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Circular Dichroism and Absorption Spectra of Mono- and Di-Aminoacridines Complexed to DNA

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The absorption and circular dichroism spectra of the intercalation complexes with DNA of the 9-aminoacridine chromophore in mono- and bisfunctional forms have been determined in the visible/near-ultraviolet region. The experimental spectra were deconvoluted into contributions from three (π^*, π) transitions and the dipole and rotatory strengths of one long-axis and one short-axis polarized transition were determined for each intercalating species. The experimental results were compared with theoretical calculations of the induced CD of intercalation complexes and a series of inferences were drawn concerning the geometry of the complexes.

INTRODUCTION

A great many planar aromatic systems bind to DNA by non-covalent intercalation between adjacent base-pairs of the double helix. Such materials are invariably biologically active and, as a result, elucidation of their mode of interaction with DNA has received much attention.[1] More recently interest has turned to compounds which incorporate two or more potential DNA intercalating regions since such polyfunctional intercalators may be expected to possess enhanced affinity for DNA.[2] Although bis- and poly-intercalation of DNA has been demonstrated conclusively, there are clear indications that intercalation complexes formed between DNA and the same intercalating moiety of mono- and polyfunctional intercalators differ; for example, the unwinding of the DNA helix or its lengthening by a bis-intercalator is not twice that produced by the corresponding mono-intercalator.[2,3]

In this work we address the problem of the comparative geometry of the intercalation complexes of DNA with the 9-aminoacridine moiety in its mono- and bisfunctional forms by the determination and analysis of the induced circular dichroism. This chromophore is particularly suitable for our purpose since its electronic spectrum is well understood[4] and since, in the

range which does not overlap the DNA spectrum, there exist transitions polarized both along the short and long axes of the dye with a consequent increase in the information content of the electronic spectra compared with that of just a single transition.

MATERIALS AND METHODS

9-Aminoacridine hydrochloride (9AA) was obtained from A. G. Fluka; it was recrystallized twice from ethanol followed by drying under vacuum. *N,N'*-Di(acridin-9-yl)hexane-1,6-diamine (diacridine) was a gift of Dr. R. G. Wright;[5] this material, with a hexane chain linking the two aminoacridine moieties, is a well-established bis-intercalator of B-DNA.[2,3] The DNA used and the preparation of the solutions have been described previously.[6]

The absorption and circular dichroism (CD) spectra were determined by the use of a Zeiss DMR 10 spectrophotometer and a Jasco J-40CS spectropolarimeter as described before.[6] All data refer to room temperature measurements near 21 °C. Data reduction and fitting procedures were carried out by the use of a Sun 4/280 computer. Molar absorptivities, ε, and molar circular dichroism, $\Delta\varepsilon$, are expressed in terms of aminoacridine moieties throughout.

Details of the method of calculating the induced circular dichroism in model transition moments of the nucleic acid bases and that of the intercalator were evaluated by the London method of monopoles[8] and the geometrical data for the intercalation complexes have been given previously.[7] The interactions between the complexes were based on x-ray diffraction data.[9,10] The calculated induced CD was found to be insensitive to relatively substantial changes in pitch and pitch angle of the bases in DNA.[7]

RESULTS

The absorption and circular dichroism spectra of 9AA in the presence of DNA in solutions containing 0.001 mol dm^{-3} of sodium chloride have been described previously.[6] These spectra depend on the ratio $\beta = C_T/C_{DNA}$ where in practice the total dye concentration, C_T, is held constant and C_{DNA}, the DNA concentration expressed as DNA phosphorous per dm^3, is varied. The dependence of these spectra on β has been quantitatively accounted for in terms of the AKS binding model[11] involving intercalated dye species, externally bound dye species and the interaction between them. The spectra shown by the points in Figures 1a and 2a have been obtained by extrapolation of the β-dependent spectra to the limit of infinite DNA concentration, $\beta = 0$, so that these spectra are, therefore, those of the intercalated species only. The spectra of the diacridine/DNA complex shown in Figures 3a and 3b correspond, similarly, to the species in the limit of $\beta = 0$.

We have also shown previously, by means of the linear dichroism of 9AA in stretched polymer films[4], that the absorption spectrum of this dye above about 300 nm is the result of three separate (π^*, π) transitions: one relatively intense transition polarized along the molecular short axis, a low-intensity long-axis polarized transition at lower wavelengths overlapped largely by the short-axis transition and another, relatively free-standing, long-axis polarized transition below about 340 nm. The analysis of the resolved short- and long-axis polarized spectra of the dye was based on their quantitative fit to harmonic vibronic progressions which is also the method used here and is, therefore, summarized below.

We take that each vibronic progression consists of bands of intensities ε_{m0} centred at $\tilde{\nu}_{m0}$ cm^{-1}, where m is the vibrational quantum number associated

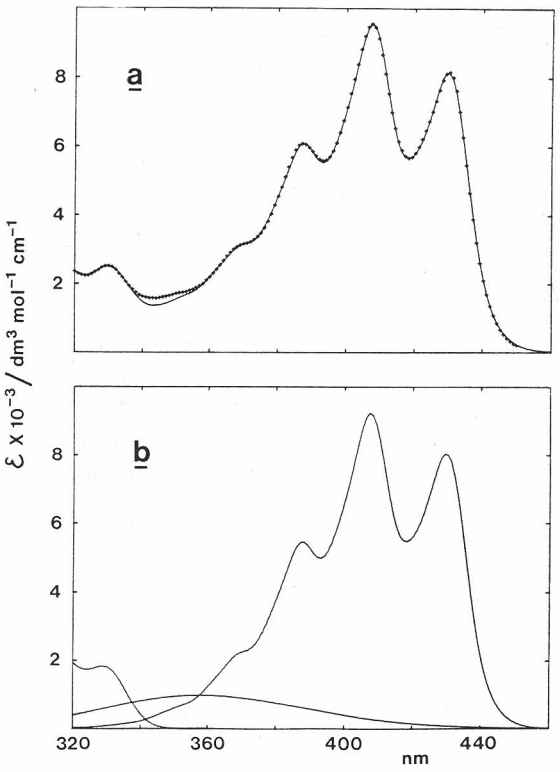

Figure 1. Absorption spectrum of the 9AA/DNA intercalation complex in 0.001 mol dm^{-3} sodium chloride solution (a) Points: experimental spectrum; solid line: fitted spectrum. (b) The deconvoluted individual transitions.

with the upper electronic state while the vibrational quantum number of the absorbing molecules in their ground states is generally zero. If the vibration is harmonic and the force constant does not change on excitation, the following relations hold[12]

$$\tilde{\nu}_{m0} = \tilde{\nu}_{00} + mV \tag{1}$$

$$\varepsilon_{m0} = \frac{\varepsilon_{00}}{\nu_{00}} \frac{X^m}{m!} \tilde{\nu}_{m0} \tag{2}$$

where V is the separation between bands and X is a quantity related to the difference in the equilibrium nuclear conformations in the two electronic states. Further quantities of interest are: the total integrated intensity, D_0, of the progression or its oscillator strength[13], f, its centre of gravity or first moment, D_1 and its dipole strength[13] $\mathcal{D}$, given by[14] (cgs units):

$$D_0 = \Sigma \varepsilon_{m0} = \varepsilon_{00} e^X (\tilde{\nu}_{00} + XV)/\tilde{\nu}_{00} \tag{3}$$

$$f = 4.319 \times 10^{-9} D_0 \tag{4}$$

$$D_1 = \Sigma\,(\varepsilon_{m0}\,\tilde{\nu}_{m0})/D_0 = \tilde{\nu}_{00} + XV \tag{5}$$

$$\mathcal{D} = 9.180 \times 10^{-39}\, D_0/D_1 \tag{6}$$

In deriving these expressions use was made of the identity

$$\sum_{m=0}^{\infty} (X^m/m!) = e^X \tag{7}$$

Finally, it is assumed that each vibronic band is represented satisfactorily by a Gaussian shape so that at any wavenumber, $\tilde{\nu}$, the contribution of the $(m, 0)$ band to the spectral intensity, $I_m(\tilde{\nu})$, is given by

$$I_m(\tilde{\nu}) = I_{m0} \exp\{-2.7726\, b^{-2}\,(\tilde{\nu} - \tilde{\nu}_{m0})^2\} \tag{8}$$

where I_{m0} is the intensity at the maximum and b is the bandwidth at $I_{m0}/2$. The value of ε_{00} to be used in Equations 3—6 then becomes

$$\varepsilon_{00} = \int_{-\infty}^{\infty} I_m(\nu)\,d\nu = 1.0645\, b\, I_{00} \tag{9}$$

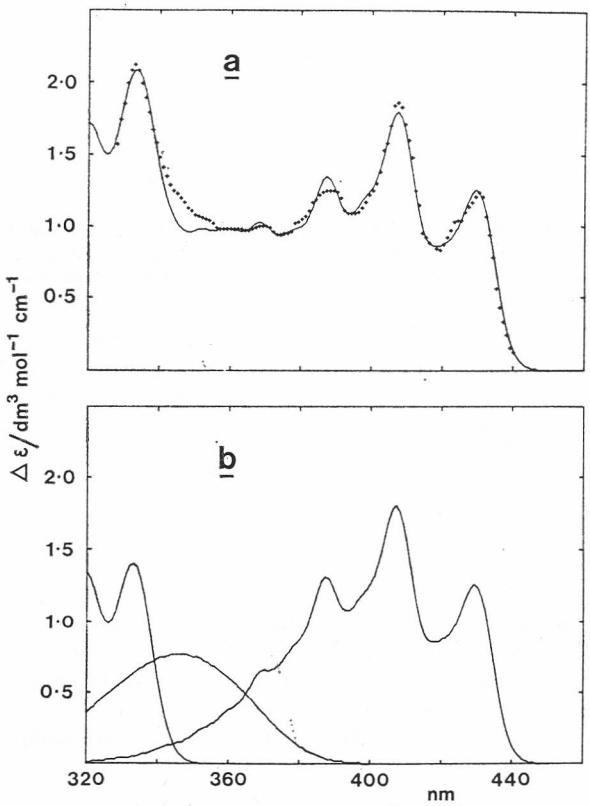

Figure 2. Circular dichroism of the 9AA/DNA intercalation complex in 0.001 mol dm^{-3} sodium chloride solution. Notation as in Figure 1.

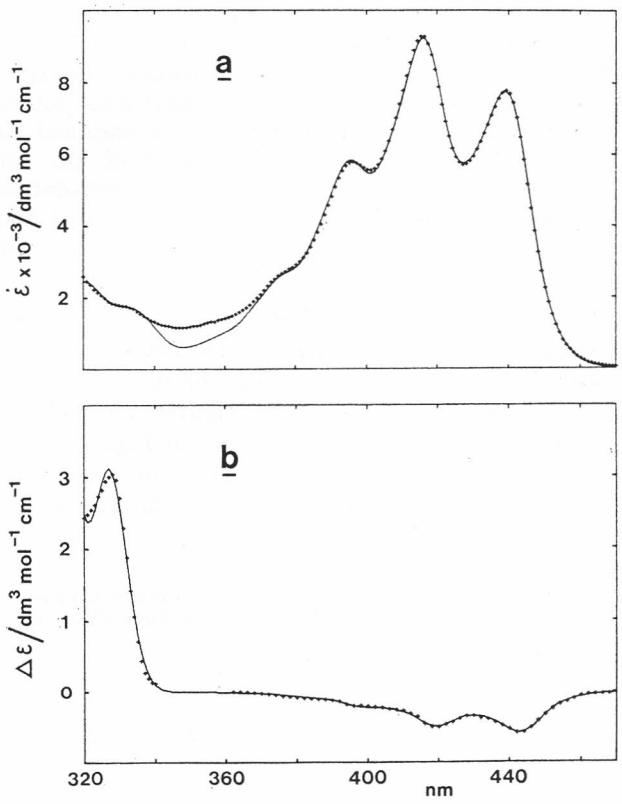

Figure 3. Absorption spectrum (a) and circular dichroism (b) of the diacridine/DNA complex in 0.001 mol dm^{-3} sodium chloride solution. Notation as in Figures 1(a) and 2(a).

and the total spectrum due to the progression may be written as

$$I(\tilde{\nu}) = I_{00} \Sigma \{(X^m/m!)(1 + mV/\tilde{\nu}_{00}) \exp[-2.7726\, b^{-2} (\tilde{\nu} - \tilde{\nu}_{00} - mV)^2]\} \qquad (10)$$

The fit of the experimental spectrum to $I(\tilde{\nu})$ involves, accordingly, 5 adjustable parameters for each progression, I_{00}, $\tilde{\nu}_{00}$, V, X and b which may then be used to calculate the quantities characterizing the progressions involved in each transition through Equations 3—6. The corresponding formulae valid for the description of the circular dichroism due to a progression are identical to the above expressions except that intensities refer now to the relevant $\Delta\varepsilon$ values and that the rotatory strength, R, is given by

$$R = 2.443 \times 10^{-39}\, \Delta\varepsilon_{00}\, b\, e^X/\tilde{\nu}_{00} \qquad (11)$$

In considering low resolution electronic spectra in solution of a number aromatic chromophores in the past we found that in most instances each electronic transition could be described to a good approximation by a single

harmonic progression.[14-20] There are, however, exceptions[4,21,22] and the short-axis lowest energy transition of 9AA is one of these. In accordance with our previous finding[4,22] in fitting the experimental spectra of the 9AA chromophore to Equation 10 the lowest energy short-axis polarized transition in each of the spectra shown in Figures 1—3 was assumed to require two progressions separated by about 400 cm^{-1}. In addition, the same short-axis polarized spectra of both 9AA and diacridine show well-defined vibrational structures with a separation between the observed peaks of about 1300 cm^{-1}; we have, accordingly, taken this as the fixed value of V in Equation 10 for all the progressions.

The results of the deconvolution of the spectra are summarized in terms of the quantities relevant to the discussion in Table I. In the case of the 9AA/DNA complex, the separate contributions of the three transitions to the absorption and CD spectra are shown in Figures 1b and 2b; their sums, simulating the experimental spectra, are shown in Figures 1a and 2a by the solid lines. For the diacridine/DNA complex only the total calculated spectra shown by the solid lines are displayed in Figures 3a and 3b.

TABLE I

Characteristics of the Transitions of the DNA Intercalation Complexes of 9AA and Diacridine above 320 nm in 0.001 mol dm^{-3} Sodium Chloride Solution

	9-aminoacridine			Diacridine		
Transition	1	2	3	1	2	3
Polarization axis	short	long	long	short	long	long
Absorption						
D_1 (nm)	404	(354)	315	413	(372)	313
f	0.062	(0.013)	0.016	0.064	(0.003)	0.022
$Đ \times 10^{36}$ (cgs)	5.33		1.08	5.57		1.45
Circular dichroism						
D_1 (nm)	396	340	320	419	(390)	309
$R \times 10^{40}$ (cgs)	5.22	2.72	3.14	—1.83		10.2
»Geometric factor«						
$(R/Đ) \cdot \Delta \times 10^4$	0.9		1.2	—0.3		2.5

NOTES: 1. The precision of the numbers enclosed in brackets is comparatively low.
2. The values of f, $Đ$ and R are calculated per mole of the acridine moiety.

The significance of the final entry in Table I, $(R/Đ)\ \Delta$, is as follows. The induced CD in the dye, R, arising from the coupling between the transition moment of the acridine with those of the DNA bases is proportional to the corresponding dipole strength, $Đ$, and to the sum[23]

$$\Sigma\, \mu_B^2\, GF/\Delta$$

where GF is a factor depending on the geometrical arrangement between the transition moments of the dye and of the bases, μ_B, and we define;

$\Delta = (\tilde{\nu}^2_B - \tilde{\nu}^2_{dye}/\tilde{\nu}_B\,\tilde{\nu}_{dye}$. If we replace the collection of transition moments in the bases by an average square moment, $\langle \mu^2 \rangle$, we may write

$$(R/\bar{D})\,\Delta \;\alpha\; \langle \mu^2 \rangle \cdot GF = \text{constant} \cdot GF \qquad (12)$$

Thus, the quantity $(R/\bar{D})\,\Delta$ enables a comparison of the data, approximately at least, in terms of relative geometries only. In these calculations we equated ν_{dye} with the corresponding value of D_1 and we took $\tilde{\nu}_B = 38460$ cm^{-1}, the position of the maximum of the absorption spectrum of DNA nearest in energy to the dye spectra considered.

DISCUSSION

The deconvolution of the intercalation complexes in solution into three separate transitions follows closely the result of the deconvolution of the polarized spectra of 9-aminoacridine in stretched poly(vinyl alcohol) films.[4] Using linear dichroic spectra in the previous work, the relatively weak long-axis transition in the long wavelength region could be physically separated from the dominant short-axis transition of this region and its contribution to the total spectrum determined with confidence. Such a separation of transitions of mutually perpendicular polarizations is not possible in isotropic solution and, as a consequence, there are considerable uncertainties associated with the position of the first moment, D_1 and of the dipole strength, $\bar{D}$, in absorption of the low-intensity long-wavelength long-axis polarized transition in both 9AA and diacridine; for this reason, in Table 1 the relevant D_1 values are placed in brackets and the corresponding $\bar{D}$ values omitted.

The deconvolution of the circular dichroism of both compounds is fully consistent with that of the corresponding absorption spectra. It may be seen from a comparison of Figures 1b and 2b that the positions of the transitions in the 9AA/DNA intercalation complex, as determined from the pair of CD and absorption spectra, coincide and similar results apply to the corresponding spectra of the diacridine/DNA complex as well; such an agreement between the analyses of independent sets of spectral data reinforces the belief that the methods used are essentially correct. There is again a problem with the long-wavelength long-axis polarized transition: its circular dichroism in the diacridine/DNA complex is very small and, accordingly, its D_1 value in Table I is placed in brackets and the R value omitted.

We are left, most importantly, for each of the two dye/DNA complexes with one short-axis and one long-axis polarized transition whose dipole strengths and rotatory strengths have been obtained with fair confidence. We may, therefore, answer in a definite affirmative the question posed in this work of whether or not there are differences in the intercalation complexes of the 9-aminoacridine moiety in its mono- and bisfunctional forms. The evidence is compelling: the signs of the CD of the short-axis polarized transitions differ in the two compounds and, also, the magnitude of the CD of the long-axis transition is significantly greater in the diacridine/DNA than in the 9AA/DNA intercalation complex.

The next task of drawing inferences concerning the actual geometries of the dye/DNA complexes is less straightforward as was anticipated from the results of the calculations recently reported[7] on the circular dichroism of

chromophores intercalated between AT base-pairs and GC base-pairs arranged in the B-DNA form. It was found that the CD induced is a highly sensitive function of both the angular and lateral displacements of the intercalator within the intercalation plane, as well as of the type of base-pair surrounding the molecule. Thus, CD results may be used to exclude certain geometries or to test specific intercalation models rather than to attempt the assignment of a unique geometry to intercalation complexes.

We shall illustrate the approach first by consideration of the 9AA/DNA intercalation complex. One general result arising from the theoretical work was that intercalators with two mutually perpendicular transition moments in the molecular plane and those centred at or near the axis of the DNA helix will have circular dichroism of opposite signs induced in the two orthogonally polarized transitions.[7] As seen in Table I, the experimental rotatory strengths of the two transitions in the 9AA/DNA complex have the same sign: we conclude that the dye is situated off the helix centre so that one is inclined to exclude geometries for this complex such as the Lerman intercalation model[24] and other pseudosymmetric forms found in crystals of 9AA complexed to dinucleoside monophosphates.[25] The above conclusions are also in agreement with the results of NMR measurements in solution of 9AA complexed to oligonucleotide duplexes:[26] the data give a poor fit to an assumed pseudosymmetric intercalation complex but they seem to be in satisfactory agreement with two »trial« models of asymmetric complexes, both of which involve the displacement of the dye from the centre of the helix towards a more intimate contact with the bases of just one of the DNA strands. One of these models, contrary to previous conjectures,[24,27,28] places the long axis of the intercalating acridine dye parallel with the pseudodyad axis of the base-pairs. Such an arrangement appeared to show promise of fitting in with the experimental results of our CD measurements and led to the model depicted in Figure 4, used here to compare theoretical calculations with experimental data.

We consider, as before,[7] an AT or a GC duplex where the helix winding angle of B-DNA is assumed to be 10° allowing a 340 pm thick intercalator to slide between the planes of the base-pairs. In Figure 4, both base-pairs are shown; the upper pair is drawn with thicker lines. The centre of the helix and the pseudodyad axis (X) are also indicated. We place the intercalator along the Y-axis between the base-pairs with its long-axis parallel to X and calculate the induced CD for the two mutually orthogonal transitions as a function of the distance from the helix centre. We note that in Figure 4 the lengths of the double arrows represent the lengths of the molecular axes of the dye rather than the dipole strengths of the corresponding transitions. The results of the calculations in arbitrary units which are proportional to the »geometric factor«, GF, in equation 12 are given in Figure 5. We also indicated in this Figure those particular values of the Y-coordinates for the two systems which correspond to the experimentally determined relative magnitude of $(R/Đ)\Delta$ of the two transitions in the 9AA/DNA intercalation complex; these are relatively close for the two types of base-pairs giving a mean value near 360 pm.

Turning to the diacridine/DNA complex, reference to Figure 5 shows that the same model is capable of providing a satisfactory interpretation of

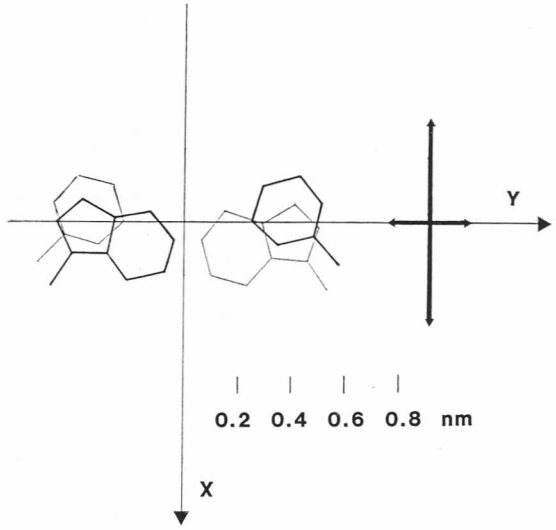

Figure 4. Model used for calculating the induced CD of the intercalated dye. The dye is situated along the Y-axis with double arrows indicating its long and short axes. X is the pseudodyad axis of the base-pairs, the top one being drawn with thick lines.

experimental results: a shift of the position of the intercalating 9AA moiety nearer to the helix axis decreases the geometric factor associated with the induced CD of the short-axis transition to negative values while increasing simultaneously that associated with the long-axis transition irrespective of the type of base-pair considered. We again indicate in Figure 5 the positions that conform to the experimentally determined sign and relative magnitude of the geometric factor; the mean distance in this case is about 300 pm.

The results just outlined provide an excellent illustration of the extreme sensitivity of the induced CD to intercalation geometry: relatively subtle changes may produce CD spectra that differ in both magnitude and sign. Clearly, intercalation geometries other than those outlined in Figure 4 may exist which lead to calculated CD consistent with the experimental data. Thus, for example, if the intercalator is assumed to be situated at positive values of the X-coordinate instead of along the Y-axis, the results of the calculations are similar to those shown in Figure 5, except that the Y-coordinates that fit the experimental results of both the 9-aminoacridine and the diacridine intercalators would need to be increased. We reemphasize that the model shown in Figure 4 should be regarded as feasible and certainly not as definitive.

CONCLUSIONS

In this work we have been able to achieve deconvolution of the absorption spectra and circular dichroism of the 9-aminoacridine moiety in its mono- and bis-intercalator forms complexed to DNA into contributions from three separate (π^*, π) electronic transitions. This has then enabled the experimental estimation of the dipole strengths and rotatory strengths with good

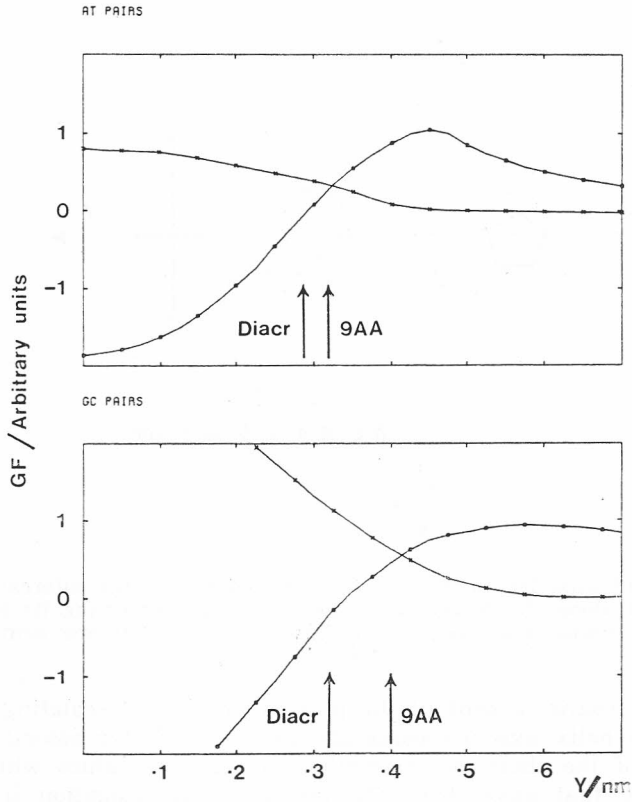

Figure 5. Magnitudes of the calculated induced CD in arbitrary units as a function of the Y-coordinate of the intercalator according to the model shown in Figure 4 for intercalation between AT and GC pairs, respectively. Squares and crosses denote short-axis and long-axis polarized transitions, respectively. The arrows indicate positions conforming best to the experimental results.

precision of two transitions, one short-axis the other long-axis, for both types of intercalators. Having determined simultaneously the characteristics of two mutually perpendicular transitions in the same intercalating chromophore provided the means for evaluating the assumed intercalation geometries using the results of theoretically calculated induced circular dichroism. We conclude, with decreasing order of confidence, the following:

1. The geometries of intercalated 9-aminoacridine moiety in the mono- and bisfunctional forms differ.
2. The intercalated 9AA moieties are situated off the centre of the DNA helix.
3. The long-axis of the intercalated 9AA moiety is likely to be approximately parallel to the pseudodyad axis of the base-pairs.
4. The intercalation geometries of the mono- and bisfunctional forms may be very similar, differing only in their relative displacements from the helix centre.

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SAŽETAK

Cirkularni dikroizam i apsorpcijski spektri mono- i di-aminoakridina kompleksiranih s DNA

Daniel Fornasiero, Tomas Kurucsev, Reidar Lyng i Bengt Nordén

Određeni su u vidljivom i bliskom ultravioletnom području apsorpcijski i spektri cirkularnog dikroizma interkalacijskih kompleksa 9-aminoakridinskog kromofora, u mono- i bifunkcionalnom obliku, sa DNA. Eksperimentalni spektri razloženi su u doprinos od tri (π^*, π) prijelaza, a dipolna i rotacijska jakost za jedan prijelaz uzduž dulje osi i jedan prijelaz uzduž kraće osi određeni su za svaku interkalacijsku vrstu. Eksperimentalni rezultati uspoređeni su sa teorijski proračunanima za inducirani CD interkalacijskih kompleksa, a izveden je i niz zaključaka o geometriji kompleksa.