BINDING OF INERT METAL COMPLEXES TO DEOXYRIBONUCLEIC ACID DETECTED BY LINEAR DICHROISM

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1. Introduction

The versatility of a sensitive linear dichroism (LD) detection system was recently demonstrated on polymers oriented by flow gradients, e.g. LD at a fixed gradient was a sensitive measure of the concentration of a complex between DNA and the ethidium cation [1]. The sensitivity was a product of several circumstances: a high stability constant, a high order parameter with respect to the orientation of the dye to the helix and to a pronounced optical anisotropy of the dye molecule. With inert metal complexes derived from cubic symmetry and without a potentiality of intercalation we have lower magnitudes in all these respects, but still LD is superior to other spectroscopic signals (circular dichroism, CD) in revealing binding. We here demonstrate by LD that complexes are formed between DNA and the trigonal inert Fe(II)tris(dipyridyl) and Fe(II)tris-(glyoxal-bis-*N*-methylimine) cations. The planar Cu(II)bis(dipyridyl) cation is also shown to give LD when added to oriented DNA.

2. Experimental method

The principle of the method may be illustrated as follows: a dilute aqueous DNA solution is subjected to a constant and stable hydrodynamic gradient ($3000\ s^{-1}$) and the linear dichroism, $LD = A_{\parallel} - A_{\perp}$ (parallel and perpendicular to the flow lines) is followed (between 200–700 nm and typically at a resolution of 10^{-6} absorbance units). For a description of the flow-cell, the instrumentation and the quantitative interpretation of LD, see [1–3]. If a molecule with anisotropic absorption, not coinciding with the intrinsic DNA absorption is bound to the oriented polynucleotide, a non-zero LD results characteristic of the absorber and its orientation to the helix axis.

The metal complexes were obtained as in [4]. Calf-thymus DNA, Sigma type I, was used without further purification, either in 0.2 M NaCl as an ionic medium or at an ionic strength approx. 10^{-3} M.

With species expected not to bind (anions, as CrO_4^{2-}, $PtCl_4^{2-}$) zero LD was observed.

3. Results and discussion

Fig.1 shows the linear dichroism developed when $Fe(dipy)_3^{2+}$ is added to an oriented DNA solution. From confident knowledge about the origin of the u.v. absorption at 294 nm in this complex [5] we can make a qualitative conclusion about the predominating orientation in the complex: slightly perturbed transitions polarized along the long-axes of the dipyridyl ligands yield by dipole coupling two exciton components with mutually perpendicular polarisations, one with A_2 symmetry (285 nm) and one with E symmetry (303 nm) (the complex belongs to the point group D_3). The A_2 band is hidden in the large negative LD band of DNA from the purine–pyrimidine π transitions, however positive sign of the E component proves that the $Fe(dipy)_3^{2+}$ complex is associated with its C_3-axis preferentially perpendicular to the DNA helix-axis (I). This conclusion is supported by the observation of positive signs on the 520–480 nm bands which are theoretically [4]

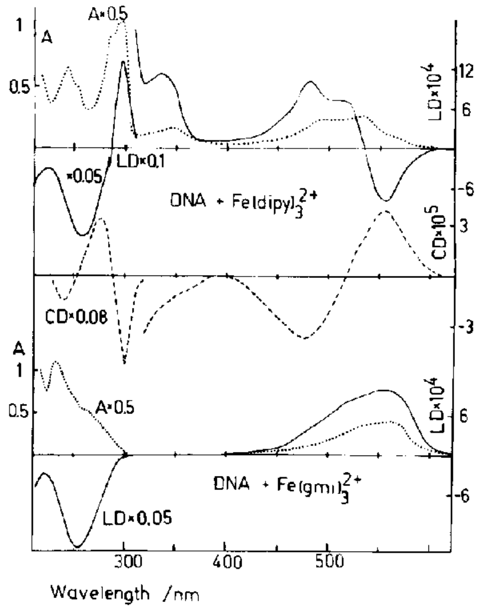


Fig.1. Linear dichroism (———), 3000 s^{-1}. Absorbance (. . . . .) and circular dichroism (- - - - -) spectra of (calf thymus) DNA + Fe(dipy)$_3^{2+}$, DNA + Fe(gmi)$_3^{2+}$. (cell length 0.1 cm, DNA concentration 0.3 mg ml^{-1}, ionic strength below 10^{-3} M, complex concentration according to absorbance, linear dichroism LD = $A_{\parallel} - A_{\perp}$ and circular dichroism, CD = $A_l - A_r$, in absorbance units).

predicted to have E symmetry. A weak A_2 transition has been supposed [6] at very low energy and the negative band at 555 nm can thus be considered as important evidence for the existence of a transition with this symmetry.

LD/A is very low, formally implying a low order, however A includes also free metal complex and not until the stability constants have been determined it is possible to make any quantitative structural estimates. An estimate employing the observations in fig.2 suggests the stability be 3×10^3 M^{-1} when considered for a N–M unity (N = nucleotide residue, M = Fe(dipy)$_3{}^{2+}$). This indicates that the maximum |LD/A| for the complex is still less than approx. 0.01 or that the orientation of the metal complex to the helix axis is just accidentally deviating from random as expected with non-directed electrostatic interaction with the peripheral phosphates.

Also with Fe(gmi)$_3{}^{2+}$ a non-vanishing LD shows an association to DNA. This LD spectrum is

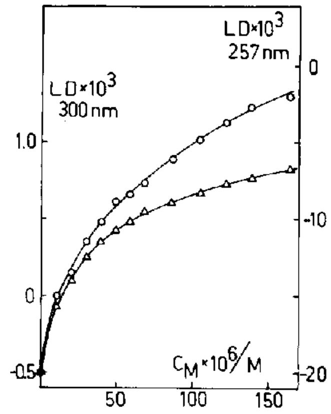


Fig.2. LD versus total metal complex concentration (C_M) at 257 nm (DNA conformation) (△——△) and at a wavelength (300 nm) characteristic for the concentration of DNA–M, (M = Fe(dipy)$_3^{2+}$) (○——○). Ionic strenght $1\cdot10^{-3}$ M NaCl. DNA concentration $0.24\cdot10^{-3}$ M.

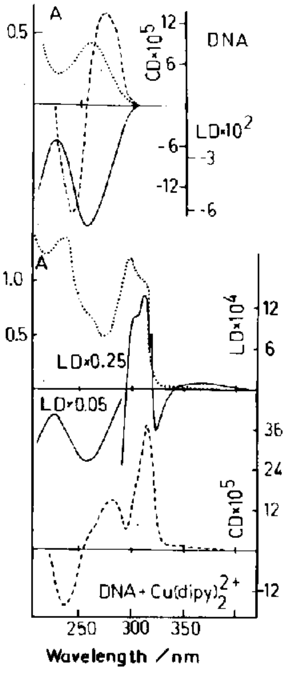


Fig.3. LD (3000 s^{-1}), A and CD of free DNA and DNA + Cu(dipy)$_2^{2+}$. Conditions and notations as in fig.1.

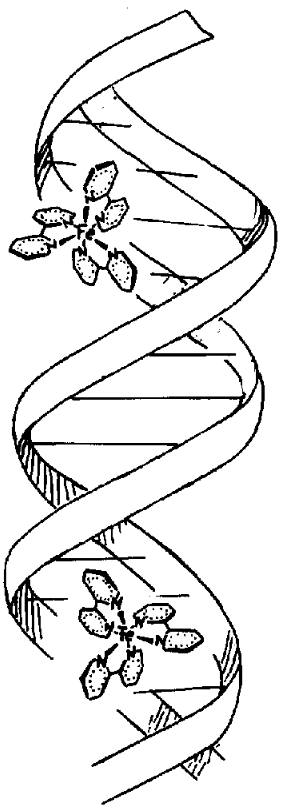
less differentiated than the one with the dipy compound with no excition band to reveal the predominant orientation. On the basis of the positive bands at low energy (E symmetry) [4] we suggest that the orientation may be similar to that in the Fe(dipy)$_3$–DNA complex. On the other hand the complete lack of CD may suggest a further lower order within the complex and a larger average N–M distance.

When the planar Cu(dipy)$_2{}^{2+}$ is added to the oriented DNA (fig.3) a series of bands arises between 300–350 nm (the visible bands have unfortunately too low intensity to be observed). If the bands at 300 nm are due to unperturbed long-axis polarized $\pi \rightarrow \pi^*$ in the dipy ligand the positive LD must be interpreted in terms of an orientation with the CuN$_4$ plane parallel to the helix-axis. However, it is reasonable to assume intercalation with this molecule and we can therefore not rule out that the LD is due to some charge-transfer transition with polarisation parallel to the helix axis.

As is visualized in the figures, weak CD effects are induced in two of the metal complexes when mixed with the chiral DNA, which may be taken as an independent evidence for the association, though it does not give any further clues about the structures. Fe(dipy)$_3$–DNA exhibites a CD spectrum resembling that of the (+)Fe(dipy)$_3^{2+}$ enantiomer. This must be simply referred to an inversion lability of this compound yielding a certain net excess of the enantiomer preferably bound ('Pfeiffer effect'). Hence we may directly conclude that the Δ-Fe(dipy)$_3^{2+}$ enantiomer (IUPAC notation), the upper one in (I), yields the best fitting, which is interesting since the Δ configuration implies the same screw sense as the DNA helix.

LD of the intrinsic DNA chromophore (257 nm) is successively decreased when increasing the metal concentration. The very small relative change in ionic strength when adding complex and the simultaneously increasing LD (fig.2) from oriented Fe(dipy)$_3^{2+}$ (300 nm) suggest that the effect is not due to a general denaturation but rather to local effects at the occupation of sites on the helix (changing the base-pair conformation).

When the ionic strength is increased (0.2 M NaCl) the LD is completely extinguished in the cases of the Fe complexes while with Cu(dipy)$_2^{2+}$ a certain intensity (5%) remains, thus proving the somewhat larger stability of this complex, reasonable in light of possibly hydrophobic interactions in this case.


(I)

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