CIRCULAR DICHROISM SPECTRA OF PUTATIVE TRANSCRIBED AND REPRESSED CHROMATIN

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SUMMARY: Circular dichroism (CD) spectra have been determined for chromatin fractions obtained by ECTHAM-cellulose chromatography. The molecular ellipticity at the positive long wavelength maximum is about 3000 deg cm^2/dmol for early-eluted chromatin fractions, thought to be relatively repressed *in vivo*, and 5000-6000 deg cm^2/dmol for late-eluted chromatin fractions, those thought to be preferentially transcribable *in vivo*. CD bands in the peptide bond spectral region also differ for the two chromatin fractions, early-eluted chromatin having a more helical conformation for proteins. In addition to previously known differences in protein content, the biological activity of a native chromatin fraction can now be correlated with the conformation of its DNA.

We have previously reported a fractionation of sonicated chromatin on ECTHAM-cellulose (1). Early-eluted fractions are depleted of low melting segments, while late-eluted fractions contain up to 50% of their DNA as sequences melting below 75° (1). Late-eluted fractions contain up to four times as much *in vivo* synthesized, chromatin bound RNA as early-eluted fractions, suggesting that the late-eluted, low melting material is preferentially transcribable while the early-eluted, higher melting chromatin is relatively repressed (2). We now report on the spectroscopic properties of these two fractions and their constituent DNA.

METHODS

Chromatin preparations were made from rabbit liver and calf thymus as previously detailed (3). Chromatography at a level twenty-fold greater than that previously reported (1) was performed on ECTHAM-cellulose prepared by the method of Peterson and Kuff (4). The thermal denaturation properties

of the chromatin fractions from this scaled-up separation were essentially identical to those reported in a prior publication (1).

DNA was prepared from chromatin fractions by repeated extractions with chloroform:isoamyl alcohol (27:1) of solutions made 2-3 M in NaCl and 1% in sodium dodecyl sulfate. The deproteinized DNA was dialyzed exhaustively to remove organic solvents and detergent before being used in further procedures.

Thermal denaturation analyses were performed as described previously (1) using 0.25 mM EDTA, pH 7.0, as solvent. Circular dichroism measurements were performed in the same solvent using a Cary Model 61 circular dichroism spectrometer. The authors are indebted to Dr. H. Shichi for the use of this instrument. Samples, having an absorbance of 1.0 at 260 nm, were scanned in 1 cm path length cells for the spectral range above 240 nm and in 2 mm path length cells for the spectral range below 260 nm. All data are reported as molecular ellipticities based on the concentration of DNA-phosphate.

RESULTS

Chromatography of calf thymus chromatin on ECTHAM-cellulose leads to a separation of types of nucleoprotein particles similar to that previously reported for rabbit liver chromatin (1). Thus, the early-eluted chromatin is composed of only high melting material, while the late-eluted material is markedly enriched in low melting sequences. In contrast to these differences for the chromatin fractions themselves, the DNA of early and late fractions of both rabbit liver and calf thymus have identical behavior on thermal denaturation analysis with T_m = 45° in 0.25 mM EDTA, pH 7.0. Early and late-eluted chromatin DNA is also indistinguishable for both tissue sources in terms of its CD spectrum in the spectral range from 210 - 300 nm. Thus, differences in spectroscopic properties between early and late chromatin fractions must arise from differences in the conformation of DNA in the two chromatin fractions.

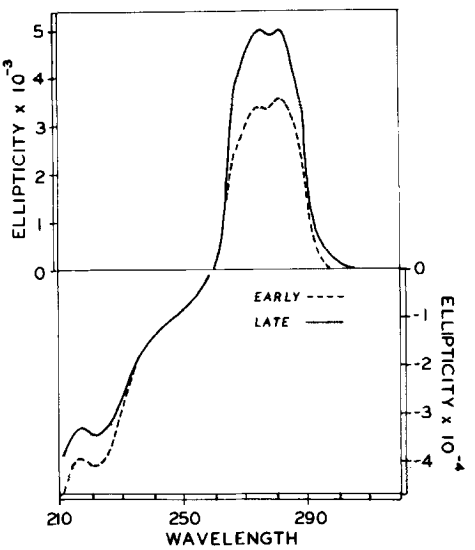

Figure 1 Circular dichroism spectra of early-eluted (- - - -) and late-eluted (————) calf thymus chromatin fractions from ECTHAM-cellulose chromatography. The molecular ellipticity of the unfractionated chromatin used in this experiment was 4000 deg cm^2/dmol at 280 nm and the ellipticity of the DNA isolated from both fractions was 9500 deg cm^2/dmol at 280 nm. Note the difference in the scales for the spectral regions above and below 260 nm. The total protein content of both early- and late-eluted chromatin was 1.25 gm/gm DNA.

Unfractionated calf thymus chromatin has a molecular ellipticity at 280 nm of about 4000-4500 deg cm^2/dmol. The high melting chromatin segments eluted at the front of the peak have an even lower ellipticity than bulk chromatin, about 3000 deg cm^2/dmol (Figure 1). In striking contrast, the material eluted late in the chromatographic peak, enriched in low melting segments, has a markedly enhanced ellipticity in this spectral region, 5000 deg cm^2/dmol (Figure 1). The two fractions differ but slightly in the region of the negative ellipticity band of DNA at 245 nm, but do differ significantly in the peptide bond chromophore region around 225 nm (Figure 1). Early eluted chromatin proteins appear to have a somewhat greater ellipticity than late eluted chromatin (the total protein contents of the two fractions are identical). This is consistent with a slightly greater helical content, although the presence of DNA ellipticity bands in this spectral region requires caution in arriving at this conclusion.

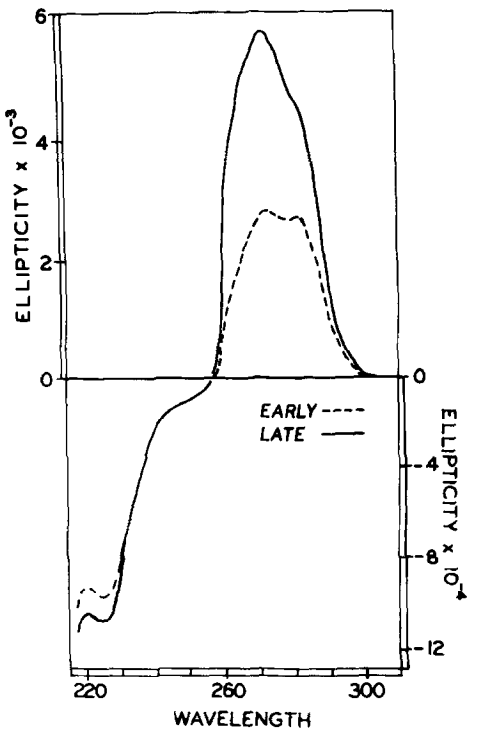

Figure 2 Circular dichroism spectra of early-eluted (- - - -) and late-eluted (————) rabbit liver chromatin fractions from ECTHAM-cellulose chromatography. The molecular ellipticity of the unfractionated chromatin used in this experiment was 4500 deg cm^2/dmol at 280 nm and the ellipticity of the DNA isolated from both fractions was 9500 deg cm^2/dmol at 280 nm. Note the difference in the scales for the spectral regions above and below 260 nm. The total protein contents of early- and late-eluted chromatin fractions was 2.0 and 3.0 gm/gm DNA respectively.

The CD spectra of fractionated rabbit liver chromatin are similar to those for calf thymus chromatin. Again, the ellipticity of the positive band is markedly reduced in the early-eluted fractions when compared to either DNA or unfractionated chromatin. In this case, the enhancement of ellipticity for the late-eluted material is even more striking than for calf thymus, 6000 deg cm^2/dmol (Figure 2). Changes similar to calf thymus chromatin are also seen in the low wavelength region, although the rotations are much greater for liver chromatin, due to the larger amount of nonhistone protein present in the liver nucleoprotein.

DISCUSSION

A number of lines of investigation, including circular dichroism spectroscopy, have convincingly demonstrated that the conformation of DNA in chromatin is not the same as the conformation of protein-free DNA. Furthermore, attempts have been made to relate this altered conformation of the nucleic acid with the repression of transcription of the bulk of the genome in animal cells (for review see 5). Slayter and collaborators (6) have suggested that the conformation of DNA in calf thymus chromatin fractions separated by gradient centrifugation differs between the rapidly and slowly sedimenting species. We have recently succeeded in reproducibly fractionating sonicated chromatin into species which differ in physicochemical properties, compositions, and biological properties (1,2,7). In particular, data on the distribution of rapidly labelled nascent RNA (presumably mostly HnRNA or mRNA) suggest that the early-eluted fractions are relatively repressed while the late-eluted fractions are preferentially transcribable. The current CD data show a strong correlation with these functional properties of the chromatin fractions. Thus, whatever the cause for the reduction in ellipticity in the 280 nm region of chromatin DNA compared to isolated DNA, it is apparent that the conformation of the early-eluted material is even less like that of free DNA than unfractionated chromatin, while the conformation of the late-eluted chromatin is altered in the direction of protein-free DNA. Furthermore, the CD spectra of the late-eluted fractions of rabbit liver and calf thymus chromatins also support this contention. Ellipticities for liver chromatin are consistently larger than those for thymus and the template activities of the two tissue chromatins for DNA-dependent RNA synthesis differ in a similar fashion (8).

If the cause of the altered CD spectrum of DNA in chromatin is indeed supercoiling, as has been suggested (9), then the current

results suggest that repressed DNA sequences in chromatin are highly supercoiled while transcribable chromatin DNA is in an extended conformation like protein-free DNA. These properties are exactly those predicted for these two species by the Paul model for the mechanism of gene regulation in eukaryotic cells (10).

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