DNA–Porphyrin Interactions probed by Induced CD Spectroscopy

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Detailed analysis by induced CD spectroscopy has revealed that the manganese porphyrin, Mn(tmpyp), binds the major groove of AT or GC sequences in DNA, as well as the reported minor groove of AT sequences, with a preference that depends on the porphyrin to DNA base-pair molar ratio.

Porphyrin–nucleic acid interactions, particularly those of tetrakis(1-*N*-methyl-4-pyridinio)porphine, H_2tmpyp, and its metal complexes have been studied extensively[1] by spectroscopic[1–5] and biochemical[1,6,7] approaches. These results have suggested that the porphyrin free base, H_2tmpyp, and square-planar complexes such as those with Cu^{2+} and Ni^{2+} intercalate between the DNA base pairs, while metalloporphyrins with axial ligands, *e.g.* Fe(tmpyp) and Mn(tmpyp), bind to the minor groove of AT regions.

Recently we[8] and others[9] have shown by DNase I footprinting and affinity cleavage experiments that Mn(tmpyp) and Fe(tmpyp) possess surprisingly high sequence preference towards the minor groove of three contiguous AT base pairs. From detailed analysis of induced CD spectroscopy we here demonstrate that Mn(tmpyp) exhibits two distinct binding modes consistent with binding to the major groove as well as the minor groove of DNA. The relative preference of each binding mode depends both on the porphyrin : DNA base-pair ratio(r) and the DNA base sequence.

Fig. 1(*a*) shows the induced CD spectra of Mn(tmpyp) in the 420–500 nm region titrated with poly(dA-dT)$_2$ DNA. Nucleic acids have no absorption bands in the visible region, however, the porphyrin exhibits a strong Soret band. Mn(tmpyp) is achiral and hence no CD is observed in the absence of DNA ($r = \infty$). On adding poly(dA-dT)$_2$, the solution exhibited two positive peaks at *ca.* 456 and 466 nm, where the longer wavelength peak was dominant. Addition of more DNA enhanced both peaks, though the short wavelength one grew more rapidly. The two peaks were of near equal intensity at r = *ca.* 0.4. At lower r values the shorter wavelength peak was dominant with a slight red shift of the peak maximum. Eventually at $r \leqslant 0.1$ only the short wavelength peak appeared. Similar spectral changes were observed in the case of Mn(tmpyp)–poly(dA)·poly(dT) DNA, although the shorter wavelength peak started to develop at lower r values of *ca.* 0.5 (data not shown). In contrast, with alternating or homo GC DNA, only one positive peak was observed during the course of DNA titration, and the intensity was much reduced compared with the case of AT containing DNA [Fig. 1(*b*)]. On adding poly(dG)·poly(dC) DNA, the peak at *ca.* 464 nm increased and then decreased in intensity with a slight blue shift of the peak maximum at lower r values. At r = 0.012 the spectrum showed a small positive band at *ca.* 459 nm.

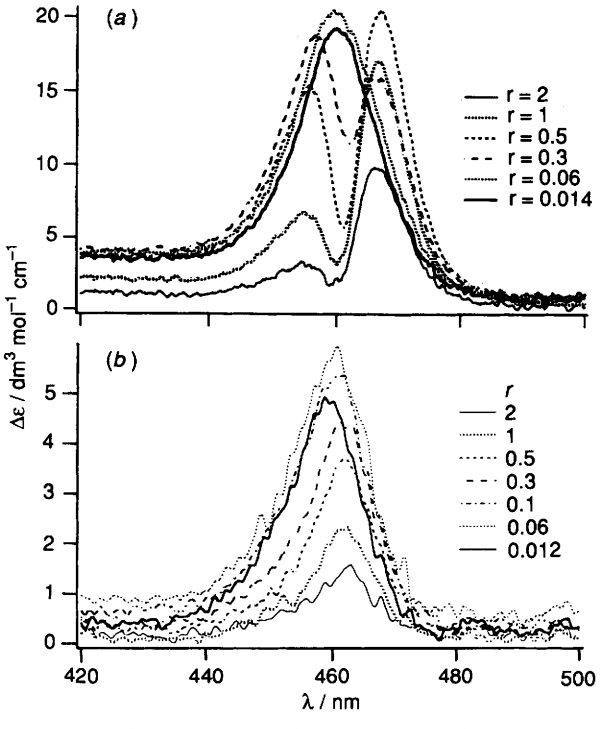

Fig. 1 Induced CD spectra of Mn(tmpyp) with poly(dA-dT)$_2$ (*a*) and poly(dG)·poly(dC) (*b*) at various r values. For clarity only the representative curves are shown. All the spectra were recorded on a Jasco J-720 spectropolarimeter and Beckman DU-64 spectrophotometer in 25 mmol dm^{-3} phosphate buffer (pH 7.1).

Induced CD experiments with calf thymus DNA gave similar results to those with poly(dA-dT)$_2$ DNA except that the intensity of the longer wavelength peak was relatively reduced (not shown). The spectral similarity seems to reflect the higher binding affinity of Mn(tmpyp) towards AT compared with GC sequences.

The two peaks observed in the case of AT or calf thymus DNA can be attributed to either of the following reasons: (*i*) It is due to splitting of the degenerate B_x and B_y bands as a result of DNA binding through a particular mode, or (*ii*) the two peaks correspond to two different binding modes. To clarify the origin of the two bands, distamycin or berenil was added to the Mn(tmpyp)-DNA solution and the CD spectra were measured. Berenil and distamycin are well established minor groove binding drugs.[10,11] Fig. 2 shows that the addition of distamycin or berenil decreased the intensity of the shorter wavelength peak and at the same time increased that of the longer wavelength one in the case of poly(dA-dT)$_2$ DNA. The change was more prominent at higher antibiotic concentrations. The likely interpretation is that preferential binding of these antibiotics to their high affinity AT sites in the minor groove of DNA displaces Mn(tmpyp) molecules and these excluded porphyrins are then available to bind the major groove of DNA.

The single positive peak observed in the case of poly (dG-dC)$_2$ or poly(dG)·poly(dC) DNA can be assigned as a peak corresponding to the major groove binding mode, as the presence of 2-amino group of guanine base may prevent the

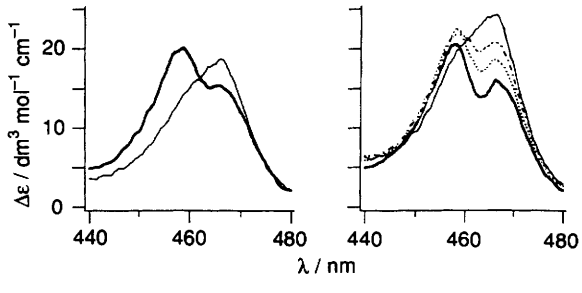

Fig. 2 Induced CD spectra of Mn(tmpyp) with poly(dA-dT)$_2$ at r = 0.38 in the presence or absence of distamycin (*a*) or berenil (*b*): $r_{distamycin}$ = 0 (—) or 0.6 (—); $r_{beranil}$ = 0 (—), 0.4 (---), 0.6 (-.-.-) or 1.0 (—)

binding of Mn(tmpyp) in the minor groove. This was confirmed by induced CD spectra titrated with poly(dI-dC)$_2$. Inosine is identical to guanine except it lacks the 2-amino group. Induced CD spectra of Mn(tmpyp) with poly(dI-dC)$_2$ resemble those with poly(dA-dT)$_2$ showing two positive peaks in the Soret band [Fig. 3(*a*)]. Addition of berenil to the poly(dI-dC)$_2$ DNA–Mn(tmpyp) solution again decreased the shorter and increased the longer wavelength peak [Fig. 3(*b*)]. These results clearly indicate that the two peaks observed in the Soret band of Mn(tmpyp) with calf thymus, poly(dA-dT)$_2$, poly(dA)·poly(dT) or poly(dI-dC)$_2$ DNA correspond to two different binding modes. The shorter and the longer wavelength peaks are assigned as originating from the minor and the major groove binding modes, respectively.

Biochemical experiments carried out in the range r = 0.1–0.4 (pH 7.5, 6 mmol dm^{-3} TRIS) have revealed that Mn(tmpyp) or Fe(tmpyp) preferentially binds in the minor groove of three contiguous AT sequences.[8,9] Our induced CD experiments have shown that the binding of Mn(tmpyp) is more complex than suggested by the biochemical studies. It is consistent with the idea that Mn(tmpyp) binds the major and the minor groove of poly(dA-dT)$_2$, poly(dA)·poly(dT) DNA as well as the major groove of poly(dG-dC)$_2$ or poly(dG)·poly(dC) DNA. The most preferred binding site is the minor groove at AT sequences, although binding to the major groove of GC sequences cannot be neglected even at lower r values. Interestingly, a recent theoretical study[12] predicts that the electronegative potential of DNA grooves in water is in the order of GC major (most negative) ≥ AT minor ≫ GC minor ≥ AT major (least negative). The result indicates that positively charged porphyrins may be attracted to the maximally electronegative major groove of GC sequences. This is in good agreement with our observations.

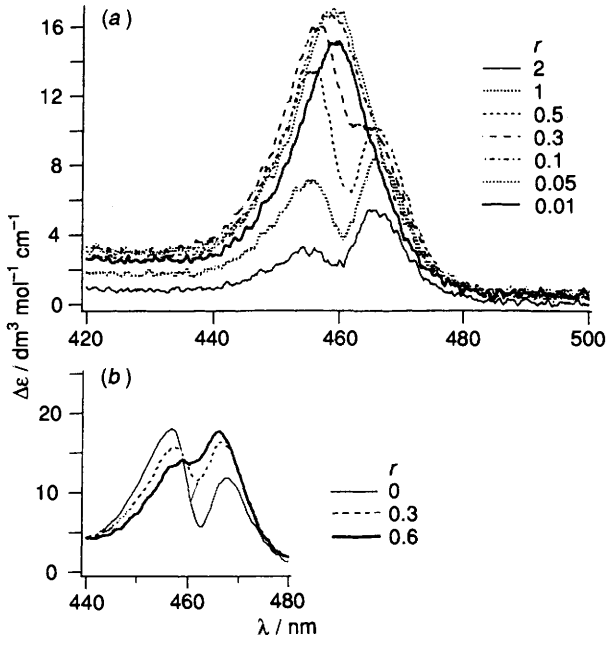

Fig. 3 Induced CD spectra of Mn(tmpyp) with poly(dI-dC)$_2$ at various r values (*a*) and the effect of addition of berenil on the induced CD at r = 0.38 (*b*)

DNA binding of Mn(tmpyp) appears to be explained by the two principal binding modes of major groove binding to AT or GC sequences and the minor groove binding to AT sequences. Of course, we have not entirely excluded the possibility of other binding modes *e.g.* 'outside binding', or induced CD resulting from the interaction between porphyrins aggregated on a chiral DNA molecule. They appear to give minor effect on CD under the experimental conditions employed. Our simple interpretation is consistent with CD result in the presence of antibiotics and with poly(dI-dC)$_2$ (Figs. 2 and 3). The induced CD titration spectra of the porphyrin free base H_2tmpyp, are more complex and exhibit an additional negative peak due to an intercalative mode of binding (not shown).

CD is highly sensitive to the electronic environment. During the titration with synthetic and natural DNA, absorption spectra exhibited little change (data not shown). Thus induced CD is very useful in understanding DNA–achiral ligand interactions. Mathematical analysis based on either Gaussian or Lorentzian curve fitting is under way to obtain semi-quantitative information on the binding-mode and sequence preference of H_2tmpyp and Mn(tmpyp). Our studies show that the combination of spectroscopic and biochemical approaches can provide unique and detailed information on the nature of DNA–ligand binding.

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