CIRCULAR DICHROISM PROPERTIES OF ETHIDIUM BROMIDE-DEOXYRIBONUCLEIC ACID COMPLEXES

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Summary: Ethidium bromide (EB) exhibits upon interaction with DNA induced optical activity between 290 to 400mμ and 440 to 540mμ. The dependence of circular dichroism on temperature and EB to DNA molar ratios indicates that "nearest-neighbor" interactions between ethidium molecules positioned within segments of the helix are the main source of optical activity in the 290 to 350mμ region. Optical activity between 440 to 540mμ may be induced by isolated ethidium molecules, interacting with double stranded DNA.

Ethidium bromide interferes with nucleic acid synthesis (1,2,3) by inhibiting the function of DNA polymerase and DNA-dependent RNA polymerase (4). Spectrophotometric (5) and fluorescence (6) methods indicate that DNA forms well-defined complexes with EB. The interaction apparently involves intercalation of the planar phenanthridinium ring between adjacent base-pairs in the double helix (7). The ability of EB to form complexes with DNA and the inhibitory effect of this dye on the process of nucleic acid synthesis are undoubtly related (8). In this report we are presenting results of circular dichroism studies in the region above 290mμ on the EB-DNA complex.

Experimental

Aliquots of Calf Thymus DNA (50mg. Worthington in 25 ml of 0.04M tris·HCl) were added to EB stock solution (40mg. Sigma in 10 ml of buffer) that was usually diluted further before addition. Ethidium to DNA ratios, EB/P, were calculated on the basis of EB and DNA phosphate concentrations using molar extinction coefficients of 5600 at 480mμ (5) and 6600 at 260mμ respectively (9). Ratios of EB bound to DNA, r, were calculated from absorbancies at 460mμ (5).

Circular dichroism and absorption measurements were carried out with a Jasco ORD/UV/CD 5 Recording Spectropolarimeter equipped with an electrically heated cell compartment.

Results

The circular dichroism of DNA-EB at r=0.144 (Complex I) containing DNA at 0.60μM/ml (EB/P=0.15) shown in fig. 1b exhibits positive dichroism in the 290 to 360mμ region which clearly consists of several overlapping bands. Additional bands of lower intensity are present at wavelengths above 360mμ. These bands, which contain both positive and negative components, are also observed in DNA-EB at r=0.01 (Complex II) containing DNA at 5.0μM/ml (fig. 1a).

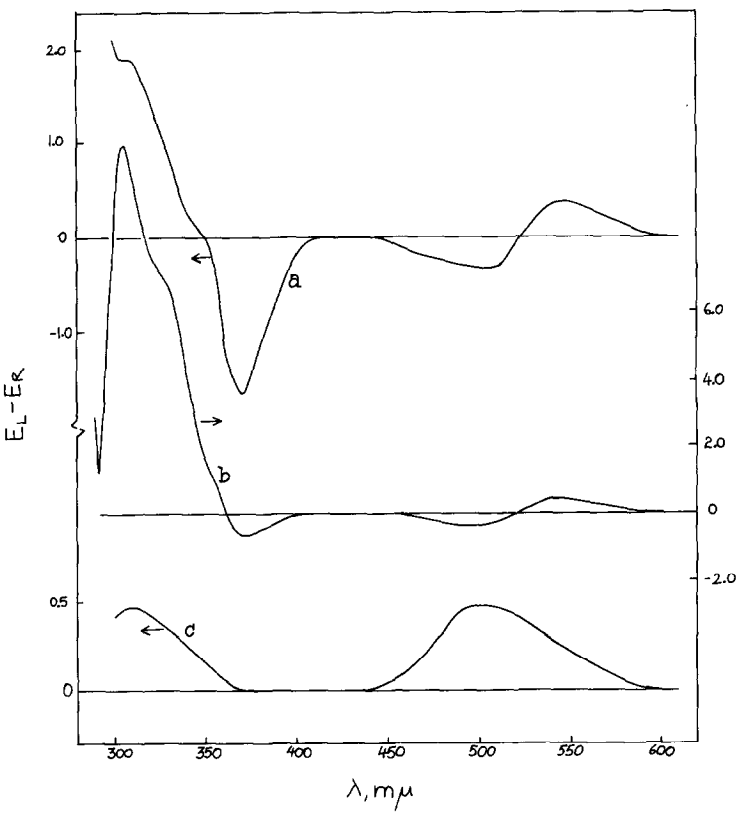

Figure 1: Circular Dichroism of DNA-EB and EB in 0.04M Tris·HCl pH 7.9 obtained at ΔOD of 0.001. (a) DNA-EB in a 5.0cm cell ([P] = 5.0 μM/ml EB = 0.05 μM/ml) (b) DNA-EB in a 1.0cm cell ([P] = 0.60 μM/ml, [EB] = 0.09 μM/ml (c) EB in a 1.0cm cell (0.50 μM/ml); a and c left ordinate; b right ordinate.

The E_L-E_R values for the positive maxima near 310mμ vary from 11.0 in Complex I to 1.9 in Complex II. By contrast magnitudes in the 440 to 600mμ region are comparable. The CD consists of a negative component between 440 and 540mμ characteristic of the interaction between polynucleotide and EB which appears superimposed on a dichroic band originating from EB (fig. 3). Free EB (0.5μM/ml) exhibits positive dichroism below 370mμ and between 450 and 590mμ with maxima near 310 and 505mμ respectively (fig. 1c). The latter band appears responsible for small differences in ellipticities between Complexes I and II in this region.

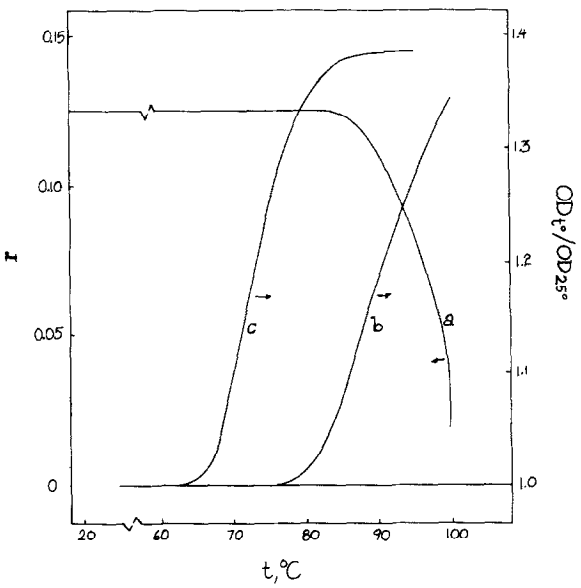

Figure 2: Effect of Temperature on DNA-EB at 0.20 μM/ml DNA EB/DNA = 0.15 in 0.04M Tris·HCl pH 7.9 (a) Variation of r with Temperature (b) Temperature-Optical Density Profile at 260mμ obtained vs DNA-EB at 25°. (c) Temperature-Optical Density Profile at 260mμ for DNA. a, left ordinate; b and c right ordinate.

The results shown in fig. 2 indicate that for DNA at 0.20μM/ml (r=0.125) binding remains unaffected at increasing temperatures up to 85°C and is subsequently sharply decreased to about 10% of its initial value. As indicated by the temperature optical density profile

at 260mμ this sharp decrease in binding occurs over a region that corresponds closely to the temperature range of DNA strand separation in the complex. This change is accompanied by a decrease in E_L-E_R near 310mμ from 10.9 to values that approach zero at 100°C.

Examination of the CD of Complex I at 97°C further indicates that the negative contributions in the 440 to 540mμ region have nearly dissappeared and the positive component originating from EB is largely restored (fig. 3). The positive dichroic band for free EB is not influenced under these conditions.

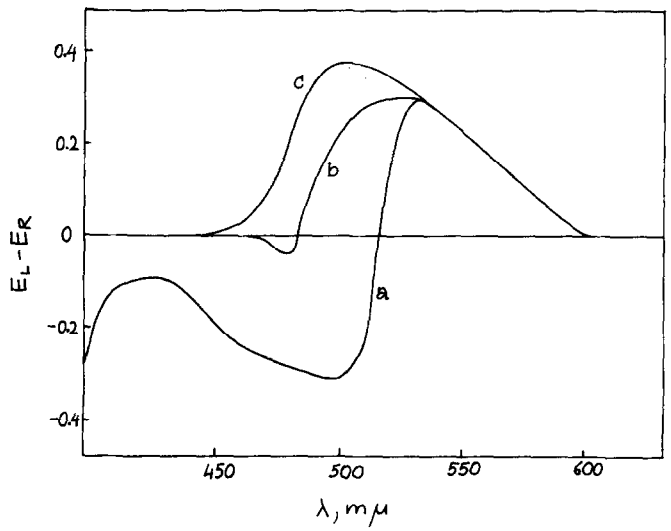

Figure 3: Circular Dichroism of DNA-EB and EB in 0.04M Tris·HCl pH 7.9 obtained at ΔOD of 0.001 in a 10cm cell (a) DNA-EB ([P] = 0.60μM/ml, [EB] = 0.09μM/ml) at 25°C (b) Same as in a at 97°C (c) EB (0.09μM/ml).

Heat-denatured DNA (0.60μM/ml) which contains short helical segments (10) and binds EB as effectively as native DNA (4) also exhibits upon interaction with EB at r=0.144 strong induced optical activity over the 290 to 540mμ region. The E_L-E_R near 310mμ is actually increased from 11.0 in native DNA to 15.4.

The exact dependence of the additional negative band or bands observed between 350 and 400mμ on EB to DNA ratios is obscured by

the strong overlap with the positive dichroism present at lower wavelengths.

Discussion

The association of EB with DNA induces strong optical activity in the resulting complexes. At low D/P, which results in an extensive distribution of the individual dye molecules over the polynucleotide chain and a concommitent minimization of effective interaction of one dye with the other, a strongly diminished effect is observed in the CD between 290 and 360mμ as compared to that found at higher D/P. Furthermore, both EB binding and optical activity in this region are virtually eliminated with random coil DNA formed when the complex is brought to elevated temperatures. This behavior is quite analogous to the disappearance of induced circular dichroism noted for the DNA-proflavine complex at low D/P and also at pH below 2.5 (11). At this pH, as the case is with elevated temperatures, hydrogen bonds between complimentary DNA bases are disrupted.

Although the assignment of dichroic bands in DNA-EB must await the analysis of each region into individual components, it appears likely that the primary source of optical activity between 290 and 360mμ is due to the interaction between the electric dipole moments of two or more regularly ordered dye molecules (12). For this "nearest-neighbor" interaction to occur a rigid hydrogen bonded polynucleotide must hold the dye molecules at close distance and at a fixed orientation. The induced optical activity would therefore, be expected to decrease at low D/P and disappear with random coil DNA as noted. An alternative model for optical activity based on the formation of an "extended helical array" (13) must also be given consideration until bands have been resolved and the exact relation between the ellipticities of individual bands and EB/P ratios has been determined. It must be noted, however, that dichroism in this

region is enhanced perceptively rather than decreased with denatured DNA. On the basis of the higher stacking coefficient indicated for denatured as compared to native DNA (14) this would appear to favor "clusters" as the main cause for optical activity below 360mμ. A further possibility, ie., the nearest-neighbor interaction between nucleic acid-bound dye dimers, which may contribute to the optical activity in the DNA-acridine orange (12) complex, cannot, in view of the small tendency of EB to aggregate (5), be significant in this system.

The appearance of optical activity in DNA-EB between 440 to 540mμ at very low D/P ratios, the insensitivity of the obtained dichroism to D/P values and the dependance of binding and the induced dichroism to temperature noted, suggest that optical activity over this region depends on EB molecules with the proper orientation interacting with an ordered polynucleotide. Optical activity may result from assymetry induced by a monomeric chromophore band within the assymetric environment of the polymer (15). This condition is fulfilled when isolated EB molecules are intercalated between bases without the necessity of invoking "nearest-neighbor" interactions.

The possibility of distingishing among different modes of interaction between DNA and EB by circular dichroism may be of value in understanding the biochemical properties of EB at the molecular level. The binding of EB to supercoiled DNA is enhanced at low EB concentratio in comparison to non-supercoiled DNA whereas at high concentrations the reverse is true (16). Variation in binding specificities at different EB concentrations is undoubtly relevent to the pharmacologically selective effect of EB on the double stranded circular DNA of trypanosomes. In this as well as other instances the biochemical effects of certain substances known to influence nucleic acid function could be illuminated by a better understanding of the nature of their binding interactions with nucleic acids.

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