CIRCULAR DICHROISM SPECTRA AND ETHIDIUM BROMIDE BINDING OF 5-DEOXYBROMOURIDINE-SUBSTITUTED CHROMATIN

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SUMMARY

Changes in CD spectra of 5-deoxybromouridine (brUdRib)-substituted chromatin depend on the extent of thymidine replacement by brUdRib. With 20 and 14 per cent replacement, a blue shift and a marked increase in positive ellipticity are reported in the CD spectra of brUdRib-substituted chromatin, while with 4.9 per cent replacement little effect is noticed. BrUdRib-substitution does not affect the number of ethidium bromide primary binding sites of chromatin.

It has recently been reported (1,2) that replacement of the thymidine moiety by 5-deoxybromouridine (brUdRib) causes dramatic changes in circular dichroism (CD) spectra of both DNA and chromatin. Thus, Augenlicht et al., (1) reported a blue shift and a marked increase in positive ellipticity at 260 nm in CD spectra of DNA and chromatin from 3T6 cells, in which thymidine had been 20 per cent replaced by brUdRib. Similar results were obtained by Simpson and Seale (2) with HeLa cells, in which thymidine had been extensively substituted (~45 per cent) by brUdRib. In both cases, the brUdRib effect was more evident in chromatin than in protein-free DNA. Opposite results were reported by Lapeyre and Bekhor (3), who found a decrease in positive ellipticity of CD spectra of brUdRib-substituted chromatin. In addition, these authors found a 4 - 15 per cent decrease in the number of primary binding sites for ethidium bromide in brUdRib-substituted chromatin.

A possible explanation for the discrepancy between the results of Augenlicht et al., (1) and Simpson and Seale (2) on one side, and those of Lapeyre and Bekhor (3) on the other, may be found in the fact that the latter authors used chromatin from ascites tumor cells, in which the maximum extent of thymidine

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replacement by brUdRib was 6.7 per cent. The present communication shows that, indeed, the changes in CD spectra depend upon the extent of thymidine replacement by brUdRib. In addition, it shows that brUdRib-substitution does not affect chromatin primary binding sites for ethidium bromide when the latter are measured by sensitive spectropolarimetric methods.

MATERIALS AND METHODS

The methodology for the growth of 3T6 cells in brUdRib-containing medium, for the isolation of DNA and chromatin and for the determination of the extent of brUdRib-substitution have been given in previous papers (1,4,5). Three concentrations of brUdRib were used, 72 μM, 36 μM and 7.2 μM, which gave, respectively, 20 per cent, 14 per cent and 4.9 per cent, replacement of thymidine moieties by brUdRib. Circular dichroism of chromatin in 10 mM Tris was measured in a Jasco Model J-40 recording spectropolarimeter, as previously described (1). We have used unsheared chromatin (correcting the CD spectra for light scattering artifacts), but it should be noted that the results reported by Simpson and Seale (2) were obtained with sheared chromatin.

Ethidium bromide binding was measured by circular dichroism, using the methodology of Dalgleish et al., (6), Williams et al., (7) and Nicolini and Baserga (8). This method assumes that only intercalated dye molecules acquire optical activity, while weakly bound dye molecules, so-called secondary sites (7-11) are not optically active. This assumption has been proved to be analytically and quantitatively correct (12), so that the spectropolarimetric method can be used to give the number of primary binding sites (in either DNA or chromatin) for ethidium bromide (8).

RESULTS

Fig.1 shows that the changes in CD spectra between 250-300 nm of brUdRib-substituted chromatin are dependent upon the extent of replacement of the thymidine moieties. Under our experimental conditions, the dramatic changes in CD spectra described by Augenlicht et al., (1) and by Simpson and Seale (2) occurred only at brUdRib concentrations of 72 and 36 μM. At 7.2 μM (4.9 per

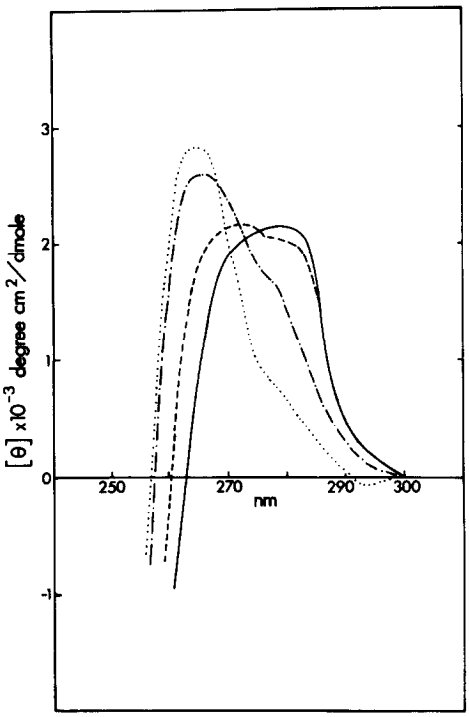

Figure 1. Circular dichroism spectra between 300 and 250 nm of chromatin from untreated 3T6 mouse fibroblasts (———) and of brUdRib-substituted 3T6 chromatin at final brUdRib concentrations of 7.2 μM (-----), 36 μM (—·—·—) and 72 μM (· · · · ·) in growth medium.

cent substitution) the CD spectrum is slightly shifted but has the same shape as unsubstituted chromatin. Between 200-255 nm, where the contribution of proteins is most important the CD spectra of chromatin are the same as that of control chromatin, regardless of the concentration of brUdRib used (Fig.2).

Fig.3 shows the ellipticity at 308 nm of chromatin-ethidium bromide complexes as a function of the ratio added dye/DNA phosphate. The amount of ethidium bromide intercalated is the same for unsubstituted chromatin and for chromatin isolated from cells grown in 72 μM brUdRib (20 per cent replacement). Both saturation curves are only 20-25 per cent that of protein-free DNA. The CD spectra between 300-350 nm of chromatin-ethidium bromide complexes are shown in Fig.4. No difference is detectable among chromatins, either unsubstituted or brUdRib-replaced to a maximum of 20 per cent.

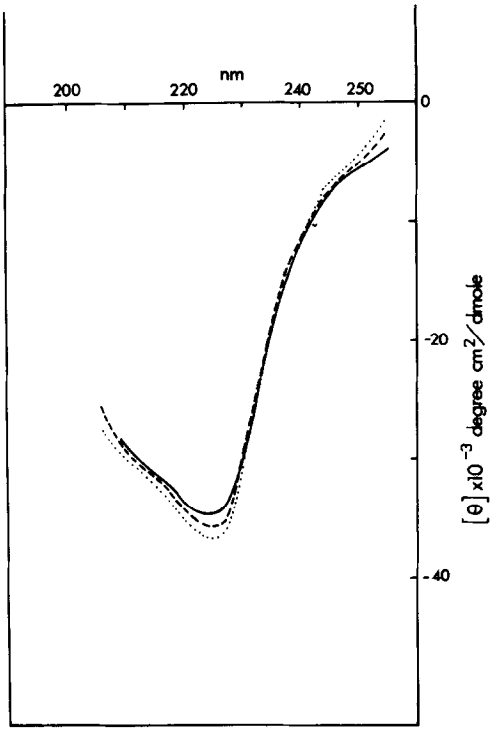

Figure 2. Circular dichroism spectra, between 200 and 255 nm, of chromatin from untreated 3T6 mouse fibroblasts (———), and brUdRib-substituted 3T6 chromatin at final concentrations of brUdRib of 7.2 μM (-----) and 72 μM (· · · · ·) in growth medium. The solvent was 0.01 M Tris-HCl, pH8.

DISCUSSION

The results reported in the present communication seem to offer an explanation for the discrepancy between the findings of Augenlicht et al. (1) and Simpson and Seale (2) on one side, and those of Lapeyre and Bekhor (3) on the other side. The increase in positive ellipticity and the change in shape of CD spectra of chromatin are dependent on the extent of brUdRib replacement. In the experiment of Lapeyre and Bekhor (3) the extent of replacement was 6.7 per cent, only slightly above the 4.9 per cent substitution which, in our laboratory, gave only a modest shift without an increase in positive ellipticity of CD spectra (Fig.1). Lapeyre and Bekhor (3) could not increase the extent of brUdRib substitution because they were injecting brUdRib in experimental animals, a methodology that results only in a modest incorporation of brUdRib into DNA, unless

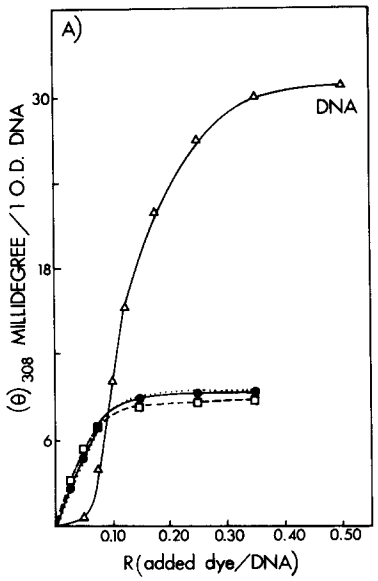

Figure 3. Ethidium bromide ellipticity at 308 nm as a function of the ratio of added dye/DNA $(\theta)_{308}$ of ethidium bromide complexed with calf thymus DNA (▲—▲), chromatin from untreated 3T6 mouse fibroblasts (●—●) and brUdRib-substituted 3T6 chromatin at final concentrations of brUdRib 7.2 μM (□---□), and 72 μM (· · · · ·) in growth medium. CD spectra were obtained in 0.01 M Tris-HCl pH8. Ellipticity is expressed in arbitrary units, i.e. millidegree per optical density of DNA (see text).

special techniques are used (13-14). Interestingly, a brUdRib-substitution of 15-30 per cent is necessary in order to obtain the well-known effect of brUdRib on differentiation (Selma Silagi, personal communication).

It is more difficult to explain the discrepancy between the effect of brUdRib-substitution on the binding of ethidium bromide by chromatin, reported by Lapeyre and Bekhor (3) and the lack of effect reported in the present communi cation. A possible explanation could be found in the methodology used by Lapeyre and Bekhor (3), spectrophotometry, that has several pitfalls (i.e. the secondary sites are only quantitatively but not qualitatively different from the intercalation sites (15)), while the spectropolarimetric method (7,8) is more accurate and measures only primary binding sites (12). Our present finding that brUdRib-substitution does not affect ethidium bromide binding, together with the report that ethidium bromide and RNA polymerase compete for the same

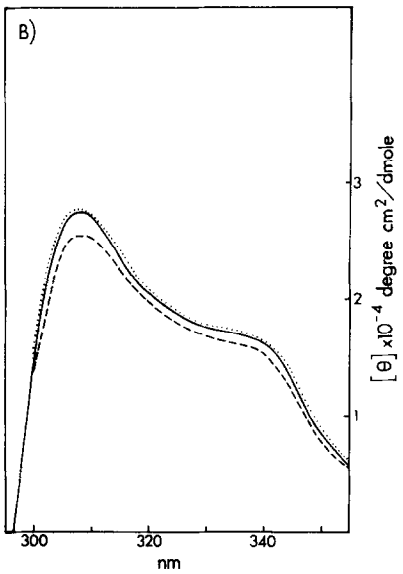

Figure 4. Circular dichroism spectra of ethidium bromide bound to chromatin from untreated 3T6 mouse fibroblasts (———) and brUdRib-substituted 3T6 chromatin at final concentrations of brUdRib of 7.2 μM (-----) and 72 μM (· · · · ·) in growth medium.
The dye/DNA ratio was always 0.25 ± 0.01. CD spectra were obtained in 0.01 M Tris-HCl pH8. The ellipticity is expressed in decimoles of ethidium bromide.

binding sites (16), are in agreement with previous findings that brUdRib substitution does not affect the number of chromatin binding sites for RNA polymerase (5).

ACKNOWLEDGEMENT

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