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Deoxyribonucleic acid binding and photocleavage studies of rhenium(I) dipyridophenazine complexes

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The intercalative binding interaction of $[Re(dppz)(CO)_3(py)][O_3SCF_3]$ (dppz = dipyrido[3,2-*a*:2′,3′-*c*]phenazine, py = pyridine) and $[Re(dppn)(CO)_3(py)][O_3SCF_3]$ (dppn = benzo[*i*]dipyrido[3,2-*a*:2′,3′-*c*]phenazine) with double-stranded calf thymus DNA, and synthetic oligonucleotides poly(dA)·poly(dT) and poly(dC)·poly(dG) has been studied with spectroscopic methods. The complexes have been found to promote cleavage of plasmid pBR322 DNA from the supercoiled form I to the open circular form II upon irradiation. The crystal structure of $[Re(dppz)(CO)_3(py)][O_3SCF_3]$ has also been established.

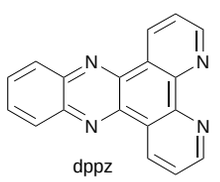
A number of transition-metal complexes have been utilized to probe nucleic acid structures and in the development of DNA-cleaving agents.[1–12] Barton and co-workers[3c–f] employed a number of chiral ruthenium(II) polypyridine complexes as DNA chirality probes. The complex $[Ru(bipy)_2(dppz)]^{2+}$ (bipy = 2,2′-bipyridine, dppz = dipyrido[3,2-*a*:2′,3′-*c*]phenazine) termed as a molecular 'light-switch' for DNA is non-emissive in aqueous buffer, but a luminescence enhancement of $>10^4$ is observed in the presence of double-stranded DNA with the appropriate chirality.[3g–h] The interaction is intercalative in nature, with the extended planar dppz ligand stacking into the double helix.

Although the photophysics and photochemistry of rhenium(I) complexes have been well documented, reports on the interactions of related complexes with DNA have been very limited.[11,12] We recently communicated DNA-binding studies of two rhenium(I) complexes $[Re(dppz)(CO)_3(py)][O_3SCF_3]$ **1** and $[Re(dppn)(CO)_3(py)][O_3SCF_3]$ **2** (dppn = benzo[*i*]dipyrido-[3,2-*a*:2′,3′-*c*]phenazine, py = pyridine) with extended diimine ligands dppz and dppn.[12] Herein we describe DNA-binding studies of these complexes with double-stranded calf thymus DNA and synthetic oligonucleotides poly(dA)·poly(dT) and poly(dC)·poly(dG) (dA, dT, dC, dG = deoxy-adenosine, -ribosylthymine, -cytidine, -guanosine). The photoexcited complexes have also been found to cleave plasmid pBR322 DNA. Moreover, the crystal structure of **1** has also been determined.

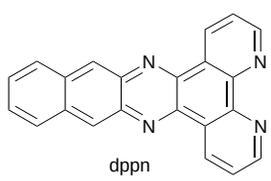
Experimental

Complexes **1** and **2** were prepared as described.[12] Autoclaved Milli-Q water was used for the preparation of the aqueous solutions. Calf thymus DNA was obtained from Sigma Chemical Company and purified by phenol extraction as described.[13] Synthetic polynucleotides poly(dA)·poly(dT) and poly(dC)·poly(dG) were obtained from Pharmacia Biotech Ltd. and used as received.

The UV/VIS spectra were obtained on a Hewlett-Packard 8452A diode-array spectrophotometer, steady-state excitation and emission spectra on a Spex Fluorolog 111 spectrofluorometer. All spectroscopic titrations were carried out in aerated 5% aqueous buffered [20 mmol dm^{-3} tris(hydroxymethyl)methylamine (Tris)–HCl, pH 7.0] methanolic solutions. The DNA concentrations per nucleotide were determined by absorption spectroscopy using the following molar absorption coefficients[14] (dm^3 mol^{-1} cm^{-1}): calf thymus DNA, 6600 at 260 nm; poly(dA)·poly(dT), 6000 at 260 nm; and poly(dC)·poly(dG), 7400 at 253 nm.



dppz



dppn

Plasmid pBR322 DNA was extracted from pBR322-transformed *Escherichia coli* DH5α and the supercoiled DNA (form I) purified with a Qiagen Plasmid Maxi Kit. Superoxide dismutase (SOD) from bovine erythrocyte was obtained from Sigma Chemical Company. Solutions for photocleavage studies were irradiated at room temperature with RPR-3500 Å lamps (Rayonet photochemical chamber reactor, model RPR-100) in Pyrex tubes immersed in a water-bath to cut off both UV and IR radiation. Solutions were electrophoresed for 3 h at 40 V on a 0.8% agarose gel in Tris–acetate buffer, pH 8. The gel was stained with ethidium bromide (3,8-diamino-5-ethyl-6-phenylphenanthridinium bromide) and photographed under UV light.

Crystallography

Single crystals of complex **1** were grown from diffusion of diethyl ether vapour into a concentrated acetonitrile solution of the complex.

Crystal data. $2C_{26}H_{15}N_5O_3Re^+$ $2CF_3SO_3^-\cdot H_2O$, M_r = 1579.43, monoclinic, space group $P2_1/n$ (no. 14), a = 14.361(6), b = 20.878(7), c = 18.906(6) Å, β = 90.99(3)°, U = 5667(3) $Å^3$, Z = 4, D_c = 1.851 g cm^{-3}, μ(Mo-Kα) = 44.36 cm^{-1}, $F(000)$ = 3064, T = 301 K. A yellow crystal of dimensions 0.20 × 0.15 × 0.35 mm was used for data collection at 28 °C on a Rigaku AFC7R diffractometer with graphite-monochromatized Mo-Kα radiation (λ = 0.710 73 Å) using ω–2θ scans with ω-scan angle (0.73 + 0.35 tan θ)° at a scan speed of 16.0° min^{-1} [up to six scans for reflection $I < 10\sigma(I)$]. Intensity data ($2\theta_{max}$ = 45°; h 0–15, k 0–22, l −20 to 20; three standard reflections measured after every 300 showed decay of 10.64%), were corrected for decay and Lorentz-polarization effects, and empirical absorption corrections based on the ψ scan of four strong reflections (minimum and maximum transmission factors 0.936 and 1.000). Upon averaging the 8037 reflections, 7678 of which were uniquely measured (R_{int} = 0.027), 4450 with $I > 3\sigma(I)$ were considered observed and used in the structural analysis. The space group was uniquely determined from systematic absences and the structure was solved by Patterson

Table 1 Selected geometric data (bond lengths in Å, angles in °) for $[Re(dppz)(CO)_3(py)][O_3SCF_3]$

Re(1)–N(1)	2.18(1)	Re(1)–N(2)	2.16(1)
Re(1)–N(3)	2.20(1)	Re(1)–C(1)	1.91(1)
Re(1)–C(2)	1.85(2)	Re(1)–C(3)	1.92(2)
Re(2)–N(6)	2.18(1)	Re(2)–N(7)	2.19(1)
Re(2)–N(8)	2.17(1)	Re(2)–C(27)	1.90(1)
Re(2)–C(28)	1.89(2)	Re(2)–C(29)	1.89(2)
N(1)–Re(1)–N(2)	87.3(4)	N(1)–Re(1)–N(3)	85.1(4)
N(1)–Re(1)–C(1)	175.9(5)	N(1)–Re(1)–C(2)	94.5(6)
N(1)–Re(1)–C(3)	92.4(6)	N(2)–Re(1)–N(3)	75.4(4)
N(2)–Re(1)–C(1)	92.2(5)	N(2)–Re(1)–C(2)	95.8(6)
N(2)–Re(1)–C(3)	175.6(5)	N(3)–Re(1)–C(1)	90.8(5)
N(3)–Re(1)–C(2)	171.1(6)	N(3)–Re(1)–C(3)	100.2(5)
C(1)–Re(1)–C(2)	89.6(7)	C(1)–Re(1)–C(3)	87.9(6)
C(2)–Re(1)–C(3)	88.7(7)	N(6)–Re(2)–C(7)	84.8(4)
N(6)–Re(2)–N(8)	86.0(4)	N(6)–Re(2)–C(27)	178.2(5)
N(6)–Re(2)–C(28)	91.8(5)	N(6)–Re(2)–C(29)	92.0(6)
N(7)–Re(2)–N(8)	75.3(4)	N(7)–Re(2)–C(27)	93.5(5)
N(7)–Re(2)–C(28)	172.4(5)	N(7)–Re(2)–C(29)	98.4(6)
N(8)–Re(2)–C(27)	93.1(5)	N(8)–Re(2)–C(28)	97.8(5)
N(8)–Re(2)–C(29)	173.6(6)	C(27)–Re(2)–C(28)	89.9(6)
C(27)–Re(2)–C(29)	88.7(7)	C(28)–Re(2)–C(29)	88.4(7)

methods and expanded using Fourier methods[15] and refinement by full-matrix least squares using the software package TEXSAN[16] on a Silicon Graphics Indy computer. A crystallographic asymmetric unit consists of two complex cations and two $CF_3SO_3^-$ anions and one water molecule. All non-H atoms of the complex cations and the S atoms of the anions were refined anisotropically; C, F and O atoms of the anions and the O atom of the water molecule have large thermal motions and were refined isotropically. The hydrogen atoms of the water molecule were not located. The other 30 H atoms at calculated positions with thermal parameters equal to 1.3 times that of the attached C atoms were not refined. Convergence for 709 variable parameters by least-squares refinement on F with $w = 4F_o^2/\sigma^2(F_o^2)$, where $\sigma^2(F_o^2) = [\sigma^2(I) + (0.035F_o^2)^2]$ for 4450 reflections with $I > 3\sigma(I)$ was reached at $R = 0.049$ and $R' = 0.065$ with a goodness of fit of 1.93; $(\Delta/\sigma)_{max} = 0.04$ for atoms of the complex cation. The final Fourier-difference map was featureless, with maximum positive and negative peaks of 1.63 and 0.91 e $Å^{-3}$ respectively. Selected bond distances and angles are summarized in Table 1.

Atomic coordinates, thermal parameters, and bond lengths and angles have been deposited at the Cambridge Crystallographic Data Centre (CCDC). See Instructions for Authors, *J. Chem. Soc., Dalton Trans.*, 1997, Issue 1. Any request to the CCDC for this material should quote the full literature citation and the reference number 186/490.

Results and Discussion

Even though there have been reports on the crystal structures of rhenium(I) diimine complexes,[17] those of the molecular structures of related complexes with extended planar ligands have been exceptionally rare.[12] The perspective drawings of the complex cation $[Re(dppz)(CO)_3(py)]^+$ with atomic numbering schemes are illustrated in Fig. 1(*a*). It is interesting that although the molecules of complexes **1** and **2** are similar, the unit cell of **1** contains two independent cations and anions while there is only one of each for that of **2**.[12] The perspective view of the unit cell of **1** is illustrated in Fig. 1(*b*). Selected bond distances and angles of **1** are listed in Table 1. The rhenium(I) centres adopt a distorted octahedral geometry with the carbonyl groups co-ordinated in a *facial* manner. The average bond distances and angles are comparable to typical values for similar rhenium(I) diimine complexes.[12,17] As expected, all the atoms of the dppz ligand lie on an essentially perfect plane, with the distance from the phenazine edge of the plane to the

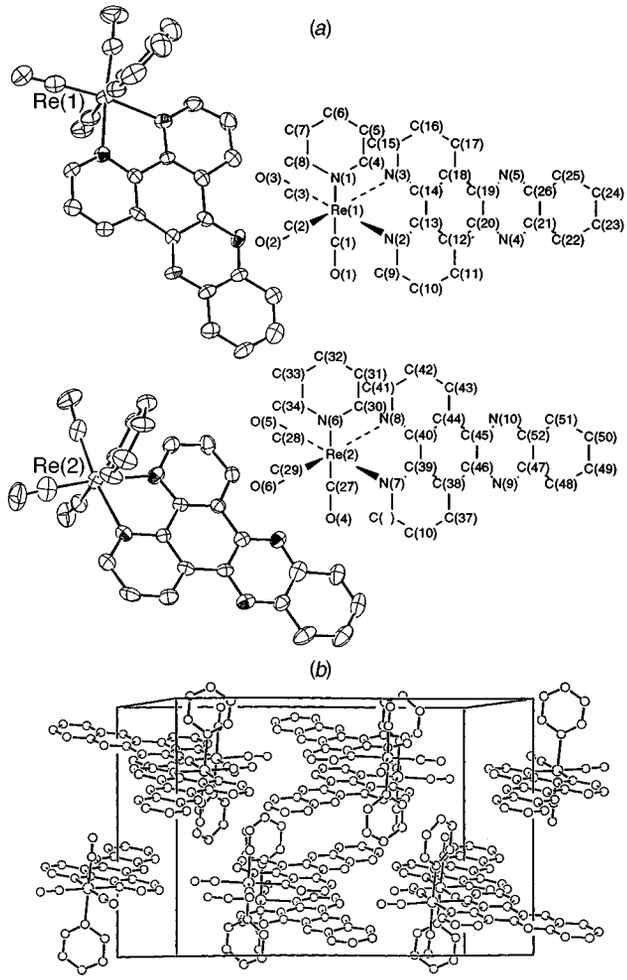


Fig. 1 (*a*) Perspective views of the two independent complex cations of complex **1**. Thermal ellipsoids are shown at the 35% probability level. (*b*) Perspective view of the unit cell of **1**

metal centre being *ca.* 10.1 Å. The distances between the ideal ring planes of the two dppz ligands on adjacent cations are found to be *ca.* 3.47 Å, showing some stacking interaction [Fig. 1(*b*)]. These values are similar to the base-pair stacking distance in DNA[18a] and the intercalator–base pair stacking distance in oligonucleotide intercalator complexes.[18b,c] Similar interplanar separations have also been observed in other dppz complexes such as $[Ru(OH_2)(dppz)(terpy)]^{2+}$ (terpy = 2,2′:6′,2″-terpyridine)[4d] and $[Ru(\eta^5\text{-}C_5Me_5)(NO)(dppz)]^{2+}$.[18d]

DNA Binding

Electronic absorption titrations. In 5% aqueous buffered (20 mmol dm^{-3} Tris–HCl, pH 7.0) methanolic solutions, the low-energy absorption bands of **1** at 366 and 384 nm and **2** at 320, 400 and 422 nm exhibit hypochromism upon addition of double-stranded calf thymus DNA. The electronic absorption spectral traces for the titration are illustrated in Fig. 2. A small bathochromic shift is observed. These findings suggest the binding of the complexes to the biopolymers, most likely through a non-covalent intercalative mode.[19] Similar observations have also been reported for other platinum(II),[2c,10a,c,20] copper(I),[9] ruthenium(II)[3c,d,f–h,5–7] and osmium(II)[3k] metallointercalators.

The intrinsic binding constants K of the rhenium(I) complexes with calf thymus DNA have been determined from the equation[21] $D/\Delta\varepsilon_{ap} = (D/\Delta\varepsilon) + (\Delta\varepsilon K)^{-1}$ through a plot of $D/\Delta\varepsilon_{ap}$ *vs.* D, where D is the concentration of DNA in base pairs, $\Delta\varepsilon_{ap} = (\varepsilon_a - \varepsilon_f)$ and $\Delta\varepsilon = (\varepsilon_b - \varepsilon_f)$. The apparent absorption coefficient, ε_a, was obtained by calculating A_{obs}/[Re]; ε_b and ε_f are the absorption coefficients of the bound and free form of the rhenium(I) complex, respectively. The slope and y intercept

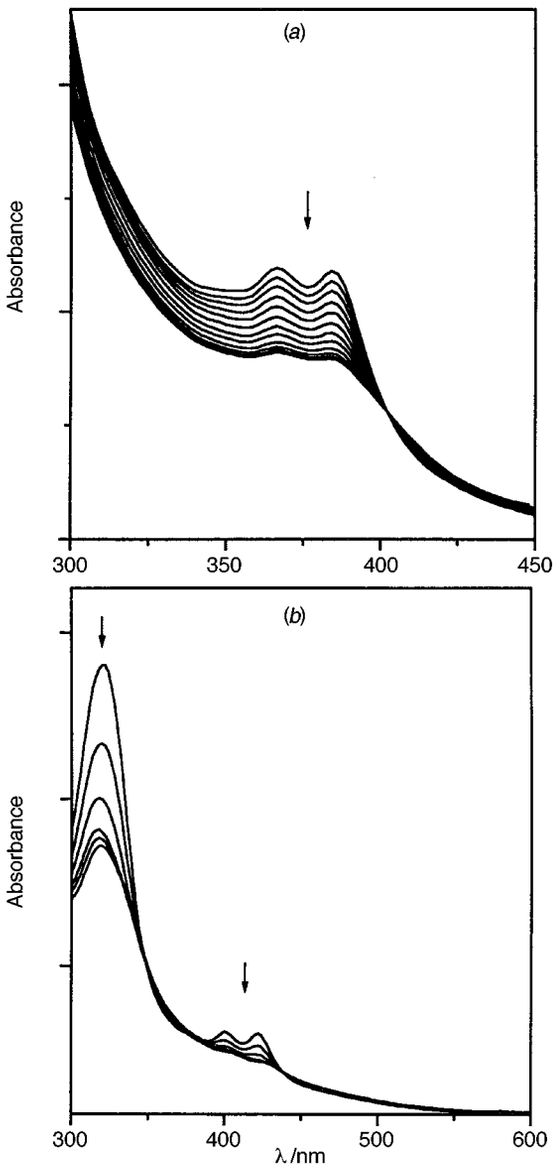


Fig. 2 Electronic spectral traces of complexes **1** (*a*) and **2** (*b*) in 5% aqueous buffered (20 mmol dm^{-3} Tris–HCl, pH 7.0) methanolic solution upon addition of double-stranded calf thymus DNA

of the linear fit of $D/\Delta\varepsilon_{ap}$ *vs.* D give $(1/\Delta\varepsilon)$ and $1/(\Delta\varepsilon K)$, respectively. The intrinsic binding constant K can be obtained from the ratio of the slope to the y intercept.

An intrinsic binding constant K of 6.4×10^4 dm^3 mol^{-1} was determined from the decay of the absorbance of complex **2** monitored at 310 nm upon addition of double-stranded calf thymus DNA. This value is comparable to those observed for $[Pt(phen)(en)]^{2+}$ (5×10^4 dm^3 mol^{-1})[22] and $[Ru(phen)_2(phi)]^{2+}$ (4.7×10^4 dm^3 mol^{-1})[3f] (phen = 1,10-phenanthroline, en = ethane-1,2-diamine and phi = 9,10-phenanthrenequinonediimine). However, for **1**, a linear fit was not obtained for the absorption data.

Emission titrations. In aqueous MeOH–buffer (20 mmol dm^{-3} Tris–HCl, pH 7.0) solutions the low-energy emission of complex **1** is enhanced upon addition of double-stranded calf thymus DNA. Despite the structural similarity of the complexes, their interactions with double-stranded calf thymus DNA are significantly different, as revealed by the luminescence behaviour in the presence of DNA. The results of the emission titrations for both complexes with DNA are illustrated with the titration curves in Fig. 3. Upon addition of calf thymus DNA the emission intensity of **1** grows steadily to around 13 times

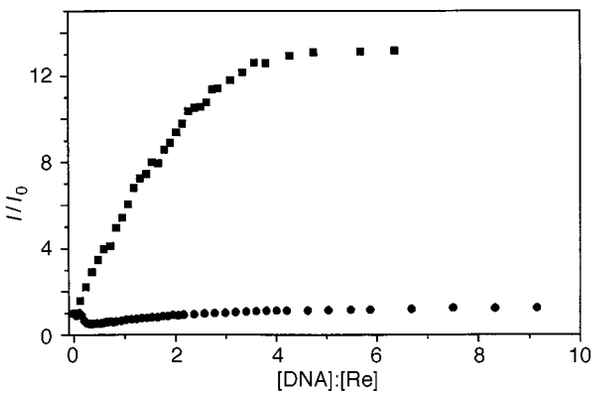


Fig. 3 Emission titration curves for complexes **1** (■) and **2** (●) with calf thymus DNA in aqueous buffered methanol. The emission intensities were monitored at 560 and 603 nm, respectively

larger and saturates at a [DNA phosphate]:[Re] ratio of *ca.* 4.5:1. However, in the case of **2**, the emission intensity drops at low [DNA phosphate]:[Re] ratios (minimum at around 0.4:1) before it gradually approaches saturation with an overall gain of approximately 1.3 times. Similar variation in emission intensity in the presence of double-stranded DNA has been observed for the related complex $[Ru(phen)_2(dppn)]^{2+}$.[3h] The enhancement in the luminescence intensities of the complexes, together with the hypochromicity observed in the electronic absorption spectra, can be ascribed to intercalation of the rhenium(I) complexes to the double helix. This is in line with the DNA-binding affinities of other transition-metal complexes with a planar heterocyclic ligand.[3c,d,f–i,k,5–7,9–11,20]

Data from the emission titrations were also employed to determined the binding constants of the rhenium(I) complexes with DNA. The concentration of the free complex, c_F, was obtained using equation (1) where c_T is the sum of the

$$c_F = c_T[(I/I_0) - P]/(1 - P) \quad (1)$$

concentrations of the free and bound forms of the complex, I and I_0 are the emission intensities in the presence and absence of DNA and P is the ratio of the observed emission intensity of the bound complex to that of the free one. The limiting emission intensity is the y intercept of a plot of I/I_0 *vs.* 1/[DNA phosphate] and the value of P can then be determined. The concentration of the bound complex, c_B is equal to $c_T - c_F$. A plot of r/c_F *vs.* r, where r is c_B/[DNA], was constructed according to the modified Scatchard equation by McGhee and von Hippel,[23] equation (2) where K is the intrinsic binding constant

$$r/c_F = K(1 - nr)\{(1 - nr)/[1 - (n - 1)r]\}^{n-1} \quad (2)$$

of the complex with the DNA and n is the binding site size in base pairs.

The binding data were fitted using the above equation to obtain the binding parameters. An intrinsic binding constant K of 4.2×10^4 dm^3 mol^{-1} for complex **1** was determined. The size of the binding site, n, was 2. This indicates that two base pairs of calf thymus DNA are occupied after binding of a single $[Re(dppz)(CO)_3(py)]^+$ unit. This value is close to those reported for other copper porphyrin intercalators.[24a]

Synthetic DNA binding

In order to gain more insight into the possibility of preferential binding of the DNA with the rhenium(I) diimine complexes, absorption and emission titrations using synthetic oligonucleotides poly(dA)·poly(dT) and poly(dC)·poly(dG) have also been carried out.

The low-energy absorption bands of both complexes experience a hypochromism and small red shift in the presence of the synthetic oligonucleotides. This is similar to the case of double-

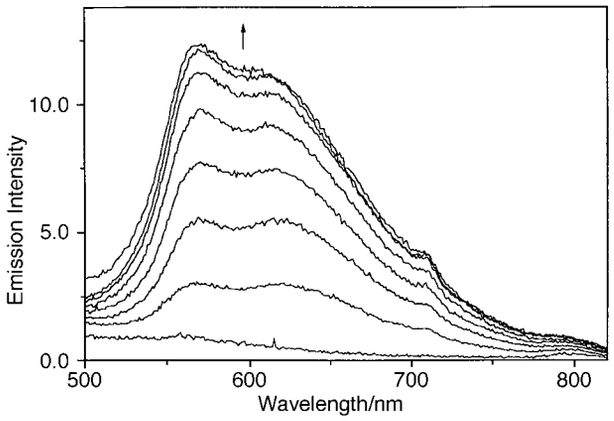


Fig. 4 Emission spectral traces of complex **1** (62 μmol dm^{-3}) in aqueous MeOH–buffer (20 mmol dm^{-3} Tris–HCl, pH 7.0) at 298 K in the presence of 0, 11, 22, 33, 45, 56, 67 and 134 μmol dm^{-3} poly(dA)·poly(dT)

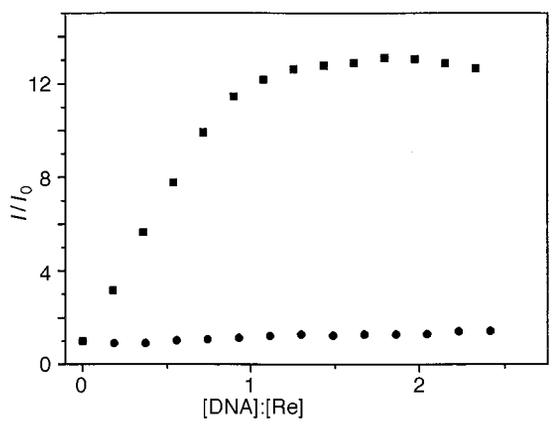


Fig. 5 Emission titration curves for complexes **1** with poly(dA)·poly(dT) (■) and poly(dC)·poly(dG) (●), respectively, in aqueous buffered methanol. The emission intensities were monitored at 560 nm.

stranded calf thymus DNA and suggests a binding mode of intercalation.

In addition, emission titrations have also been carried out for the complexes with poly(dA)·poly(dT) and poly(dC)·poly(dG), respectively. Fig. 4 illustrates the emission spectral traces for complex **1** upon addition of poly(dA)·poly(dT) in 5% methanol–buffer (20 mmol dm^{-3} Tris–HCl, pH 7.0). The emission intensity shows a dramatic enhancement and saturates at a very low [DNA phosphate]:[Re] ratio of around 1.3:1, with an overall 13-fold gain. Fig. 5 displays the titration curves for **1** with poly(dA)·poly(dT) and poly(dC)·poly(dG), respectively. In sharp contrast, with poly(dC)·poly(dG), the emission intensity of **1** does not show any considerable enhancement. Owing to the similar luminescence behaviour of the complex in the presence of calf thymus DNA and poly(dA)·poly(dT), it is suggested that the complex has a higher affinity towards the AT sites of double-stranded calf thymus DNA. Similar specificity at the adenine and thymine pairs has also been observed for $[Cu(bcp)_2]^{+}$ [9b] (bcp = bathocuproine = 2,9-dimethyl-4,7-diphenyl-1,10-phenanthroline) and $[Ru(phen)_3]^{2+}$.[24b] This preferential binding for poly(dA)·poly(dT) polymer can be explained by the fact that propeller twisting of the base pairs is relatively facile for a dA·dT sequence,[24c] and this could alleviate steric effects associated with the non-intercalated ligands about the metal centre.

Surprisingly, in contrast to the case with calf thymus DNA, complex **2** reveals a considerable enhancement in luminescence intensity in the presence of poly(dA)·poly(dT) (Fig. 6). The intensity of the band at 603 nm shows a steady increase and saturates at [DNA phosphate]:[Re] of *ca.* 5:1 with a total intensity gain of *ca.* five-fold. The titration curves for **2** with poly-

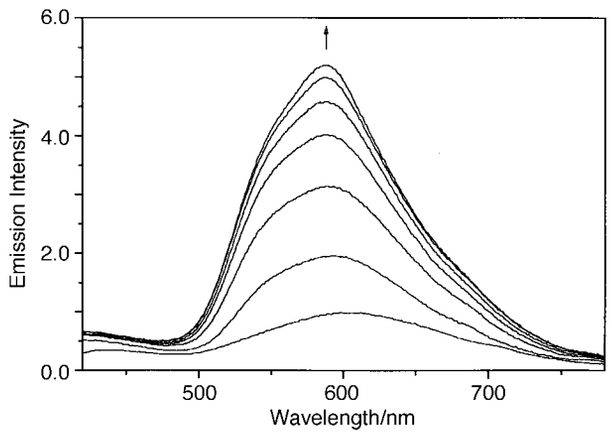


Fig. 6 Emission spectral traces of complex **2** (40 μmol dm^{-3}) in aqueous MeOH–buffer (20 mmol dm^{-3} Tris–HCl, pH 7.0) at 298 K in the presence of 0, 33, 67, 100, 134, 167 and 201 μmol dm^{-3} poly(dA)·poly(dT)

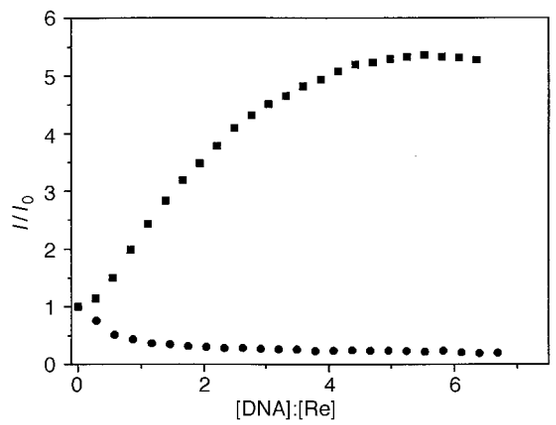


Fig. 7 Emission titration curves for complex **2** with poly(dA)·poly(dT) (■) and poly(dC)·poly(dG) (●), respectively, in aqueous buffered methanol. The emission intensities were monitored at 603 nm

(dA)·poly(dT) and poly(dC)·poly(dG) are illustrated in Fig. 7. It is interesting that in the presence of poly(dC)·poly(dG) the emission of the complex is quenched. The emission intensity becomes steady at [DNA phosphate]:[Re] = 3:1 and the final intensity is *ca.* 0.2 times the original. Similar observations have also been reported for other ruthenium(II) metallointercalators such as $[Ru(tap)_3]^{2+}$ (tap = 1,4,5,8-tetraazaphenanthrene) and the luminescence quenching may be attributable to photooxidation of the guanine by the excited complex.[6a–c,e]

It appears that the combined effects of (1) quenching of the luminescence associated with the poly(dC)·poly(dG) sites and (2) emission enhancement occurring with the poly(dA)·poly(dT) sites can explain the characteristic emission titration curve of complex **2** with calf thymus DNA (Fig. 3).

Photocleavage

Irradiation of complex **1** or **2** and plasmid pBR322 DNA in 1.7% methanolic buffer (20 mmol dm^{-3} Tris–HCl, pH 7.0) at $\lambda > 350$ nm for 30 min under aerobic conditions results in cleavage of the supercoiled form (I) of the plasmid pBR322 DNA to the nicked form (II) [Fig. 8(*a*), 8(*b*)]. No DNA cleavages are observed for controls in which the complexes are absent [lanes A, Fig. 8(*a*), 8(*b*)] or incubation of the plasmid with either complex in the dark [lanes H, Fig. 8(*a*), 8(*b*)]. In the case of **2**, a [DNA]:[Re] ratio of 10:1 (or smaller) causes degradation of the plasmid [lanes D–G, Fig. 8(*b*)] as a result of non-specific multiple cuts.

In order to establish the reactive species responsible for the photoinduced cleavage of the plasmid, the following experiments have been carried out. Irradiation of the plasmid pBR322 DNA in the presence of complex **1** under anaerobic

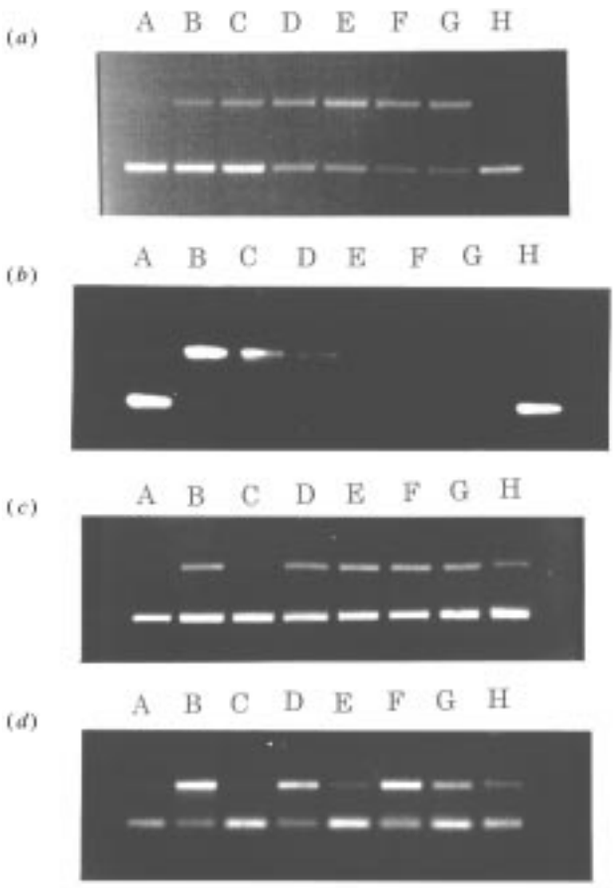


Fig. 8 0.8% Agarose gel showing the results of electrophoresis of pBR322 plasmid DNA (8.7 μmol dm^{-3}) photolysed for 30 min: (*a*) in the presence of complex **1** at 0 (A), 0.25 (B), 0.50 (C), 1.00 (D), 1.49 (E), 1.98 (F) and 2.45 μmol dm^{-3} (G), 2.45 μmol dm^{-3} in the dark (H); (*b*) in the presence of **2** at 0 (A), 0.22 (B), 0.43 (C), 0.87 (D), 1.30 (E), 1.73 (F) and 2.17 μmol dm^{-3} (G), 2.17 μmol dm^{-3} in the dark (H); (*c*) in the absence of complex (A); in the presence of 0.50 μmol dm^{-3} **1** (B), in the dark (C), in the presence of histidine (1.20 mmol dm^{-3}) (D); D_2O used instead of buffer (E); in the presence of mannitol (50.0 mmol dm^{-3}) (F), ethanol (1.70 mol dm^{-3}) (G), sodium formate (100.0 mmol dm^{-3}) (H); (*d*) in the absence of complex (A); in the presence of 0.22 μmol dm^{-3} **2** (B), in the dark (C), in the presence of histidine (1.20 mmol dm^{-3}) (D), D_2O used instead of buffer (E); in the presence of mannitol (50.0 mmol dm^{-3}) (F), ethanol (1.70 mol dm^{-3}) (G), sodium formate (100.0 mmol dm^{-3}) (H)

conditions by degassing the solution with purified nitrogen for 25 min does not cause appreciable changes (data not shown). It appears that oxygen is not involved in the photolytic cleavage. This finding is also demonstrated in Fig. 8(*c*). The cleavage of the plasmid is not inhibited in the presence of a singlet oxygen ($^1O_2$) scavenger, histidine[25] (1.20 mmol dm^{-3}) [lane D, Fig. 8(*c*)], as well as hydroxyl radical ($OH^{\bullet}$) quenchers such as mannitol[26a] (50.0 mmol dm^{-3}) [lane F, Fig. 8(*c*)], ethanol[26] (1.7 mol dm^{-3}) [lane G, Fig. 8(*c*)] and sodium formate[26b] (100.0 mmol dm^{-3}) [lane H, Fig. 8(*c*)]. Furthermore, no enhancement in photocleavage activity is observed for the reaction carried out in D_2O, in which singlet oxygen has a prolonged lifetime.[27] These findings suggest that the photoinduced cleavage is a consequence of the oxidation of the plasmid DNA biopolymer by the excited complex **1***, probably *via* oxidation at the guanine site.[6a–c,e]

In contrast, photocleavage of the plasmid pBR322 DNA is inhibited in degassed buffer solution for complex **2**. Superoxide anion radical ($O_2^{\bullet -}$) may be the reactive species as the cleavage is slightly inhibited in the presence of superoxide dismutase (SOD)[26] (1.0 μmol dm^{-3}) (data not shown), a facile superoxide radical quencher. The role played by reactive oxidants such as singlet oxygen and hydroxyl radical has been revealed by the following experiments.

In the presence of histidine (1.2 mmol dm^{-3}) [lane D, Fig. 8(*d*)] no noticeable inhibition in the cleavage activity of complex **2** is observed, indicating that singlet oxygen is not likely to be the cleaving agent. Besides, no enhancement but inhibition in the photocleavage of the plasmid DNA is observed for **2** in D_2O [lane E, Fig. 8(*d*)], which further confirms that singlet oxygen is not involved in the cleavage.

On the other hand, different degrees of inhibition in the photoinduced cleavage of the plasmid by complex **2** are observed in the presence of hydroxyl radical scavengers such as mannitol (50.0 mmol dm^{-3}) [lane F, Fig. 8(*d*)], ethanol (1.7 mol dm^{-3}) [lane G, Fig. 8(*d*)] and sodium formate (100.0 mmol dm^{-3}) [lane H, Fig. 8(*d*)]. This suggests that, in addition to superoxide anion radical, hydroxyl radical is likely to be one of the reactive species for the cleavage.

In conclusion, the complexes bind to the biopolymer by intercalation, with the planar diimine ligands stacked in between the base pairs of the DNA. It is this close proximity which renders cleavage of the DNA effective by the photoactivated complexes. The cleavage mechanism for **1** and pBR322 is likely to be direct oxidation of the DNA by the excited complex. For that between **2** and pBR322, the complex acts as an oxygen sensitizer and superoxide and hydroxyl radicals are likely to be the reactive species responsible for cleavage of the plasmid. Similar observations have also been reported for other ruthenium(II) and cobalt(III) systems.[28]

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