Orientation and Linear Dichroism Characteristics of Porphyrin–DNA Complexes†

Nicholas E. Geacintov,*,‡ Victor Ibanez,‡ Michel Rougee,§ and Rene V. Bensasson§

Chemistry Department, New York University, New York, New York 10003, and Laboratoire de Biophysique, Museum National d'Histoire Naturelle, 75005 Paris, France

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ABSTRACT: The linear dichroism spectra of complexes of tetrakis(*N*-methyl-4-pyridinio)porphine (H_2TMpyP) and its zinc(II) derivative (ZnTMpyP) with DNA oriented in a flow gradient have been investigated. The dichroism of H_2TMpyP determined within the Soret band and the Q_y band system is consistent with an intercalative conformation in which the plane of the porphyrin ring system is nearly parallel to the planes of the DNA bases. In the case of ZnTMpyP on the other hand, the porphyrin ring system is inclined at angles of 62–67° with respect to the axis of the DNA helix. The pyridyl groups in both cases are characterized by a low degree of orientation with respect to the axis of the helix. In contrast to H_2TMpyP which does not significantly affect the degree of alignment of the DNA in the flow gradient, the binding of ZnTMpyP causes a significant decrease (about 50% for a base pair/ZnTMpyP ratio of 20) in the intrinsic dichroism at 260 nm due to the oriented DNA bases; the binding of ZnTMpyP to DNA either gives rise to regions of higher flexibility or causes bends or kinks at the binding sites. Increasing the ionic strength has little influence on the linear dichroism of the ZnTMpyP–DNA complexes, but the number of molecules bound at intercalation sites diminishes in the case of the H_2TMpyP–DNA complexes; the accompanying changes in the linear dichroism characteristics suggest that external H_2TMpyP complexes are formed at the expense of intercalation complexes. Taken together, these linear dichroism results are consistent with the intercalative model for H_2TMpyP–DNA and the external binding model for ZnTMpyP–DNA complexes proposed by Fiel et al. [Fiel, R. J., Howard, J. C., & Datta Gupta, N. (1979) *Nucleic Acids Res. 6*, 3093–3118] and Pasternack et al. [Pasternack, R. F., Gibbs, E. J., & Villafranca, J. J. (1983) *Biochemistry 22*, 5409–5417].

With absorption spectrophotometry, circular dichroism, spectrofluorometry, stopped flow, temperature jump, and other techniques, it has been shown that various water-soluble, substituted, and cationic porphyrins and metalloporphyrins form strong complexes with nucleic acids in solution (Fiel et al., 1979; Carvlin & Fiel, 1983; Carvlin et al., 1983; Pasternack et al., 1983a,b, 1984, 1985; Kelly & Murphy, 1985). Tetrakis(*N*-methyl-4-pyridinio)porphine (H_2TMpyP, Figure 1) and its metallo derivatives display different affinities for GC-rich and AT-rich regions of DNA, and different types of complexes are formed with poly(dG-dC) than with poly(dA-dT) (Carvlin et al., 1983; Pasternack et al., 1983a,b, 1984). Two types of interactions, including intercalative and nonintercalative complex formation, have been recognized. Certain porphyrin derivatives readily appear to intercalate between GC base pairs, while others form external or partially intercalated complexes at AT regions. The base specificities depend on the ionic strength; for example, H_2TMpyP preferentially binds via intercalation to GC base pairs of native DNA at low ionic strengths ($\mu < 0.01$), while at higher ionic strengths binding to AT base pairs occurs preferentially (Carvlin et al., 1983; Pasternack et al., 1984, 1986).

The types of interactions with DNA also depend on the structural properties of the porphyrin derivatives. Porphyrins without axial ligands, such as H_2TMpyP, intercalate into DNA readily (Fiel & Munson, 1980; Banville et al., 1983); metalloporphyrins such as ZnTMpyP (Figure 1) do not appear to form intercalation complexes and are believed to be bound externally, because the bulky metal ligand sterically hinders the intercalative insertion of the porphyrin molecular ion between adjacent base pairs (Pasternack et al., 1983a). The apparent equilibrium association constants determined spectrofluorometrically and with the aid of the McGhee–Von Hippel method (McGhee & Von Hippel, 1974) yield similar values of $K_{app} = (1–2) \times 10^6$ M^{-1} for the intercalating H_2TMpyP in poly(dG-dC) or in poly(dA-dT) and for the externally bound ZnTMpyP in poly(dA-dT) (Kelly & Murphy, 1985).

The ability of the peripheral *N*-methylpyridine to rotate around the C_4–C_{meso} bond axis is another important structural feature that influences intercalative binding; in contrast to H_2TMpyP, tetrakis(*N*-methyl-2-pyridinio)porphine, in which the pyridyl rings are frozen perpendicular to the porphine ring system, does not form intercalation complexs with either DNA or poly(dG-dC) (Carvlin & Fiel, 1983; Carvlin et al., 1983; Pasternack et al., 1983a).

Linear dichroism methods (Norden, 1978) can provide information on the orientations of drug molecules with respect to the DNA bases and distinguish between classical intercalation complexes (e.g., proflavin bound to DNA) and other types of conformations (Geacintov et al., 1978). In order to clarify the differences in the geometries of the complexes formed when H_2TMpyP and ZnTMpyP bind to DNA, we have compared the flow linear dichroism properties of these porphine complexes with those of the proflavin–DNA intercalation complexes. The porphyrin derivatives are characterized by two different transitions in the visible region of the spectrum, which are oriented along the *x* and *y* directions within the plane of the planar ring system (Weiss, 1972). Consequently, by determining the orientations of these two transitions relative to those of the DNA bases that absorb below 300 nm, the relative orientations of the porphine ring system relative to those of the DNA bases can be estimated.

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‡ New York University.

§ Museum National d'Histoire Naturelle.

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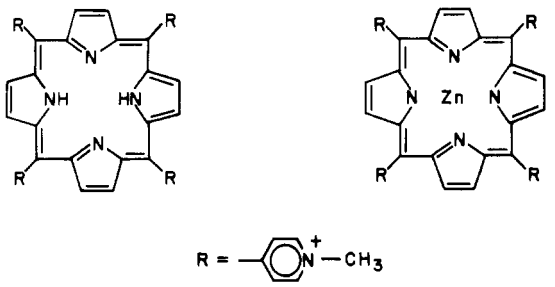

FIGURE 1: Structures of H_2TMpyP (left) and ZnTMpyP (right).

The pyridyl substituent groups also contribute rather strongly to the absorbance of the complexes around 260 nm (Carvlin & Fiel, 1983), and thus, their degree of orientation can also be probed.

Materials and Methods

Free base H_2TMpyP tosylate was obtained from Midcentury (Posen, IL) and used as received; the molar extinction coefficients in water were determined to be 2.5×10^5 M^{-1} cm^{-1} (422 nm) and 1.6×10^4 M^{-1} cm^{-1} (518 nm). The insertion of Zn was carried out at room temperature by reaction of a 10^{-3} M solution of the free base with zinc acetate (10%) excess); the molar extinction coefficients for the Zn derivative were determined to be 2.31×10^5 M^{-1} cm^{-1} (436 nm) and 1.9×10^4 M^{-1} cm^{-1} (563 nm), in good agreement with the values reported by Nata (1981). Reagent-grade proflavin was obtained from Aldrich Chemical Co. (Milwaukee, WI) and was used without further purification.

Calf thymus DNA (Worthington Biochemicals, Freehold, NJ) solutions were prepared as described previously (Geacintov et al., 1982). The solutions were sonicated at 4 °C for 3 min at 40% power of the sonifier (Model 200, Branson, Danbury, CT). After exhaustive dialysis against the buffer solution (5 mM sodium cacodylate, 10 mM NaCl, pH 7.0), the solutions were centrifuged to remove any residual solid particles. The hyperchromicities of the DNA solutions were in the range of 1.36–1.38. Stock solutions of the porphyrins and proflavin in the buffer solution were prepared, and the required aliquots were added to the DNA solutions. The concentrations of the chromophores were determined by absorption spectrophotometry on a Perkin-Elmer 320 (Norwalk, CT) spectrophotometer.

In the flow linear dichroism experiments, the DNA molecules in the aqueous buffer solution are subjected to a velocity gradient in a Couette cell. The latter consists of two concentric quartz cylinders, a stationary outer cylinder and a rotating (400 rpm) inner cylinder. The inside diameter of the outer cylinder is 23 mm, while the outside diameter of the inner cylinder is 22 mm; thus, the annular gap is 0.5 mm, and the total optical path length in the Couette cell is 1 mm. As the inner cylinder is rotated, the DNA molecules in the solution within the annular gap tend to align along the flow lines. The degree of alignment of the DNA bases and the complexed porphyrins is probed by utilizing polarized light with its propagation axis oriented at a 90° angle with respect to the axis of rotation; the absorbances determined with the polarization vectors of the light oriented either in a parallel or vertical orientation with respect to the flow lines are designated by $A_\parallel$ and $A_\perp$, respectively. The linear dichroism is defined as the difference between these two quantities:

$$\Delta A = A_\parallel - A_\perp \quad (1)$$

In our home-assembled linear dichroism system, the light source is a 150-W Cermax xenon arc (ILC Technology, Sunnyvale, CA) focused on the entrance slit of a 200-mm J&Y monochromator (Instruments SA, Inc., Metuchen, NJ) whose wavelength drive is controlled by a microprocessor control unit. The light issuing from the monochromator is modulated with a photoelastic modulator (Model PM3, Hinds International, Inc., Portland, OR), which, after passing through a crystal polarizer, produces linearly polarized light with alternately vertical and linear polarization at a frequency of 100 kHz. The light transmitted through the sample is detected with a Hamamatsu R376 photomultiplier tube (Hamamatsu Corp., Middlesex, NJ) whose output is detected with a PAR Model 126 lock-in amplifier (Princeton Applied Research, Princeton, NJ). The output of the lock-in amplifier is fed to a DATA 6000 waveform analyzer (Data Precision Analogics Corp., Medford, MA). After appropriate manipulation of the data, the wavelength dependence of the linear dichroism ΔA is obtained with a digital plotter. The wavelength dependence of the system was carefully checked in the 240–700-nm region by utilizing a Suprasil quartz plate inclined at a small angle with respect to the incident light beam to simulate a dichroic sample. The concentration dependence of the ΔA signal was linear up to an absorbance of at least 0.7 in the 1-mm annular optical path length in the Couette cell. With velocity gradients of 1000–1300 s^{-1}, the absolute value of the reduced linear dichroism $\Delta A/A$ at 260 nm (where A is the isotropic absorbance of the DNA sample) is about −0.10, which is close to the maximum value obtainable by this flow orientation method (Wada & Kozawa, 1964; Norden & Tjerneld, 1976).

Results

The absorbance and linear dichroism spectra of porphyrin–DNA solutions were measured at different concentrations of drugs and at constant DNA concentration (3.8×10^{-4} M calculated in terms of the concentration of base pairs). The concentration of porphyrins is expressed in terms of the ratio r_0 (concentration of base pairs/total concentration of porphyrins), and experiments with r_0 in the range of 40–400 were performed.

Addition of porphyrin stock solutions to DNA solutions resulted in all cases to a volume increase of only 0.5% (addition of 5 μL of stock solution/mL of DNA solution). Thus, the absorption and linear dichroism spectra of the initial DNA solutions and the porphyrin–DNA complexes can be directly compared to one another.

The linear dichroism and absorption spectra of DNA complexes of H_2TMpyP and ZnTMpyP for $r_0 = 20$ are depicted in Figures 2A and 3A, respectively. In Figure 2A the absorption spectrum and linear dichroism spectra of DNA, both measured just before the addition of H_2TMpyP, are also shown. The linear dichroism spectra of DNA alone and of the ZnTMpyP–DNA complexes are compared to one another, and to the absorption spectra of the complexes, in Figure 3A.

The base lines in the absence of rotation of the Couette cell are wavelength-dependent, particularly at high sensitivities of the instrument. These base lines were always subtracted from the linear dichroism spectra determined with the Couette cell under rotation.

In the case of DNA alone, the negative values of ΔA are due to the inclination of the base planes perpendicular to the flow direction [see, for example, Wada and Kozawa (1964) and Norden and Tjerneld (1976)]. The negative linear dichroism observed within the absorption bands of the porphyrin derivatives complexed with the DNA indicates that the transition moments, which are oriented within the planes of the molecules (Weiss et al., 1972), are also tilted closer to the flow lines then perpendicular to the flow lines.

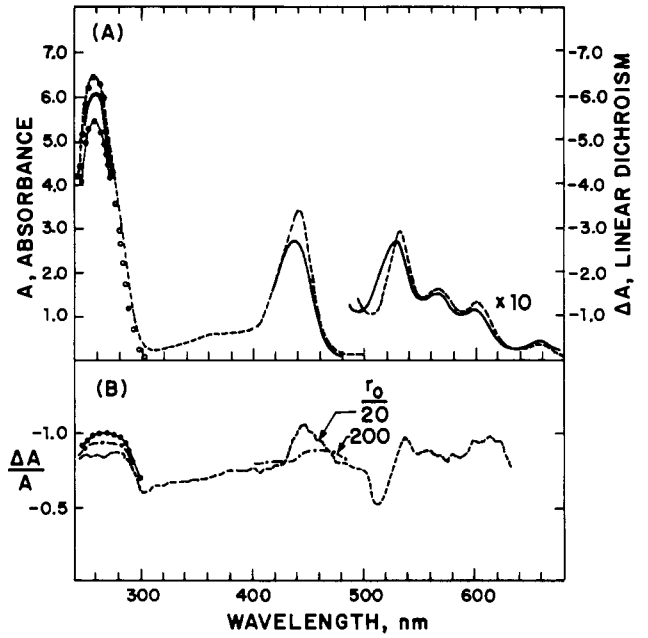

FIGURE 2: Absorption (A), linear dichroism (ΔA), and reduced linear dichroism ($\Delta A/A$) of DNA and of H_2TMpyP–DNA complexes. All absorbance values are expressed in terms of a 10-mm path length; ΔA values are expressed in arbitrary units. DNA concentration: 4.1 $\times 10^{-4}$ M expressed in concentration of base pairs. (A) (●) Absorption spectrum of DNA alone and (○) the corresponding ΔA spectrum. (—) Absorption spectrum of $r_0 = 20$ complexes and (– –) the corresponding ΔA spectrum. (B) Reduced linear dichroism spectra: (●) DNA; (–·–) $r_0 = 200$ complexes; (– – –) $r_0 = 20$ complexes.

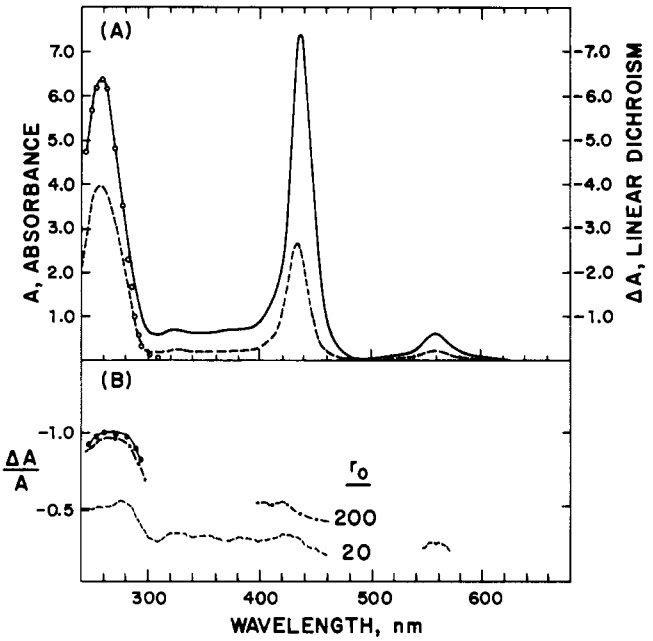

FIGURE 3: Absorption (A), linear dichroism (ΔA), and reduced linear dichroism spectra ($\Delta A/A$) of DNA and of ZnTMpyP–DNA complexes. All other indications are as in Figure 2. (A) (—) Absorbance of the $r_0 = 20$ complexes (the absorption spectrum of DNA is the same as the one shown in Figure 2A); (○) linear dichroism spectrum of DNA alone and (– –) ΔA spectrum of $r_0 = 20$ complexes. (B) Reduced linear dichroism spectra: (●) DNA; (–·–) $r_0 = 200$ complexes; (– – –) $r_0 = 20$ complexes.

In Figures 2B and 3B the wavelength dependence of the reduced dichroism $\Delta A/A$ of DNA, and of the porphyrin–DNA complexes ($r_0 = 20$ and 200), is shown. If only one oriented transition moment is present in the oriented sample, then the reduced dichroism is expected to be constant and independent of wavelength. In Figures 2–4, the values of $\Delta A/A$ have been

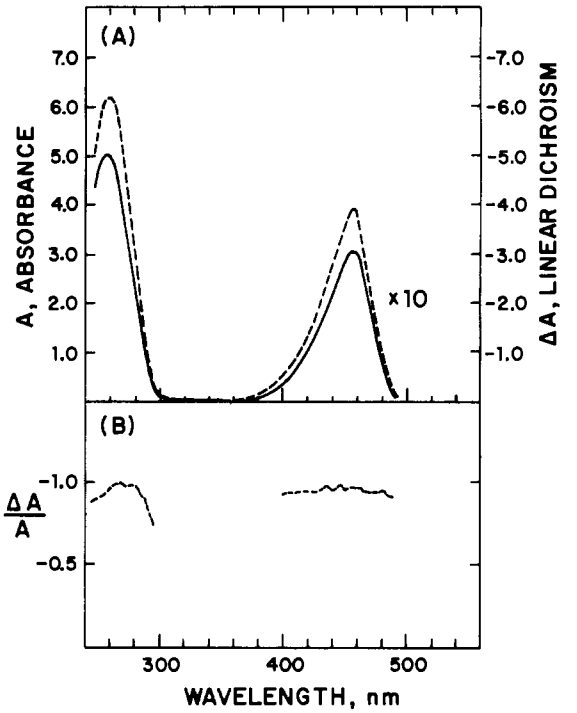

FIGURE 4: Absorption (A), linear dichroism (ΔA), and reduced linear dichroism ($\Delta A/A$) spectra of proflavin–DNA complexes. DNA concentration 3.8 $\times 10^{-4}$ M, expressed in concentration of base pairs. (A) (—) Absorption spectrum of $r_0 = 50$ complexes and (– –) linear dichroism spectrum of the same complexes. (B) Reduced linear dichroism of the $r_0 = 50$ complexes.

normalized to a value of −1.0 within the DNA absorption maximum at 258 nm. Gaps in the $\Delta A/A$ spectra occur in those wavelength regions in which the values of the ΔA and A signals are so close to the base lines (especially for $r_0 = 200$) that it is impossible to determine these ratios accurately.

The increase in absorbance in the 240–280-nm region (Figure 2A) upon addition of H_2TMpyP is attributable to the porphyrin molecules; a nearly identical increase in absorbance in this wavelength region is observed upon the addition of ZnTMpyP. In buffer solution, the absorption maximum of this porphyrin derivative is located at 260 nm, which is quite close to the DNA absorption maximum of 258 nm. In contrast to the increase in the absorbance in this wavelength region, the magnitude of the linear dichroism remains almost unchanged upon the addition of the porphyrin (Figure 2A); this indicates that the H_2TMpyP molecules contribute only to a minor extent to the ΔA spectrum in this wavelength region.

In the visible region of the absorption spectrum of H_2TMpyP–DNA complexes, a significant dichroism due to the Soret band, peaking at 442 nm and slightly red shifted with respect to the absorption maximum at 438–439 nm, is observed. Above 500 nm, a linear dichroism signal due to the weaker Q_y absorption band is also evident.

The linear dichroism characteristics of the ZnTMpyP–DNA complexes are different from those of the H_2TMpyP–DNA complexes. In the latter case, the values of $\Delta A/A$ are close to −1.0 within the Soret band at the 437–438-nm maximum and within the Q_y absorption band above 500 nm. In the case of the ZnTMPpyP–DNA complexes, the $\Delta A/A$ values within the Soret band are 50% smaller than those within the DNA absorption band below 300 nm. Furthermore, there is a significant decrease in $\Delta A/A$ values in the 250–300-nm region upon addition of ZnTMpyP (Figure 3B), this effect being larger for $r_0 = 20$ than for $r_0 = 200$; this result suggests that the ability of the DNA molecules to orient along the flow lines is decreased upon binding of ZnTMpyP.

Discussion

Interpretation of Linear Dichroism Spectra. Flexible, long-chain molecules such as DNA are deformed, as well as oriented in a hydrodynamic field. Several models have been utilized to derive theroretical expressions for the reduced linear dichroism (Wada, 1964; Norden, 1978; Wilson & Schellman, 1978). Generally, the reduced dichroism is expressed in terms of two factors:

$$\Delta A/A = (3/2)[\langle \cos^2 \theta \rangle - 1]F(G) \qquad (2)$$

The first factor defines the intrinsic dichroism of a chromophore where θ is the angle between the transition dipole moment and the orientation axis (assumed to be the axis of the helix). The second factor is the orientation factor, which has values in the range $0 < F(G) < 1.0$, and is a function of the velocity gradient G, the extent of deformation of the DNA molecule in the flow field, and an average of the projections of the axes of the partially oriented local DNA segments onto the laboratory axes.

The π–π^* transition moments of the DNA bases are oriented in-plane, and thus, $\Delta A < 0$ below 300 nm. The exact angle of tilt of the bases with respect to the axis of the helix has been a matter of controversy (Houssier, 1981; Lee & Charney, 1982; Edmondson & Curtis Johnson, 1985), and most recently, a value of 76° has been deduced from an analysis of the wavelength dependence of the reduced dichroism (Edmondson & Curtis Johnson, 1985). In estimating the angles of orientation of the transition dipole moments of drug and other molecules complexed with DNA, these problems are usually bypassed by simply comparing the $\Delta A/A$ values in the absorption bands of the chromophores and the DNA bases (Norden & Tjerneld, 1976; Geacintov et al., 1978; Houssier, 1981). This approach can be tested by analyzing the linear dichroism spectra of DNA complexes of molecules that are known to intercalate between the base pairs of DNA.

Proflavin is a well-known intercalating agent [see, for example, Neidle (1979) and Patel (1979)]; the wavelength dependence of ΔA and of the reduced linear dichroism measured in our flow dichroism system are depicted in Figure 4. It is evident that the reduced dichroism due to the proflavin chromophore in the 400–500-nm range is, within experimental error, equal to the values of $\Delta A/A$ at 250–280 nm due to the π–π^* transitions of the DNA bases (Figure 4); thus, with eq 2, this result suggests that the in-plane transition moment of the acridine ring system is parallel to the transition moments of the nucleic acid bases, as expected for intercalation complexes. Since similar results are obtained with electrically oriented proflavin–DNA complexes (Ramstein et al., 1973; Geacintov et al., 1978; Houssier, 1981), we conclude that the orientation by flow does not introduce any unusual distortions of the DNA molecules that would invalidate the approach utilized here to estimate θ. The decreases in the values of $\Delta A/A$ for DNA alone below 255 and above 285 nm (Figure 2A) are well-known and may be due to n–π^* transitions with out-of-plane polarization (Norden, 1978).

H_2TMpyP–DNA Complexes. At $r_0 = 200$, the change in the absorbance at 260 nm upon addition of H_2TMpyP is negligibly small (Figure 2A); for $r_0 = 20$, there is a 10% increase in the absorbance upon addition of this porphyrin derivative (a similar increase is observed in the case of ZnTMpyP, Figure 3A). However, at both concentrations, the values of ΔA of the H_2TMpyP–DNA complexes are the same as those in the case of DNA alone. This porphyrin derivative therefore does not contribute significantly to the linear dichroism of the complexes around 260 nm; this suggests that the pyridyl groups, which absorb significantly in this wavelength range, are characterized by either a low degree of orientation or orientation angles close to 55° (for which ΔA is close to zero according to eq 2).

The reduced linear dichroism below 285 nm is somewhat lower for the complexes than for DNA alone (Figure 2B). One obvious reason for this behavior is that H_2TMpyP increases the absorbance at 260 nm, without contributing substantially to the dichroism; thus, for $r_0 = 20$, the reduced dichroism is reduced by about 14% at 260 nm, as compared to the increase in absorbance of about 12%. However, the nearly 7% decrease in $\Delta A/A$ for $r_0 = 200$ cannot be accounted for in this manner, and other, unknown effects may account for these small changes in the reduced linear dichroism below 285 nm.

The linear dichroism spectra obtained with $r_0 = 20$ and with $r_0 = 200$ (data not shown) are similar. In the $r_0 = 200$ case, the ΔA spectrum closely resembles the absorption spectrum, and thus, the reduced linear dichroism, which could be determined with accuracy only within the Soret band (Figure 2A), is nearly constant as a function of wavelength. Above 440 nm, $\Delta A/A$ is only about 10% lower than the reduced dichroism within the DNA absorption band in the 260-nm range (DNA without H_2TMpyP); this suggests that above 440 nm the transition moments of the Soret band are nearly parallel to those of the DNA bases. According to Pasternack et al. (1983a), H_2TMpyP molecules intercalated at GC sites are characterized by an absorption maximum at 444 nm, and our linear dichroism data are consistent with a dominance of an intercalated species in this wavelength range.

At higher porphyrin/DNA ratios ($r_0 = 20$), the external binding at AT sites should become more prominent according to Carvlin et al. (1983). In the case of $r_0 = 20$, the linear dichroism maxima within the 440-nm Soret band and within the 525–530-nm absorption band are slightly red shifted (by about 3–4 nm) with respect to the absorption maxima. Because of this effect, the reduced linear dichroism is not constant as a function of wavelength in the visible portion of the spectrum. The red shift in the ΔA spectrum relative to that in the absorption spectrum is consistent with the existence of a species that has an absorption maximum at a wavelength lower than that of the intercalated species at 444 nm and that is characterized by a lower dichroism. Both of these criteria are consistent with the increased prominence of an externally bound species as suggested by Carvlin et al. (1983) and Pasternack et al. (1983a).

The absorption band system above 500 nm is oriented along the y direction of the ring system, while the Soret band is characterized by a mixed x and y polarization (Weiss et al., 1972). At $r_0 = 20$, in which case $\Delta A/A$ is measureable (Figure 2B), the generally high values of the reduced linear dichroism in the 400–620-nm range support the previous conclusion, based on the $\Delta A/A$ values observed within the Soret band, that the orientation of the plane of the H_2TMpyP porphyrin ring system is parallel or nearly parallel to the planes of the DNA bases.

The decreased values of $\Delta A/A$ in certain wavelength regions is attributable to the presence of externally bound H_2TMpyP molecules. When more than one type of oriented chromophore contributes to a linear dichroism spectrum in the same wavelength interval, the reduced dichroism can vary as a function of wavelength; maxima or minima in the $\Delta A/A$ spectra are observed at wavelengths where the dichroism is dominated by one of the oriented species (Geacintov et al., 1978). In the $r_0 = 20$ case in Figure 2B, this occurs at 444 nm, which corresponds to the absorption maximum of inter-

Table I: Linear Dichroism (ΔA) and Absorption (A) Maxima of the Soret Band of Porphyrin–DNA Complexes (r_0 = 20) at Two Different Ionic Strengths[a]

	H_2TMpyP			ZnTMpyP		
μ	A (nm)	ΔA (nm)	fwhm (nm)	A (nm)	ΔA (nm)	fwhm (nm)
0.01	438	441	35	437	435	23
0.20	428	440	40	439	437	23
% change		−38			−11	

[a] DNA concentrations was 4.1×10^{-4} M in base pairs. fwhm denotes full width at half-maximum height of the Soret band. Ionic strength was varied by changing the NaCl concentrations. Percent change in ΔA was calculated for the Soret maxima and upon increasing μ from 0.01 to 0.20.

calated H_2TMpyP molecules at GC sites (Pasternack et al., 1983a).

ZnTMpyP–DNA Complexes. In the r_0 = 200 case, the magnitude of the ΔA signal in the DNA region is only slightly smaller than that of the DNA alone. In the Soret band region, in contrast to the characteristics of the H_2TMpyP–DNA complexes, the reduced linear dichroism is about 50% smaller than that in the DNA region (Figure 3B. Furthermore, the $\Delta A/A$ values are not constant across this absorption band, suggesting that the x and y directions of the porphyrin ring system with respect to the planes of the DNA bases are somewhat different. On the basis of eq 2 and with methods outlined elsewhere (Norden & Tjerneld, 1976; Geacintov et al., 1978; Houssier, 1981), orientation angles of the porphyrin transition moments relative to the orientation axis (the axis of the helix) can be calculated. In this calculation, a tilt angle of the DNA bases relative to this axis of either 76° (Edmondson & Curtis Johnson, 1985) or 90° (B-DNA model) is assumed (Geacintov et al., 1978). With a tilt angle of 76°, porphyrin orientation angles of 65° (utilizing $\Delta A/A$ values in the 400–420-nm range) or θ = 62° (utilizing the reduced dichroism values in the 420–460-nm range) are obtained. If a base tilt angle of 90° is assumed, then angles of 67° and 64°, respectively, are calculated. Because of the properties of the $\langle \cos^2 \theta - 1 \rangle$ function, the calculated orientations of the x and y directions of the plane of the porphyrin ring system are not very sensitive to the tilt angle of the DNA bases relative to the axis of the helix. The r_0 = 200 data are assumed to be most suitable for calculating orientation angles since the contributions of free molecules to the absorbance A is minimized.

In summary, the plane of ZnTMpyP is oriented at an angle of 62–67° with respect to the axis of DNA. This conclusion is reenforced by the $\Delta A/A$ values obtained for the y-polarized transition in the 550–570-nm range in the case of r_0 = 20; the reduced linear dichroism in this wavelength range is similar in magnitude to the values determined withint the Soret band.

The calculated orientation angles of the complexed ZnTMpyP molecules are clearly inconsistent with those of intercalation complexes and in agreement with earlier conclusions that these molecules are bound to the DNA externally (Fiel et al., 1979; Carvlin et al., 1983; Pasternack et al., 1983a).

A striking feature of the linear dichroism data of ZnTMpyP–DNA complexes is the sharply diminished reduced linear dichroism within the DNA absorption band below 280 nm in the case of r_0 = 20 (Figure 3B). This indicates that the ability of the DNA molecules to orient along the flow lines is reduced upon complex formation with ZnTMpyP molecules. Such a tendency is not apparent in the dichroism of H_2TMpyP–DNA complexes (Figure 2B). Reduction in the $\Delta A/A$ values has been previously observed in the binding of certain peptides to DNA (Gabbay et al., 1976) and is probably due to the formation of bends, kinks, or regions of higher flexibility at the porphyrin binding sites. These results suggest that the preferred binding at ZnTMpyP molecules at AT sites disrupts the local DNA structure, perhaps by interfering with AT hydrogen bonding.

Effect of Ionic Strength on Linear Dichroism Spectra. The nature of the binding of H_2TMpyP to DNA is known to be ionic strength dependent (Pasternack et al., 1983a, 1984; Carvlin et al., 1983). The apparent binding constant decreases and the porphyrin molecules are increasingly shifted from GC intercalation sites to external AT sites as the ionic strength is increased. It was therefore of interest to determine the effect of increasing ionic strength on the linear dichroism spectra. In 0.2 M NaCl, there are only relatively minor changes in the linear dichroism spectra (data not shown), even though there are large changes in the absorption spectra as reported by Pasternack et al. (1984) and Cavlin et al. (1983). The relevant data are summarized for both H_2TMpyP and ZnTMpyP in Table I. The most prominent changes are observed in the case of H_2TMpyP, where the position of the Soret absorption maximum shifts from 440 nm (μ = 0.01) to 429 nm (μ = 0.20); the absorbance, measured at the respective Soret maxima, is increased by 25%; this hypochromic effect is consistent with a diminished number of H_2TMpyP molecules intercalated at GC sites (Fiel et al., 1979; Pasternack et al., 1984). The maximum in the linear dichroism spectrum on the other hand does not change significantly, even though the amplitude is diminished by 37% at the higher ionic strength. The full width at half-maximum (fwhm) of the Soret ΔA band increases from 35 to 40 nm at the higher ionic strength; this is consistent with a lower dichroism contributed by a species of H_2TMpyP molecules that absorb at a lower wavelength, as expected for porphyrin molecules bound externally at AT sites. Thus, the conclusions of Fiel et al. (1979) and Pasternack et al. (1983a, 1984) regarding the effects of ionic strength on the characteristics of the binding of H_2TMpyP to DNA are supported by the linear dichroism data.

In the case of ZnTMpyP, the effects of ionic strength are much less pronounced (Table I). Only minor changes are observed in the positions of the A and ΔA maxima of the Soret band, and the magnitude of the ΔA signal changes by only 11%. Since the fwhm of the ΔA band of the Soret band does not change either, all of these observations, taken together, are consistent with the nonintercalative binding of ZnTMpyP to DNA.

Conclusions

Linear dichroism techniques can provide information on the characteristics of porphyrin–DNA complexes that could not be determined by the other methods previously utilized to study these complexes. These characteristics include the orientations of the different transition moments (the Soret and Q_y bands) relative to those of the DNA bases, the orientation (or lack thereof) of the pyridyl side groups, and the bending or kinking of the DNA induced by the binding of the ZnTMpyP derivative. The changes in the binding characteristics of H_2TMpyP as a function of increasing ionic strength can be followed directly.

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Time Dependence of Near-Infrared Spectra of Photodissociated Hemoglobin and Myoglobin

Massimo Sassaroli and Denis L. Rousseau*
AT&T Bell Laboratories, Murray Hill, New Jersey 07974
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ABSTRACT: The near-infrared charge-transfer transitions at ~760 nm in photodissociated hemoglobin and myoglobin display very different time dependences. In photodissociated myoglobin at room temperature the transition has fully relaxed to its deoxymyoglobin value by 10 ns. In photodissociated hemoglobin, the transition is shifted by 6 nm to longer wavelengths at 10 ns. It relaxes about halfway back to the deoxyhemoglobin value by about 100 ns but subsequently changes very slowly out to about 100 μs when the signal intensity becomes too small to follow any further. The intensity of this transition, present in only five-coordinate hemes, is found to follow the same time dependence as the wavelength change. Consequently, there appears to be a correlation between a structural property of the heme (as inferred from the wavelength of the charge-transfer transition) and a functional property (the CO recombination) of the protein (as inferred from the intensity of the transition). Possible origins for this correlation are considered.

Hemoglobin and myoglobin have a well-known charge-transfer transition in the near-infrared at 759 and 761 nm, respectively (Eaton & Hofrichter, 1981). Several years ago Iizuka et al. (1974) studied the properties of this transition in the photoproducts obtained by photodissociating the CO-bound complexes of these proteins at 4 K. In both hemoglobin and myoglobin they found a substantial shift of this line to lower energy in the photoproducts as compared to the deoxy preparations under the same conditions. Recently, other groups have confirmed these low-temperature differences (Ansari et al., 1985; Fiamingo & Alben, 1985). Moreover, since these seminal studies of the low temperature stabilized photoproducts, studies of other spectroscopic (Ondrias et al., 1983b; Rousseau & Ondrias, 1985; Rousseau & Argade, 1986) and structural (Chance et al., 1983; Powers et al., 1984) properties have been reported. In addition, many studies of the properties of the photoproducts generated at ambient temperatures with transient techniques have been carried out (Henry et al., 1983a,b; Hofrichter et al., 1983; Scott & Friedman, 1984; Findsen et al., 1985a,b; Friedman, 1985; Dasgupta et al., 1985; Rousseau & Argade, 1986). From these many studies it has been found that at room temperature the

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