The binding of binuclear platinum(II)–terpyridine complexes to DNA

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The binding of platinum (II)–terpyridine complexes to DNA was studied by using equilibrium dialysis. Optical absorption methods were used to measure the ability of the ligands to aggregate in aqueous buffer. Scatchard plots for the binding of the monomeric $[\text{Pt}(\text{terpy})\text{SC}_4\text{H}_9]^+$ cation to DNA at 1.0 M are curvilinear, concave upwards, suggesting two modes of binding. The association constant decreases at higher ionic strengths, consistent with polyelectrolyte theory, and 1.1 cations are released per bound ligand molecule. The association constants of the binuclear ligands $[\text{Pt}(\text{terpy})\text{S}(\text{CH}_2)_4\text{S}(\text{terpy})\text{Pt}]^{2+}$ and $[\text{Pt}(\text{terpy})\text{S}(\text{CH}_2)_6\text{S}(\text{terpy})\text{Pt}]^{2+}$ are 8 and 23 times larger respectively than the affinity of the monomer. For the latter binuclear derivative the increase may be ascribed to bifunctional reaction. Differential dialysis experiments with DNAs of differing base composition show that $[\text{Pt}(\text{terpy})\text{SC}_4\text{H}_9]^+$ has a requirement for a single G·C base-pair at the highest-affinity site. However, in the binuclear ligands chromophore specificity is severely compromised. Similar experiments indicate that 9-aminoacridine and selected methylene-linked diacridines show no significant sequence selectivity.

INTRODUCTION

Polyfunctional intercalating agents with the capacity to bind to DNA in a sequence-dependent manner are of interest because of their potential for development as specific modulators of gene expression. To date attempts to enhance nucleotide selectivity within this class of compound have focused largely on the synthesis of ligands incorporating a bridging chain that is itself likely to impart selectivity as a result of specific interactions with the DNA base-pairs (Wakelin, 1986). For example, there have been several attempts to take advantage of the proven specificity of proteins for DNA by incorporating oligopeptides into the linker chain of diacridines (Bernier *et al.*, 1981; Kelly *et al.*, 1985; Helbecque *et al.*, 1985). An alternative approach to improved sequence selectivity is to attempt to exploit the known intrinsic specificity of the intercalating chromophores themselves. Although several classes of heteroaromatic (Muller & Crothers, 1975) and platinum(II)-containing intercalators (Howe-

Grant & Lippard, 1979; Wakelin *et al.*, 1984) are known to bind to G+C-rich regions of DNA selectively, the idea that their inclusion in polyfunctional agents may further enhance selectivity (Kosturko *et al.*, 1979) has received little attention. However, this latter notion provided a principal motivation for our synthesis (McFadyen *et al.*, 1986) of a model monomer and a series of binuclear ligands formed by linking two (2,2':6',2''-terpyridine)platinum(II) moieties via $\alpha\omega$ -dithioalkanes (see Fig. 1). Among these ligands we found that those with $n = 5, 6$ and 7 function as bisintercalators whereas those with $n = 8$ and 10 bind in a mixed mono-functional/bifunctional mode (McFadyen *et al.*, 1986). We were not able to assign unambiguously the binding mode of the homologues with $n = 4$ and 9 .

Here we report the findings of equilibrium binding studies of the interaction of these bisplatinum–terpyridine ligands with natural and synthetic DNAs. Initially, equilibrium-dialysis measurements were made with M-4, D-4, D-6 and calf thymus DNA to investigate to what

Fig. 1. Structures of the (butane-1-thiolato)-(2,2':6',2''-terpyridine)platinum(II) ligand (M-4) and of the dimeric dithiolato-linked 2,2':6',2''-terpyridineplatinum(II) ligands (D-n)

The monomeric compound is referred to by the pseudonym M-4 and the dimers by D-n, where n represents the number of carbon atoms in the linker chain.

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extent affinity for DNA is increased by bifunctional reaction within this series of compounds. Where possible, studies were carried out at a range of ionic strengths. Optical measurements were used to assess the ability of ligands to self-associate, so that Scatchard (1949) plots for binding to DNA might be corrected for dimerization of the free ligand. To investigate whether bifunctional platinum-terpyridine dimers do indeed bind with enhanced specificity, we have used the partition-dialysis technique of Muller & Crothers (1975) to measure the distribution of ligands between DNA samples of different G+C content. We have also performed differential dialysis measurements with 9-aminoacridine and selected diacridines for the purposes of comparison with bisintercalators not expected to show sequence selectivity as a result of chromophore-base-pair interactions (Wakelin, 1986).

MATERIALS

Buffers

Experiments with the platinum-terpyridine ligands were conducted in Hepes/KOH buffers at pH 7.0 containing 2 mM-Hepes (Calbiochem) and sufficient KF to yield the required ionic strength. This buffer system is referred to below as KFH buffer. Measurements with the acridines were carried out in 2 mM-Hepes/NaOH buffers at pH 7.0 containing NaCl as supporting electrolyte (designated as SH buffer). Ultrapure water from a Barnstead Nanopure system was used throughout.

Nucleic acids

Micrococcus lysodeikticus DNA, *Clostridium perfringens* DNA and calf thymus DNA were purchased from Sigma Chemical Co. Solutions containing 2 mg/ml in the appropriate buffer of *I* 0.2 were sonicated in a Branson B15 150 W sonicator. For calf thymus DNA the large probe was used at power level 5 with a duty cycle of 30% sonication for a total period of 6 min at 0 °C. For the bacterial DNAs the microprobe was used at power level 5, duty cycle 30%, for a total period of 4 min. These sonication procedures produce DNA fragments of M_r about 1×10^6 . DNA solutions were subsequently dialysed against buffer of the required ionic strength, clarified by filtration through Whatman GF-C glass-fibre filters, and stored frozen at -20 °C. DNA concentrations are based on an assumed ϵ_{260} 260 (molar absorption coefficient with respect to nucleotide pairs) of $13200 \text{ M}^{-1} \cdot \text{cm}^{-1}$ for calf thymus and *Cl. perfringens* DNA and on the measured value of $12600 \text{ M}^{-1} \cdot \text{cm}^{-1}$ for *M. lysodeikticus* DNA (Tubbs *et al.*, 1964). Poly(dA-dT)·poly(dA-dT) and poly(dG-dC)·poly(dG-dC) were purchased from Boehringer Mannheim as the freeze-dried sodium salts and were prepared for use by dissolving in the appropriate buffer. Concentrations are based on an ϵ_{254} of $16800 \text{ M}^{-1} \cdot \text{cm}^{-1}$ for poly(dG-dG)·poly(dG-dC) and an ϵ_{262} of $13400 \text{ M}^{-1} \cdot \text{cm}^{-1}$ for poly(dA-dT)·poly(dA-dT) (Wells & Wartell, 1974).

Ligands

The platinum ligands were prepared as previously described (McFadyen *et al.*, 1986). The acridine ligands were synthesized and purified as the crystalline hydrated hydrochlorides by Dr. B. Elmes, Division of Applied Organic Chemistry, C.S.I.R.O., Melbourne, Vic., Australia, by the method of Deshpande & Singh (1972). They

are referred to by the pseudonym C-*n*, where *n* represents the number of carbon atoms in the linker chain.

METHODS

Dimerization constants

The dimerization constants, K_d , for M-4, D-4 and D-6 were determined by using the observed deviations from Beer's-Law plots at the wavelengths 343 nm, 345.5 nm and 346 nm respectively. Absorbance measurements were made as a function of ligand concentration in 2 mm-, 10 mm- and 50 mm-light-path quartz cuvettes thermostatically controlled at 20 °C in a Cary 219 spectrophotometer. Data were fitted to a simple dimerization model and K_d values determined by a least-squares method as reported previously (Wakelin *et al.*, 1984).

Equilibrium dialysis

Binding curves were derived from equilibrium-dialysis measurements made with an MSE Dianorm apparatus. Dialysis cells having two 5 ml compartments separated by a Spectrapor 2 regenerated cellulose membrane (M_r cut-off 12000–14000) were loaded with 4.5 ml of calf thymus DNA in the concentration range 140–160 μM in one chamber and an equal volume of ligand solution in the other. To establish equilibrium the cells were rotated in a water bath at 20 °C for 20 h for M-4 and 96 h for D-4 and D-6. Then 0.4 ml of a 10% solution of the sodium salt of *N*-lauroylsarcosine in water was added to 3.6 ml of sample from each chamber and the total and free ligand concentrations in equilibrium with the DNA were determined spectrophotometrically, by using the molar absorption coefficients shown in Table 1. The appropriate control experiments were performed to verify complete dissociation of the DNA-ligand complexes by detergent over the range of degrees of binding studied. The concentration of bound ligand, equal to the difference between concentrations of total and free drug, was divided by the nucleic acid concentration to yield the binding ratio, *r*, expressed with respect to nucleotide pairs. Concentrations of free ligand were corrected to yield the concentration of non-associated ligand, *c*, by using the dimerization constants given in Table 1. Binding isotherms were constructed in the form of Scatchard (1949) plots (*r/c* versus *r*). For measurements at *I* 0.01 for M-4 and for all the measurements for D-4 the association constants were determined by linear extrapolation of binding data to the Scatchard-plot ordinate. In all other cases binding parameters were evaluated by a non-linear least-squares fit, by using the computer program of Duggleby (1981), to eqn. (10) of McGhee & von Hippel (1974):

$$r/c = K_{(0)}(1 - Nr) \left(\frac{(1 - Nr)}{[1 - (N-1)r]} \right)^{N-1}$$

where $K_{(0)}$ is the intrinsic association constant to an isolated binding site and *N* is the site size (lattice units occluded by one bound ligand).

Specificity determinations

In experiments to measure the distribution of ligands between *Cl. perfringens* DNA and *M. lysodeikticus* DNA three-compartment dialysis cells were used in which the chambers were separated by regenerated Spectrapor 2 membranes (M_r cut-off 12000–14000). The outer

Table 1. Molar absorption coefficients and dimerization constants for M-4, D-4 and D-6

Molar absorption coefficients were measured in KFH buffer at a ligand concentration of 5 μ M with the use of 50 mm quartz cuvettes at the wavelength maxima given in parentheses.

Compound	(I)	$10^{-4} \times \epsilon$ (KFH buffer) ($\text{M}^{-1} \cdot \text{cm}^{-1}$)	$10^{-4} \times \epsilon$ (KFH buffer containing 1% <i>N</i> -lauroylsarcosine) ($\text{M}^{-1} \cdot \text{cm}^{-1}$)	K_d (M^{-1})
M-4	0.01	1.32 (343 nm)	1.31 (347 nm)	1.0×10^4
	0.10	1.28 (343 nm)	1.30 (347 nm)	1.6×10^4
	0.50	1.23 (343 nm)	1.29 (347 nm)	1.9×10^4
D-4	0.50	1.49 (345.5 nm)	2.04 (347.5 nm)	4.4×10^3
	1.00	1.48 (345.5 nm)	2.08 (347.5 nm)	9.4×10^3
D-6	0.50	1.50 (346 nm)	2.16 (347 nm)	6.2×10^4

compartments were loaded with 4 ml of the DNAs at the same concentration (150 μ M) and the central compartment with 4 ml of ligand solution in the appropriate buffer. After equilibration at 20 °C for 20 h for M-4 and the acridines and for 96 h for the bisplatinum–terpyridine complexes, 0.4 ml of a 10% solution of the sodium salt of *N*-lauroylsarcosine in water was added to 3.6 ml of sample from each chamber. The absorbance of each DNA solution was measured against the similarly treated sample from the free-drug compartment at the appropriate wavelength maximum in the range 345–350 nm for the platinum ligands and in the range 400–415 nm for the acridines. The quotient of the measured absorption values is the distribution ratio of drug bound to the two DNAs. This quantity was evaluated for at least three input ratios at and below 0.1 drug molecule per DNA base-pair, and for all ligands examined was independent of degree of binding. The measured distribution ratios thus correspond to the parameter α (Muller & Crothers, 1975) defined as the ratio of the affinity constants of a ligand for two DNAs of differing G+C content. The differential dialysis experiments involving poly(dA-dT)·poly(dA-dT) and poly(dG-dC)·poly(dG-dC) were carried out in three-compartment dialysis cells of 1 ml capacity per compartment. Volumes of reagents were adjusted accordingly, and the procedure outlined for the bacterial DNAs was followed.

RESULTS

Dimerization constants

The propensity of M-4, D-4 and D-6 to aggregate in aqueous buffers was studied for the set of experimental conditions used to investigate ligand binding. Since the solubility of these ligands is limited in chloride-containing buffers, KF was used as the supporting electrolyte. The measured dimerization constants are shown in Table 1. For M-4 and D-4 the K_d values increase with ionic strength, a situation also found to apply for the structurally related [Pt(terpy)SC₆H₅]⁺ cation (Wakelin *et al.*, 1984) and for ethidium and proflavine (Quadri-foglio *et al.*, 1974). Strong intermolecular aggregation of the dimeric ligands, as reflected in the dimerization constants, is also accompanied by intramolecular stacking of the terpyridine chromophores (McFadyen *et al.*, 1986). Spectral data (McFadyen *et al.*, 1986) indicate that both interactions are disrupted in buffers containing detergent and in organic solvents.

Interaction with calf thymus DNA

Scatchard plots for the interaction of M-4 with calf thymus DNA in KFH buffer of *I* 0.01, *I* 0.1 and *I* 0.5 at 20 °C are shown in Fig. 2. The markedly concave-upwards binding isotherm at *I* 0.01 extending to binding ratios above 0.6 is indicative of two types of binding: a strong intercalative mode and a weaker non-intercalative secondary interaction. This notion is supported by the effects of M-4 on the contour length of sonicated fragments of calf thymus DNA (McFadyen *et al.*, 1986), where a linear increase in helix extension is observed up to $r \sim 0.2$ followed by a marked decrease in extension at higher degrees of binding. The data at *I* 0.01 are not amenable to simple Scatchard analysis, nor could the results be fitted to eqn. (10) of McGhee & von Hippel (1974), which corresponds to an excluded-site model. In this situation the intrinsic association constant to an isolated binding site, $K_{(0)}$, can be estimated by extrapolating to the ordinate axis (Crothers, 1968; Muller & Crothers, 1968, 1975; McGhee & von Hippel, 1974), yielding a value for $K_{(0)}$ of 4.5×10^5 (Table 2). As the ionic strength is increased the binding of M-4 to DNA is weakened, and the data at *I* 0.1 and *I* 0.5 (Figs. 2*b* and 2*c*) now fit the McGhee & von Hippel (1974) equation. The binding parameters so derived are given in Table 2. For M-4 there is a linear relationship between $\log K_{(0)}$ and $\log[K^+]$ (Fig. 2*a* inset), in accord with polyelectrolyte theory as developed by Record *et al.* (1978) and Friedman & Manning (1984). The former treatment predicts a slope in this plot of -0.88 , the latter a value of -1.24 . The observed slope of -1.0 falls between these two extremes, and by following the treatment of Record *et al.* (1978) may be used to estimate that the binding of M-4 is associated with the release of 1.1 bound K^+ ions per molecule from the condensation sheath of DNA. $K_{(0)}^{1M}$, the affinity of M-4 for DNA in the absence of ion condensation effects, can be calculated by extrapolating the plot of $\log K_{(0)}$ versus $\log[K^+]$ to the ordinate axis and using eqn. 7.17 of Record *et al.* (1978):

$$\ln K_{\text{obs.}} = \ln K_T^0 + Z \cdot \xi^{-1} \cdot \ln \gamma_{\pm} \delta - Z \cdot \psi \cdot \ln[M^+]$$

where $K_{\text{obs.}}$ is the observed association constant, K_T^0 is the association constant in the absence of ion condensation effects, Z is the slope of the plot of $\ln K_{\text{obs.}}$ versus $\ln[M^+]$ and $\gamma_{\pm}$ is the ionic activity coefficient. $\xi = e^2/\epsilon k T b$ [eqn. 2.7 of Record *et al.* (1978)], where e is the protonic charge, ϵ is the bulk dielectric constant of solvent, k is Boltzmann's constant, T is the Kelvin temperature and b is the average axial charge spacing

Fig. 2. Interaction between (butane-1-thiolato)-(2,2':6',2''-terpyridine)platinum(II), M-4 and calf thymus DNA in buffer of I 0.01 (a), I 0.10 (b) and I 0.50 (c) at 20 °C

The continuous curves are theoretical, computed to fit eqn. (10) of McGhee & von Hippel (1974) with the use of the parameters for $K_{(0)}$ and N in Table 2. Inset to (a): plot of $\log K$ versus $\log [K^+]$ for M-4.

along the helical axis. $\delta = 0.33b$ and $\psi = 1 - (2\xi)^{-1}$ [eqn. 2.9 of Record *et al.* (1978)]. The value obtained, $4.1 \times 10^3 \text{ M}^{-1}$, is similar to the $K_{(0)}^{1M}$ values reported (Wakelin *et al.*, 1984) for the structurally related cations hydroxyethylthiolato(terpyridine)platinum(II) ($2.3 \times 10^3 \text{ M}^{-1}$) and phenylthiolato(terpyridine)platinum(II) ($1.7 \times 10^3 \text{ M}^{-1}$).

Attempts to determine binding parameters for D-4 were made difficult by precipitation of the drug-DNA complex above binding ratios of about 0.07:1. In this circumstance the range of r values that could be spanned was so small that parameters derived from curve-fitting are of dubious validity. Accordingly, the association constants for D-4 at I 0.5 and I 1.0 (Table 2)

Table 2. Parameters of binding for the interaction of M-4, D-4 and D-6 with calf thymus DNA at 20 °C

For M-4 (I 0.01) and D-4 (I 0.5 and 1.0) association constants were determined by linear extrapolation of binding data to the Scatchard-plot ordinate. Otherwise binding parameters were evaluated by using a non-linear least-squares fit to eqn. (10) of McGhee & von Hippel (1974). The uncertainty in values of $K_{(0)}$ is estimated to be $\pm 10\%$.

Compound	I	$10^{-4} \times K_{(0)}$ (M^{-1})	N (nucleotide pairs per binding site)
M-4	0.01	45	—
	0.10	4.3	2.5
	0.50	0.84	2.8
D-4	0.50	6.5	—
	1.00	3.0	—
D-6	0.50	19	2.5

Fig. 3. Interaction between μ -1,6-dithiolatohexanebis-[2,2':6',2''-terpyridineplatinum(II)], D-6 and calf thymus DNA in 1.050 buffer at 20 °C

For details see the legend to Figure 2.

were estimated by linear extrapolation of the experimental results (not shown) to the ordinate axis of the Scatchard plot. The $K_{(0)}$ value at I 0.5 is greater than that for the monofunctional analogue under the same conditions. As expected, at I 1.0 the affinity is weakened. For D-6 a binding isotherm was obtained at I 0.5 and is shown in Fig. 3. These data were fitted to eqn. (10) of McGhee & von Hippel (1974), yielding the binding parameters given in Table 2. The strength of intercalative binding by D-6 is so substantial at I 0.5 that attempts to measure binding at lower ionic strengths were precluded because of difficulties in measurement of very low

concentrations of the free drug. At higher ionic strengths precipitation of ligand-DNA complex became a problem.

Specificity determinations

The sequence specificity of the monomer M-4 and the dimers D-4 through to D-10 was assessed by differential dialysis of the ligands against *M. lysodeikticus* DNA (72% G+C) and *Cl. perfringens* DNA (30% G+C) in 1.01 KFH buffer. The parameter α (Muller & Crothers, 1975) was determined at least in triplicate for each ligand, and the data are presented in Table 3. For M-4, the observed α of 2.4 is the expected value for intercalation in which a single side of a G·C base-pair constitutes the only binding site with appreciable affinity, and is given by the ratio of the G+C content of *M. lysodeikticus* DNA to the G+C content of *Cl. perfringens* DNA (Muller & Crothers, 1975). If this specificity of the chromophore is retained when the dimeric ligands bisintercalate, then their α values will depend sensitively on the size and nucleotide composition of the binding site. For example, if a single 'base-pair sandwich' is formed, then the most preferred sequence would constitute two adjacent G·C base-pairs with each chromophore interacting selectively with one side of each G·C pair. For this situation the theoretical value of α is 5.76, as derived from the ratio of the probability of occurrence of this sequence in *M. lysodeikticus* DNA and *Cl. perfringens* DNA. The ligands D-7 and upwards are capable of forming a two- 'base-pair sandwich', and here the most preferred sequences are likely to be G·C-(G·C or A·T)-G·C or G·C-A·T-G·C or G·C-G·C-G·C, corresponding to α values of 2.30, 5.76 and 13.82 respectively. However, if for the binuclear ligands only one chromophore influences specificity, the other binding in an entirely non-selective manner, then the value of α would be the same as that found for the monomer M-4. The measured α values for the bisplatinum dimers (Table 3), although indicating that the ligands have a preference for binding to *M. lysodeikticus* DNA, all fall well below the value observed for the monomer. It would thus appear that the chromophore specificity is severely compromised in the

Table 3. Specificity data for platinum(II)-terpyridine complexes and acridine ligand

The α values for the acridines were determined in 0.1 I SH buffer at 20 °C and for the terpyridine complexes in 0.1 I KFH buffer at 20 °C. α refers to the distribution of ligand between *M. lysodeikticus* DNA and *Cl. perfringens* DNA. Each α value is the mean of at least three determinations to a precision of at least ± 0.1 (half range). Abbreviation: 9AA, 9-aminoacridine.

Terpyridine ligand	α	Acridine ligand	α
M-4	2.4	9AA	1.1
D-4	1.1	C-4	0.9
D-5	1.4	C-6	1.0
D-6	1.3	C-8	1.0
D-7	1.7		
D-8	1.2		
D-9	1.4		
D-10	1.2		

bifunctional ligands. For the purpose of comparison, the α values for 9-aminoacridine and the C-4, C-6 and C-8 methylene-linked diacridines are also reported in Table 3 and reveal no evidence of significant sequence selectivity. The direct proportionality between binding constants and G+C content for a range of monofunctional platinum(II) metallointercalators (Howe-Grant & Lippard, 1979; Wakelin *et al.*, 1984) implies that binding of these ligands to DNA sequences containing only A·T base-pairs should be very weak or non-existent. That they do in fact have a weak affinity for A·T base-pairs is shown by the results of differential dialysis experiments with calf thymus DNA and poly(dA-dT)·poly(dA-dT), where the $K_{(0)}$ of M-4 for the synthetic polymer was found to be $1.0 \times 10^4 \text{ M}^{-1}$ in buffer of *I* 0.1. Similar measurements involving calf thymus DNA and poly(dG-dC)·poly(dG-dC) yield an affinity constant of M-4 for poly(dG-dC)·poly(dG-dC) of $1.2 \times 10^5 \text{ M}^{-1}$, a value indistinguishable from that expected for a 100% -G+C DNA at *I* 0.1 on the basis of the measured affinity for calf thymus DNA (44% G+C) at this salt concentration (Table 2).

DISCUSSION

In its binding to calf thymus DNA the monomeric ligand M-4 shows the two broad modes of interaction typical of a range of planar aromatic molecules (Wilson & Jones, 1981), i.e. there is primary intercalative binding and weaker external attachment to the helix, the latter being preferentially diminished at higher cation concentrations. The strength of intercalative binding by M-4 is substantial, the binding constant at *I* 0.01 of $4.5 \times 10^5 \text{ M}^{-1}$ being equivalent to that reported for 9-aminoacridine under similar conditions (Wilson *et al.*, 1981). On binding, the monocationic M-4 releases 1.1 K^+ ions per molecule from the condensation sheath of DNA, a value comparable with the number of cations released on binding ethidium (Howe-Grant & Lippard, 1979) and 4'-(9-acridinylamino)methanesulphon-*m*-anisidide (Wilson *et al.*, 1981). In this respect M-4 differs from the structurally related (hydroxyethanethiolato)-(2,2':6',2''-terpyridine)platinum(II) (Howe-Grant & Lippard, 1979) and (thiophenolato)-(2,2':6',2''-terpyridine)platinum(II) (Wakelin *et al.*, 1984) cations, where excess release of 1.5 and 1.4 cations respectively per bound ligand molecule has been observed.

The differential dialysis measurements clearly indicate that M-4 has a high preference for binding to one side only of a G·C base-pair. This is reflected in the concordance between the expected and measured α values and the observation that $K_{(0)}$ for binding to poly(dG-dC)·poly(dG-dC) is some 12-fold greater than the association constant for binding to poly(dA-dT)·poly(dA-dT). This degree of selectivity is characteristic of the thiophenolato (Wakelin *et al.*, 1984) and hydroxyethanethiolato (Howe-Grant & Lippard, 1979) analogues of M-4 and is also shared by a platinum(II) complex derived from 1,10-phenanthroline (Howe-Grant & Lippard, 1979). The specificity of these ligands may well be consequent upon the presence of the platinum atom, since the 2,2':6',2''-terpyridine and 1,10-phenanthroline complexes are structurally quite dissimilar. In this regard it is notable that in the crystal structure of the (hydroxyethanethiolato)-(2,2':6',2''-terpyridine)platinum(II)-deoxyCpG intercalation complex (Wang *et al.*,

1978) the thiolato tail is located in the major groove of the DNA mini-helix and the platinum atom lies sandwiched directly between the O-6 atoms of the guanine residues. As we have noted previously (Wakelin *et al.*, 1984), if G·C-base-pair specificity arises from maximum monopole-monopole interactions between the ligand and base-pair (Muller & Crothers, 1975), then it is just this orientation of the positively charged platinum atom (formal charge 2+) and the large residual negative charge on the O-6 atom of guanine (Dean & Wakelin, 1979) that would give rise to specificity.

The 23-fold enhancement in affinity of D-6 for DNA compared with the affinity of M-4 at *I* 0.5 is in keeping with the frequently made observation (Wakelin, 1986) that progression from mono- to bi-functional intercalation is accompanied by substantially enhanced affinity constants. However, the magnitude of the enhancement is well below the 10^4 -fold increase expected if the free energy of bisintercalative binding were simply obtained by doubling the free energy of binding of the monomeric ligand. This suggests that the effects of interaction of the linker chain with DNA cannot be ignored, nor can the energetic changes associated with distortion of both nucleic acid and ligand in the formation of the bisintercalated complex. For D-4 the situation is more complicated, since the hydrodynamic measurements carried out at *I* 0.01 (McFadyen *et al.*, 1986) were enigmatic. The helix-extension parameter indicated mixed-mode binding, whereas the helix-unwinding angle placed this ligand among the bifunctional intercalators. It is difficult, therefore, to know whether the 8-fold enhancement in the association constant of D-4 when compared with M-4 at *I* 0.5 reflects bifunctional reaction or mixed monofunctional/bifunctional binding. Although there is doubt about the binding mode of D-4 at *I* 0.01, it has been shown in studies with a polyamine-linked ethidium dimer (Delbarre *et al.*, 1983) that bifunctional reaction is promoted by raising the ionic strength. Certainly for the structurally related alkyl-linked diacridines in 0.5 M- NH_4F (Wakelin *et al.*, 1979) there was no marked increase in binding affinity until the first clearly bifunctional derivative, C-6, was reached. The binding mode of D-4 at *I* 0.5 could be resolved by the appropriate viscometric titrations, but these were precluded by precipitation of ligand-DNA complex above *r* $\sim$ 0.07.

The α values observed for the ligands D-4 through to D-10 indicate that these compounds have a preference for binding to the DNA of highest G+C content. However, the measured values fall short of the theoretically expected result for a monomeric compound (2.4) or for the cases of bifunctional intercalation in which each chromophore binds with the same specificity as the monomeric unit. In contrast with M-4, 9-aminoacridine shows only slight G·C-base-pair preference, a situation observed for numerous heteroaromatic chromophores (Muller & Crothers, 1975). This preference is abolished for the C-6 and C-8 diacridine derivatives, where there is no evidence of specificity. The monofunctional C-4 diacridine displays a slight preference for binding to the A+T-rich DNA. Since, in general, ligands that bind to the outside of the DNA helix show A·T-base-pair specificity (Muller & Gautier, 1975), the selectivity of the C-4 diacridine may be a consequence of external binding by its non-intercalated chromophore.

It is clear that the notion of enhancing sequence specificity by linking together sequence-selective chromophores has not been realized in this series of bisplatinum terpyridines. If our ideas of the importance of the interaction between the platinum atom and the O-6 atom of guanine are correct, the specific binding of the (2,2':6',2''-terpyridine)platinum(II) moiety may require precise orientation of the chromophore in the preferred binding site. It is possible that within this series of compounds the ligands are not able to form bisintercalated complexes in which the geometrical requirements for specific chromophore-base-pair interactions are satisfied for either chromophore. The reasons for this are not obvious to us from experiments with space-filling molecular models. However, intercalative binding causes substantial structural and electronic distortions to DNA, which are most evident in the near vicinity of the bound chromophore. Thus it may be that in the bisintercalated complexes modifications to the geometry of each intercalation site are coupled, leading to loss in specificity. If this hypothesis is correct, then, as the chromophores are separated by longer linker chains, so that the influences of binding of one upon the other are diminished, specificity should return. That this is not the case may be due to the mixed binding mode of the higher D-*n* complexes (McFadyen *et al.*, 1986) or to the fact that even for D-10 the chromophores are still only far enough apart to allow formation of a two-'base-pair sandwich' complex, which may be an insufficient distance for uncoupling the binding sites. Further work with sequence-specific chromophores in which the flexibility and length of the linker chain are varied in such a way as to impose bifunctional reaction at all chromophore separations is clearly warranted.

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