Binuclear platinum (II)–terpyridine complexes

A new class of bifunctional DNA-intercalating agent

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A series of binuclear DNA-binding ligands was prepared by linking two (2,2':6',2''-terpyridine)platinum(II) moieties via $\alpha\omega$ -dithiols of the type HS-[CH₂]_n-SH where $n = 4$ –10. A monomeric analogue was also synthesized. Compounds were characterized by elemental analysis and electronic and n.m.r. spectroscopy. Viscometric measurements with sonicated rod-like DNA fragments and covalently closed circular DNA were performed to investigate the mode of binding of these agents. The ligands with $n = 5$ and 6 function as bis intercalators and form a single 'base-pair sandwich' in violation of neighbour-exclusion binding. Bifunctional reaction occurs for the ligand with $n = 7$, whereas the ligands with $n = 8$ and 10 show a preference for mixed monofunctional/bifunctional binding. The data do not permit definitive assignment of the binding mode of the ligands with $n = 4$ and 9. All compounds are growth-inhibitory against mouse leukaemia L1210 cells in culture with IC₅₀ values in the range 2–14 μ M.

INTRODUCTION

Recently much effort has been directed towards the design of DNA-binding agents showing enhanced nucleotide-sequence selectivity. A frequently employed strategy involves the use of polyfunctional intercalating agents, where it is expected that an increase in ligand-binding site size will provide the opportunity for additional specificity in binding (Wakelin, 1986). A logical facet of this approach is to incorporate into such agents chromophores that are known to have a preference for binding at a given dinucleotide site. To this end, studies on the interaction of Pt(II)–terpyridine thiolato complexes with DNA are particularly pertinent (Howe-Grant & Lippard, 1979; Wakelin *et al.*, 1984), since they indicate that these agents have both a high DNA-binding affinity and the requirement for a single G·C base-pair at the intercalation site. Thus the Pt(II)–terpyridine chromophore is a good candidate for incorporation into bifunctional ligands designed to interact selectively with G+C-rich regions of DNA. In

addition, the presence of a heavy metal in polyfunctional agents confers on them several other properties not shared by classical homo- or hetero-aromatic intercalating ligands. For example, the presence of the electron-dense platinum atom may facilitate X-ray-diffraction studies of DNA fibres containing bound ligand and single-crystal analysis of ligand–oligonucleotide complexes. The opportunity is also provided to use ¹⁹⁵Pt n.m.r. spectroscopy as a novel probe of solution structure.

In the present study we describe the synthesis and characterization of a range of dimeric molecules formed by linking the Pt(II)–terpyridine moiety via $\alpha\omega$ -dithiols of various chain lengths. The synthesis of a related monomer is also reported. The structures of the compounds are shown in Fig. 1. As a prerequisite to investigation of the thermodynamics and sequence selectivity of these ligands we examine here their bis intercalative capacity and the relationship between ligand structure and functionality. Accordingly, viscometric titrations with sonicated rod-like fragments of calf thymus DNA were used to establish helix-extension

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

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parameters, and helix-unwinding angles were determined with covalently closed circular DNA.

MATERIALS

Buffers

Experiments were carried out in Hepes/KOH buffer, pH 7.0 at room temperature, containing 2 mM-Hepes (Calbiochem) and sufficient KF to yield I 0.01 (this buffer system is referred to below as KFH buffer). Ultrapure water from a Barnstead Nanopure system was used throughout.

Nucleic acids

Calf thymus DNA (type 1) was purchased from Sigma Chemical Co. Covalently closed circular plasmid DNA, constructed by inserting a 9.1 kilobase mini F fragment into the *EcoRI* site of plasmid pBR322, was a gift from Dr. H. E. D. Lane (Department of Cell Biology, University of Auckland, Auckland, New Zealand). The G+C content of the DNA is approx. 50%. DNA solutions used in helix-extension measurements were prepared as follows. A solution of calf thymus DNA containing 2 mg/ml in KFH buffer supplemented with KF to give I 0.5 was sonicated in a Branson B15 150W sonicator fitted with the large probe at power level 5 for a total of 10 min at 0 °C. The solution was then extensively dialysed, in Spectrapor 2 dialysis tubing of M_r cut-off 12000, against KFH buffer, I 0.01, clarified by filtration through Whatman GF-C glass-fibre filters, and stored frozen at -20 °C. DNA solutions used in equilibrium-dialysis studies were sonicated for a total of 6 min. DNA concentrations are based on an assumed value for $\epsilon_{(P),260}$ of $13200 \text{ M}^{-1} \cdot \text{cm}^{-1}$, where the molar absorption coefficient is expressed with respect to nucleotide pairs.

Thiols

Heptane-1,7-dithiol, octane-1,8-dithiol and decane-1,10-dithiol were prepared according to published procedures (Hall & Reid, 1943). All other thiols were purchased from the Aldrich Chemical Co.

Platinum complexes

The intermediate compound chloro-(2,2':6',2''-terpyridine)platinum(II) chloride dihydrate,

[Pt(terpy)Cl]Cl $\cdot$ 2H $_2$ O, was prepared as previously described (Howe-Grant & Lippard, 1980). For the sake of brevity the monomeric compound is referred to below by the pseudonym M-4 and the dimers by D- n , where n represents the number of carbon atoms in the linker chain (Fig. 1). Preparative details are given below for M-4, D-4 and D-6. All other dimers were prepared in essentially the same way as D-6. It was sometimes necessary to purify the reaction product by dissolving it in a small amount of water, filtering and then isolating the final material by acetone precipitation or freeze-drying. All complexes were dried under vacuum at room temperature. Analytical data are presented in Table 1. Microanalyses were performed by the Australian Micro-analytical Service (Melbourne, Vic., Australia).

(Butane-1-thiolato)-(2,2':6',2''-terpyridine)platinum(II) nitrate, M-4

Butane-1-thiol (25 μ l) and tetraethylammonium hydroxide (128 μ l; 1.52 M solution in methanol) in ethanol (2 ml) were added slowly under N $_2$ to a stirred solution of [Pt(terpy)Cl]Cl $\cdot$ 2H $_2$ O (0.1 g) in water (4 ml). The intense-red solution that resulted was filtered in air and the product precipitated by addition of a saturated aqueous solution of NaNO $_3$ (3 ml). The product was collected, washed sparingly with chilled 0.4 M-HNO $_3$, then dissolved in ethanol (8 ml) and finally precipitated as red crystals by slow addition of diethyl ether (30 ml). The precipitate was collected, washed with diethyl ether/ethanol (4:1, v/v) and while still wet with the wash liquid was placed in a desiccator and dried under vacuum for 2 h.

μ -Butane-1,4-dithiolato bis-[2,2':6',2''-terpyridine-platinum(II)] dichloride dihydrate, D-4

Butane-1,4-dithiol (10 μ l) and tetraethylammonium hydroxide (112 μ l; 1.52 M solution in methanol) in ethanol (1 ml) was added slowly under N $_2$ to a stirred solution of [Pt(terpy)Cl]Cl $\cdot$ 2H $_2$ O (0.096g) in water (2 ml). The intense-red solution that resulted was quickly filtered in air and on cooling the product separated as fine purple needles. After standing at 4 °C for 4 h the product was collected, then successively washed with an aqueous 0.2 M solution of tetraethylammonium chloride, acetone/ethanol (1:1, v/v) and acetone.

Table 1. Analytical data for M-4 monomer and D- n dimers

The values shown refer to elemental composition by weight.

			Analytical data (%)									
			Carbon		Hydrogen		Nitrogen		Chlorine		Sulphur	
Compound		M_r	Calc.	Found	Calc.	Found	Calc.	Found	Calc.	Found	Calc.	Found
M-4	C $_{19}$ H $_{22}$ N $_4$ O $_3$ PtS	581.6	39.23	39.6	3.81	3.5	9.63	9.5	—	—	5.51	5.4
D-4	C $_{34}$ H $_{30}$ Cl $_2$ N $_6$ Pt $_2$ S $_2$ $\cdot$ 2H $_2$ O	1083.9	37.67	37.5	2.79	3.1	7.75	7.9	6.54	6.7	5.92	6.0
D-5	C $_{35}$ H $_{32}$ Cl $_2$ N $_6$ Pt $_2$ S $_2$ $\cdot$ 4H $_2$ O	1133.9	37.08	37.4	2.85	3.1	7.42	7.3	6.25	6.1	5.66	5.6
D-6	C $_{36}$ H $_{34}$ Cl $_2$ N $_6$ Pt $_2$ S $_2$ $\cdot$ 6H $_2$ O	1148.0	37.66	37.6	2.99	3.3	7.32	7.2	6.18	6.1	5.59	5.7
D-7	C $_{37}$ H $_{36}$ Cl $_2$ N $_6$ Pt $_2$ S $_2$ $\cdot$ 6H $_2$ O	1198.0	37.09	37.2	3.03	3.1	7.01	7.0	5.92	5.9	5.34	5.3
D-8	C $_{38}$ H $_{38}$ Cl $_2$ N $_6$ Pt $_2$ S $_2$ $\cdot$ 4H $_2$ O	1175.0	38.80	38.5	3.26	3.4	7.14	6.8	6.03	6.4	5.45	5.7
D-9	C $_{39}$ H $_{40}$ Cl $_2$ N $_6$ Pt $_2$ S $_2$ $\cdot$ 4H $_2$ O	1190.0	39.35	39.2	3.39	3.7	7.06	6.9	5.96	5.7	5.39	5.2
D-10	C $_{40}$ H $_{42}$ Cl $_2$ N $_6$ Pt $_2$ S $_2$ $\cdot$ 4H $_2$ O	1202.0	39.96	40.0	3.36	3.5	6.99	6.8	5.89	5.9	5.33	5.7

μ -Hexane-1,6-dithiolato bis-[2,2':6',2''-terpyridine-platinum(II)] dichloride tetrahydrate, D-6

Hexane-1,6-dithiol (10 μ l) and tetraethylammonium hydroxide (86 μ l; 1.52 M solution in methanol) in ethanol (3 ml) were added slowly under N₂ to a stirred solution of [Pt(terpy)Cl]Cl₂·2H₂O (0.074g) in water (5 ml) to yield an intense-purple solution. Water (4 ml) was added and the solution filtered. The product was precipitated by slow addition of acetone (30 ml), then collected and washed with acetone.

(Ethylenediamine)-(3,4,7,8-tetramethyl-1,10-phenanthroline)platinum(II) dichloride and bis-(1,10-phenanthroline)platinum(II) dichloride

The former complex was prepared as described by McFadyen *et al.* (1985) and the latter as described by Hall & Plowman (1956).

METHODS**Dimerization constant**

The dimerization constant, K_d , for M-4 in KFH buffer was determined by using the observed deviation from a Beer's-Law plot at a wavelength of 343 nm. 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 was determined by the method reported previously (Wakelin *et al.*, 1984).

Equilibrium dialysis

Measurements of ligand binding were carried out by equilibrium dialysis in an MSE Dianorm apparatus. Dialysis cells having two 5 ml compartments separated by a Spectrapor 3 regenerated cellulose membrane were loaded with 4 ml of 150 μ M-DNA in KFH buffer in one chamber and 4 ml of the appropriate ligand solution in the other. The cells were rotated to establish equilibrium in a water bath at 20 °C for 20 h for M-4 and for 96 h for D-4 through to D-10. A 0.4 ml portion of a 10% solution of *N*-lauroylsarcosine (sodium salt) 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 2. Control experiments were performed to verify complete dissociation of the ligand-DNA complexes. In the case of M-4, free ligand concentrations were corrected to take account of dimerization by using the measured K_d value.

Viscometry

Measurements with covalently closed circular DNA were performed at 20 °C essentially according to the method of Revet *et al.* (1971), with the instrumentation described by Wakelin *et al.* (1984). The viscometer contained 1.0 ml of a 100 μ M solution of plasmid DNA in KFH buffer. Ligand solutions were added in increments varying between 2 and 20 μ l from an Agla precision micrometer syringe via a fine polyethylene tube. The concentration of complex being delivered from the tip of the plastic tube was determined by u.v.-absorption measurements. Solutions were freed of particulate material by passing them through 0.22 μ m-pore-size Millipore filters before use. Reduced viscosities were calculated by established methods, taking into account the dilution caused by addition of ligand solutions. Viscometric measurements on sonicated DNA were performed essentially by the method of Cohen & Eisenberg (1966, 1969), with the apparatus described above. In this case, because of the lower reduced viscosity of the sonicated fragments (M_r , 2.5×10^5 – 5×10^5), the DNA concentration was increased to 300 μ M. Values of the relative contour length of DNA in the presence of the ligand compared with free DNA, L/L_0 , were calculated as the cube root of the measured relative increase in reduced viscosity (Wakelin *et al.*, 1978).

N.m.r. measurements

N.m.r. spectra were recorded by Mr. T. J. Rook and Dr. R. T. C. Brownlee of La Trobe University (Melbourne, Vic., Australia) on a Jeol FX-200 n.m.r. spectrometer.

Growth inhibition of L1210 cells

L1210 cells were obtained from the Ludwig Institute (Sydney, N.S.W., Australia) and grown in culture in Minimum Essential Medium (Flow Laboratories, Rockville, MD, U.S.A.) supplemented with foetal-calf serum (15%, v/v), glutamine (2 mM) and gentamycin (2 mg/100 ml). Cells were incubated without agitation in an O₂/CO₂/N₂ (1:2:17) atmosphere at 37 °C, and under these conditions the average doubling time was approx. 12 h. Drugs were diluted as required in sterile water, and then 40 μ l of drug solution was dispensed into 2 ml of medium containing 5×10^4 cells/ml. Control cultures received 40 μ l of sterile distilled water. After incubation at 37 °C for 48 h, cells were counted on a Coulter model ZM counter. Cell growth as a percentage of control was plotted versus drug concentration, and the IC₅₀ value was determined. Each IC₅₀ value quoted is the mean for at least two determinations.

RESULTS**Synthesis and characterization of ligands**

All compounds in Fig. 1 were prepared under N₂ by reaction between [Pt(terpy)Cl]Cl₂·2H₂O and the appropriate thiol in ethanol/water with sufficient base present

Table 2. Spectral properties of M-4 and D-*n* dimers

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

Compound	$10^{-4} \times \epsilon$ (M ⁻¹ ·cm ⁻¹)		
	KFH	KFH/1% SDS	KFH/1% Sarkosyl
M-4	1.32 (343)	1.32 (346.5)	1.31 (347)
D-4	1.53 (345.5)	2.04 (345.5)	2.07 (347)
D-5	1.83 (346)	2.37 (346)	2.34 (347)
D-6	1.83 (346)	2.56 (346)	2.52 (347)
D-7	1.74 (347.5)	2.46 (346)	2.29 (347)
D-8	1.57 (347)	2.21 (346)	2.10 (347)
D-9	1.57 (348)	2.43 (346.5)	2.22 (347)
D-10	1.46 (347)	2.35 (346.5)	2.14 (347)

Table 3. ^{13}C and ^{195}Pt chemical shifts of M-4, D-4 and D-6

^{13}C n.m.r. spectra were recorded in $^2\text{H}_2\text{O}$ at a frequency of 50.1 MHz with the use of 10 μs pulse widths (45°) and 16000 data points. Dioxan (δ 67.2) was used as an internal reference. ^{195}Pt n.m.r. spectra were obtained at 42.7 MHz with the use of 8000 data points. ^{195}Pt chemical shifts were calculated from the measured absolute frequency of the platinum resonance. The assignments of C-2 and C-2' could be reversed, as could those of C-3 and C-3'.

Compound	Chemical shift (p.p.m.)												Pt
	C-2	C-3	C-4	C-5	C-6	C-2'	C-3'	C-4'	C-7	C-8	C-9	C-10	
M-4	159.1	125.9	142.7	129.6	152.5	153.7	124.4	142.5	30.3	37.5	22.3	13.5	1373
D-4	158.3	125.8	142.8	129.6	152.4	152.8	124.3	142.6	29.4	32.8	—	—	1424
D-6	158.2	125.7	142.7	129.5	152.4	152.6	124.1	142.0	30.6	34.2	28.7	—	1373

to account for the proton release associated with co-ordination of the thiol. The monomer M-4 was isolated with NO_3^- as counterion, whereas Cl^- served in this role for the dimers. Although dimers were initially formed in solution from reaction between $\text{HS}-(\text{CH}_2)_n-\text{SH}$ ($n = 2$ and 3) and $[\text{Pt}(\text{terpy})\text{Cl}]\text{Cl}\cdot 2\text{H}_2\text{O}$, solid complexes corresponding in structure to D-2 and D-3 could not be isolated. The ^{13}C chemical-shift assignments and the ^{195}Pt chemical shifts for the representative compounds M-4, D-4 and D-6 are presented in Table 3. The numbering scheme for the carbon atoms is shown in Fig. 1. The resonances of the thiolate ligands are readily assigned (Barbarella *et al.*, 1976) and are substantiated by the observation of Pt coupling to C-7 in the spectrum of D-6.

The electronic spectra of dilute solutions ($<10\ \mu\text{M}$) of M-4 and the dimers show a similar position and pattern of peaks, particularly in the 300–350 nm region of the spectrum. A $5\ \mu\text{M}$ solution of M-4 exhibits the following absorption maxima and molar absorption coefficients in this range: 311 nm ($10700\ \text{M}^{-1}\cdot\text{cm}^{-1}$), 328 nm ($11000\ \text{M}^{-1}\cdot\text{cm}^{-1}$) and 343 nm ($13200\ \text{M}^{-1}\cdot\text{cm}^{-1}$). When the concentration of M-4 is increased above about $20\ \mu\text{M}$, hypochromicity is observed, owing to aggregation. Its measured dimerization constant, $1.02 \times 10^4\ \text{M}^{-1}$, is of the same order of magnitude as that reported for the structurally related compounds $[\text{Pt}(\text{terpy})\text{SCH}_2\text{CH}_2\text{OH}]^+$ (Jennette *et al.*, 1976) and $[\text{Pt}(\text{terpy})\text{SC}_6\text{H}_5]^+$ (Wakelin *et al.*, 1984). The intensities of the spectral bands of the dimeric ligands are substantially less than double that of the monomeric species, and are also found to be concentration-dependent. These phenomena are presumably due to respectively intra- and inter-molecular stacking interactions. Thus the enhancement observed in the absorption coefficients of the 345.5–348 nm peaks in the spectrum of the binuclear compounds in moving from KFH buffer to KFH buffer containing 1% Sarkosyl or SDS (Table 2) is most likely attributable to disruption of aggregation (especially intramolecular) by the detergent so that the chromophores are separated on the surface of the detergent micelles. Support for this notion is provided by the observation that solutions of M-4 follow Beer's Law in the concentration range 5–1000 μM in KFH buffer containing 1% Sarkosyl but not in buffer alone. It appears that aggregation is also disrupted in organic solvents such as dimethylformamide, dimethyl sulphoxide and ethanol. Self-aggregation of Pt(II)-terpyridine systems is far more pronounced than that displayed by other intercalating chromophores, as typified by the phenanthridines (Wakelin & Waring, 1980) and the

simple acridines (Quadrifoglio *et al.*, 1974). Close stacking is also a feature of the solid-state structure of Pt(II)-terpyridine complexes (Jennette *et al.*, 1976; Dewan *et al.*, 1980).

Interaction with sonicated DNA

The extension of the DNA helix associated with binding of M-4 and D-4 through to D-10 was studied with sonicated rod-like fragments of calf thymus DNA. Fig. 2 shows representative helix-extension plots for the compounds M-4, D-4, D-6 and D-10. The increase in contour length, L/L_0 , is plotted as a function of the drug/nucleotide pair input ratio D/P , or in the case of M-4 the binding ratio r . The input ratios for M-4 have been corrected to binding ratios by using equilibrium binding parameters determined for calf thymus DNA in KFH buffer (W. D. McFadyen, L. P. G. Wakelin, I. A. G. Roos & B. L. Hillcoat, unpublished work). For D-4 through to D-10, at least up to input ratios of about 0.3, measurements of ligand binding by equilibrium dialysis indicate that virtually all added drug is bound to DNA. For each helix-extension plot the slope, or helix-extension parameter, was generated from a linear least-squares fit to the initial portion of the curve (usually up to D/P of about 0.15). For the monomer M-4 up to a binding ratio of about 0.2 there is a linear increase in helix extension of 0.2 nm ($2.0\ \text{\AA}$) per bound molecule. The decrease in the extension parameter at higher extents of binding is suggestive of additional non-intercalative binding that does not cause lengthening of the helix. The increase in contour length caused by M-4 is the same as that observed (Wakelin *et al.*, 1984) for the analogue of M-4 in which the butanethiolato anion is replaced by the benzenethiolato anion. We have also determined the helix-extension parameters of the square planar complexes (ethylenediamine)-(3,4,7,8-tetramethyl-1,10-phenanthroline)platinum(II) dichloride and bis-(1,10-phenanthroline)platinum(II) dichloride (results not shown). Despite the fact that they are structurally different ligands from the monomeric terpyridine complexes, they cause the same increase, 0.2 nm ($2\ \text{\AA}$), in contour length. It seems that the platinum(II) intercalators as a group appear to cause substantially less extension of DNA per intercalated molecule than do the classical hetero-aromatic systems typified by ethidium and proflavine, where values of 0.27 nm ($2.7\ \text{\AA}$) have been reported (Reinert, 1973). The data for the dimeric compounds (Table 4) fall into two distinct groups, with D-5, D-6, D-7 and D-9 showing approximately twice the helix extension of the monomer, consistent with bifunctional intercala-

Fig. 2. Effects of M-4, D-4, D-6 and D-10 on the relative contour length of sonicated rod-like fragments of calf thymus DNA in buffer of 1.0.01 at 20 °C

The ordinate represents the calculated ratio of contour lengths in the presence (L) or in the absence (L_0) of ligand. For M-4 the abscissa represents the binding ratio of ligand expressed with respect to base-pairs. For D-4, D-6 and D-10 the abscissa represents the molar ratio of added ligand to DNA base-pairs. The data presented for each compound are derived from two independent experiments.

tion. On the other hand, D-4, D-8 and D-10 cause an increase in helix extension that is indicative of a mixed monofunctional/bifunctional binding mode. Like M-4, the dimeric compounds also show a diminution in helix extension at higher extents of binding.

Interaction with closed circular DNA

Helix-unwinding angles were determined by viscosity titrations with supercoiled DNA, each titration being carried out at least twice. All of the compounds remove and then reverse DNA supercoiling. Results representative of the ligands studied are shown in Fig. 3 for M-4, D-4, D-6 and D-10. The shapes of the titration curves and reduced viscosities (about 28 dl/g) of the DNA-ligand complexes at equivalence, where the supercoiling is fully relaxed, are both similar and comparable with the observed behaviour of the plasmid with ethidium and 9-aminoacridine (Denny *et al.*, 1985b). Taking the unwinding angle of ethidium as 26° (Wang, 1974), the measurements yield the equivalence binding ratios and unwinding angles given in Table 4. In the case of M-4 the input ratio at equivalence was corrected to a binding

ratio by use of the binding parameters determined for interaction with calf thymus DNA (W. D. McFadyen, L. P. G. Wakelin, I. A. G. Roos & B. L. Hillcoat, unpublished work). This correction assumes that contributions from the free energy of supercoiling to the effective association constant vanish for fully relaxed circles. For the binuclear ligands equilibrium-dialysis measurements with calf thymus DNA showed binding to be quantitative at equivalence input ratios. The unwinding angle of M-4 typifies values observed for monofunctional intercalators, which fall within the range 10–26° (Wilson & Jones, 1981). For the binuclear compounds D-5, D-6 and D-7 the unwinding angles are approximately twice that of M-4, and these data, along with the helix-extension parameters, unequivocally identify these compounds as bis intercalators. The dimer D-4 behaves enigmatically, for, although the unwinding angle, 31.1°, indicates bifunctional binding, the helix-extension parameter suggests mixed functionality. A situation somewhat akin to this is found among 9-aminoacridine dimers when the 9-amino groups are linked by either a $-\text{[CH}_2\text{]}_6-$ or $-\text{[CH}_2\text{]}_2-\text{O}-\text{[CH}_2\text{]}_2-$ chain (Wakelin *et al.*, 1978; Wright

Table 4. Physicochemical and biological data for M-4 monomer and D-*n* dimers

The helix-extension parameters are defined with respect to nucleotide pairs and the increase in contour length is calculated assuming that a gradient of 1 is equivalent to an extension of 0.34 nm (3.4 Å). The equivalence binding ratio of ethidium for the circular DNA has been determined to be 0.072 with respect to nucleotide pairs under conditions comparable with those used here (Denny *et al.*, 1985b). Helix-extension parameters are quoted to a precision of ± 0.02 nm (0.2 Å) and unwinding angles to $\pm 1^\circ$. Interchromophore separations are estimated from CPK models with the linker chain in the fully extended conformation.

Compound	Helix-extension parameter	Increase in contour length [nm (Å)]	Equivalence binding ratios	Helix-unwinding angle ($^\circ$)	Interchromophore separation [nm (Å)]	Growth inhibition, IC ₅₀ (μ M)
M-4	0.60	0.20 (2.0)	0.1070	17.5	—	14
D-4	0.73	0.25 (2.5)	0.0602	31.1	0.76 (7.6)	2
D-5	1.12	0.38 (3.8)	0.0591	31.7	0.88 (8.8)	5
D-6	1.13	0.38 (3.8)	0.0520	36.0	1.00 (10.0)	5
D-7	1.14	0.39 (3.9)	0.0585	32.0	1.12 (11.2)	5
D-8	0.83	0.28 (2.8)	0.0800	23.4	1.24 (12.4)	4
D-9	1.13	0.38 (3.8)	0.0743	25.2	1.36 (13.6)	3
D-10	0.92	0.31 (3.1)	0.0817	22.9	1.48 (14.8)	2

et al., 1980). Here, unwinding angles typical of bifunctional reaction are observed, whereas helix-extension parameters suggest monofunctional intercalation. In the case of the D-9 dimer the situation is reversed, since the helix-extension parameter identifies this compound as a bifunctional ligand whereas the

unwinding angle is consistent with mixed-mode binding. Although it is difficult to reconcile these apparently conflicting parameters of intercalation, this sort of observation is not without precedent. For a polyamine-linked ethidium dimer, Gaugain *et al.* (1978) report a helix-unwinding angle suggestive of monofunctional intercalation whereas the helix-extension parameter is some 1.6-fold larger than that of ethidium. The unwinding angles of D-8 and D-10 fall far short of the value expected for bifunctional reaction, leading to the conclusion, as did their helix-extension parameters, that these homologues bind in a monofunctional or mixed-functional mode.

DISCUSSION

Experiments with CPK space-filling molecular models indicate that the dimers D-4 through to D-10 are all capable of bifunctional intercalation. All the ligands can readily form a single 'base-pair sandwich', i.e. intercalation may occur at adjacent sites with one base-pair sandwiched between the two chromophores. For D-7 and upwards, the possibility of a two 'base-pair sandwich' also arises, in which intercalation takes place across two base-pairs, leaving an empty intervening site. However, the results of the viscometric measurements indicate that, whereas D-5, D-6 and D-7 function as bis intercalators, the compounds D-8 and D-10 show a preference for a mixed monofunctional/bifunctional binding mode. For D-8 and D-10 the non-intercalated chromophore may bind externally to the DNA or associate with a chromophore from an adjacent ligand. That these are likely events is supported by the finding that M-4 (W. D. McFadyen, L. P. G. Wakelin, I. A. G. Roos & B. L. Hillcoat, unpublished work) and its benzenethiolato analogue (Wakelin *et al.*, 1984) show both a propensity to self-aggregate and bind externally to DNA. It is not possible to decide whether D-4 is genuinely bifunctional or whether it represents the interchromophore separation that marks the transition from mono- to bi-functional intercalation. In this regard it is regrettable that the ligands corresponding to D-2 and D-3 could not be isolated. Consideration of molecular models also indicates that intercalation permitting

Fig. 3. Effects of M-4, D-4, D-6 and D-10 on covalently closed circular DNA

The ordinate represents the reduced viscosity in dl/g and the abscissa shows the molar ratio of added ligand to DNA base-pairs. Panel (a) ○, D-6; ▲, M-4. Panel (b) ○, D-4; ▲, D-10.

maximum chromophore/ base-pair overlap can most readily occur when the linker chain is located in the wide groove of the B-type helix. In this connection it is noteworthy that the crystal structure of a complex between deoxyCpG and (hydroxyethanethiolato)-(2,2':6',2''-terpyridine)platinum(II) shows the $-\text{CH}_2\text{CH}_2\text{OH}$ side chain to be located in the major groove of the mini-duplex (Wang *et al.*, 1978). The platinum-terpyridine dimers are structurally similar to the simple alkyl-linked diacridines (Wakelin *et al.*, 1978), and it is interesting therefore to compare the properties of these two series of molecules. The platinum compounds evidently share with the diacridines (Wakelin *et al.*, 1978) the ability to violate neighbour-exclusion binding (Crothers, 1968), since the chromophores in D-5 and D-6 are geometrically able to span only one base-pair. For the diacridines the $-\text{[CH}_2\text{]}_6-$ -linked dimer is the first clearly bifunctional member of the series with an estimated interchromophore separation of 0.88 nm (8.8 Å) (Wakelin *et al.*, 1978). Among the bisplatinum-terpyridines definitive bifunctional intercalation also begins at about the same interchromophore separation, 0.82 nm (8.2 Å), at the D-5 homologue, the presence of the large sulphur atom in the platinum compounds apparently compensating for the shorter linker chain. One point of contrast is that, whereas bifunctional reaction is observed for all the diacridines of linker chain length six carbon atoms or more, the longer-chain-length platinum compounds show a trend towards mixed functionality of binding. The transition from monofunctional to bifunctional reaction in the diacridines is marked by an enhancement in their toxicity to tumour cells in culture and in their efficacy as anti-tumour agents in mice (Chen *et al.*, 1978; Denny *et al.*, 1985b). However, toxicity of the platinum dimers towards L1210 cells in culture appears to be independent of ligand structure, the IC_{50} values for growth inhibition by the monomer M-4 and the dimers all falling within the narrow range 2–14 μM (Table 4). All the platinum compounds produce extensive cell lysis, suggesting that these agents may exert their effects on cell membranes rather than on DNA. Since the platinum compounds are permanently charged, unlike the basic diacridines, it is not certain that they gain access to the cell nucleus.

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