molecules such as short pieces of DNA, implies that the length of the fiber is readily adjusted to changes in electrostatic stress. Hence, it seems that the chromatin fiber has a relatively large longitudinal compressibility and therefore should be easily bent.

Bending of the 30-nm fiber is required for its further coiling, for example, to form chromosomes. The nature of this folding is not yet achieved structurally: the angular orientation of nucleosomal disks relative to the fiber axis allows length changes that accompany small angular changes in disk orientation. The dichroism increase which accompanies fiber compaction in the cross-linking buffer is a reflection of the structural adaptation of the fiber to changing logitudinal stress.

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# Synthesis of the Antibacterial Peptide Cecropin A(1–33)†

R. B. Merrifield,* L. D. Vizioli, and H. G. Boman

ABSTRACT: Cecropin A(1–33) was synthesized by an improved stepwise solid-phase method. The synthesis was designed to give high coupling yields and minimal amounts of byproducts. All coupling steps were monitored for completion by a new ninhydrin procedure, and the fully protected peptide-resin was analyzed for deletion peptides by the solid-phase Edman preview technique. Both methods indicated that the average coupling yield was >99.8%. The unpurified peptide mixture resulting from HF cleavage and extraction into 10% acetic acid was analyzed by reverse-phase high-pressure liquid chromatography, and 93% of the total product was shown to be the desired [Trp(For)$^2$]cecropin A(1–33), indicating an average yield per synthetic cycle of 99.8%. Removal of the formyl group at pH 9, followed by ion-exchange chromatography, gave the purified product. Cecropin A(1–33) showed antibacterial activity against both Gram-positive and Gram-negative bacteria. Against *Escherichia coli*, the activity was only slightly lower than that of the natural 37-residue cecropin A when tested over a 100-fold concentration range; the minimum inhibitory concentration was approximately 1 $\mu$M. The formyl derivative was somewhat less effective in killing *E. coli* than the free 1–33 peptide. The antibacterial activity was discussed in terms of an amphipathic $\alpha$-helix structure and the binding of the peptide to bacterial membranes.

The cecropins are a newly discovered class of antibacterial peptides produced by the humoral immune response of certain insects (Hultmark et al., 1980; Boman & Hultmark, 1981; Boman & Steiner, 1981). Cecropins and about 10 other immune proteins are induced in the hemolymph of the pupae of the giant silk moth *Hylophora cecropia* following injection of live bacteria. The first cecropins to be purified to homogeneity and for which tentative primary structures are available are the A and B forms (Steiner et al., 1981). They each contain 37 amino acid residues, with a basic N-terminal region and a hydrophobic C-terminal region ending with a blocked carboxyl groups. Cecropins A and B are strongly homologous but differ significantly in structure and function from other known basic peptides such as melittin.

The cecropins are antibacterial against a variety of Gram-negative bacteria and therefore differ from lysozyme, which is effective only against certain Gram-positive bacteria. The range of susceptible pathogens is broader than that for melittin, and in addition, the latter is lytic for Chang liver cells whereas cecropins have no effect on these cells or on sheep erythrocytes or insect cells in tissue culture (Steiner et al., 1981).

A synthetic program on the cecropins has been undertaken with the objectives of confirming their structure, providing sufficient material to enable more extensive studies on their mode of action, and examining the role of their very interesting structures on their bactericidal activity. This paper describes in detail the solid-phase synthesis of cecropin A(1–33), which at the initiation of the work was believed to represent the complete sequence of the molecule. The structure of the synthetic protected peptide-resin I is

Boc-Lys(ClZ)-Trp(For)-Lys(ClZ)-Leu-Phe-Lys(ClZ)-Lys(ClZ)-Ile-Glu(*O*Bzl)-Lys(ClZ)-Val-Gly-Gln-Asn-Ile-Arg(Tos)-Asp(*O*cHex)-Gly-Ile-Ile-Lys(ClZ)-Ala-Gly-Pro-Ala-Val-Ala-Val-Val-Gly-Gln-Ala-Thr(Bzl)–$OCH_2$–$C_6H_4$–$CH_2CONHCH_2$–$C_6H_4$–resin

I

The synthesis followed the stepwise solid-phase strategy (Merrifield, 1963), with the acid-labile *tert*-butyloxycarbonyl (Boc)[1] group for temporary $N^{\alpha}$ protection and more acid-stable

† From the Rockefeller University, New York, New York 10021 (R.B.M. and L.D.V.), and the Department of Microbiology, University of Stockholm, S-10691 Stockholm, Sweden (H.G.B.). *Received March 17, 1982.* The work was supported by Grant AM01260 from the U.S. Public Health Service and by a grant from the Swedish Natural Science Foundation to H.G.B. (BU2453-108).

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