*Pure & Appl. Chem.*, Vol. 69, No. 4, pp. 767–772, 1997.
Printed in Great Britain.


# Photoinduced electron transfer between ruthenium complexes and nucleotides or DNA

John M. Kelly, [a] Martin M. Feeney,[a] Luc Jacquet,[a] Andrée Kirsch-De Mesmaeker,[b] Jean-Paul Lecomte.[b]

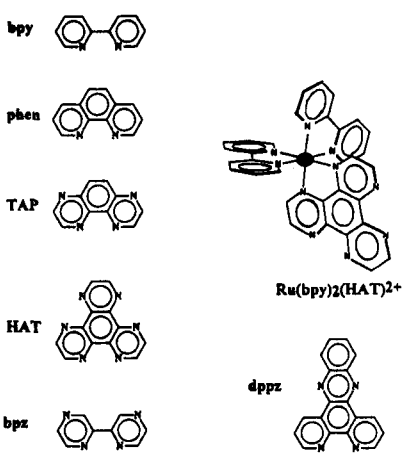
[a]*Chemistry Department, Trinity College, University of Dublin, Dublin 2, Ireland*
[b]*Chimie Organique Physique, CP160/08, Université Libre de Bruxelles, B1050 Brussels, Belgium*

*Abstract:* The quenching of the luminescence of $Ru(TAP)_3^{2+}$, $Ru(HAT)_2(bpy)^{2+}$ (TAP = 1,4,5,8-tetraazaphenanthrene; HAT = 1,4,5,8,9,12-hexaazatriphenylene; bpy = 2,2'-bipyridyl) and related oxidising complexes by DNA, polynucleotides, and purine nucleotides occurs by reductive electron transfer. Laser flash photolysis provides evidence for the formation of the reduced metal complex and the deprotonated nucleotide radical cation. This photo-oxidation leads to DNA strand-breaks and to the formation of covalent adducts with GMP or DNA. The adducts with $Ru(TAP)_3^{2+}$ or $Ru(HAT)_2(bpy)^{2+}$ are formed via a covalent bond between the C atom β to the coordinating N in the TAP or HAT ligand and the N2 of guanine.

## INTRODUCTION

The binding of ruthenium complexes to nucleic acids and especially to DNA has been extensively studied in the last decade (ref. 1-3). As a diverse range of complexes can be synthesised and as they possess convenient absorption and emission properties, these compounds offer many advantages as DNA probes. They have also been used for the study of possible long-range electron transfer processes in DNA (ref. 4).

In this paper we consider principally the properties of complexes containing the ligands 1,4,5,8-tetraaazaphenanthrene (TAP), 2,2'-bipyrazine (bpz), and 1,4,5,8,9,12-hexaazatriphenylene (HAT). The TAP complexes bind non-covalently to DNA in a manner analogous to the much studied $Ru(phen)_3^{2+}$ (ref. 5) - that is by partial insertion of one of the ligands between the base-pairs of DNA. The binding of the HAT complexes is much stronger, presumably because of a greater degree of insertion of this ligand. The electron-attracting ability of these ligands make their complexes more oxidising than related 2,2'-bipyridyl or 1,10-phenanthroline analogues and this enables a series of complexes $Ru(bpy)_nL_{3-n}^{2+}$ to be prepared whose excited state reduction potential $E^o(Ru^{2+*}, Ru^+)$ can vary by over 700 mV from that of $Ru(bpy)_3^{2+}$ (ref. 6).




## PHOTOPHYSICAL PROPERTIES AND EVIDENCE FOR ELECTRON TRANSFER

The absorption bands in the visible spectrum of the complex generally show a small red shift when the compound is bound to DNA, although the effect with the complexes $Ru(bpy)_2(HAT)^{2+}$ and $Ru(bpy)_2(TAP)^{2+}$ is more marked. Comparison of the absorption and emission spectra of $Ru(bpy)_2(HAT)^{2+}$ in different media suggests that these spectral changes upon binding to DNA are due to a combination of the changed environment of the complex and to an increased rigidity compared to aqueous solution (ref. 7, 8). Upon binding to DNA the emission of $Ru(bpy)2(HAT)^{2+}$ increases about three-fold (ref. 9). More recently the observation that the binding to DNA of $Ru(bpy)_2(dppz)^{2+}$ (dppz = dipyrido[3,2-a:2',3'-c]phenazine) increases its quantum yield by over a thousand-fold has led to the suggestion of its use as a "molecular light switch" for the detection of the bio-molecule (ref. 10). It has been conclusively demonstrated that in such complexes the dppz is intercalated between the base pairs of

DNA and that the dramatic increase in lifetime is principally due to its consequent protection from quenching by water (ref. 11).

A quite different behaviour is observed for the complexes containing two or three HAT or TAP ligands. In each of these cases the emission is quenched (ref. 6,12,13). Fig. 1 shows the behaviour of a series of $Ru(bpy)_n(TAP)_{3-n}^{2+}$ (n=0-3) complexes. Comparison of this behaviour with the redox behaviour of the excited states reveals that the complexes with the more oxidising excited states are quenched by DNA. A similar behaviour has been previously observed for various dyes, including phenothiazinium dyes such as methylene blue or thionine (ref. 14-16) and attributed to reductive quenching by the nucleobases, especially guanine. To test that this is the case here, experiments have been carried out with synthetic polynucleotides and nucleotides. Thus for all complexes where excited state quenching is observed with DNA, quenching is found for double stranded poly[d(G-C)].poly[d(G-C)]. For complexes such as $Ru(TAP)_3^{2+}$ the emission is enhanced when the complexes bind to poly[d(A-T)].poly[d(A-T)]; however the more oxidising complexes such as $Ru(HAT)_3^{2+}$ are quenched by this polynucleotide (ref. 17) This can be attributed to these latter complexes being able to oxidise the adenine base. Similar behaviour has been reported with strongly oxidising excited states of dyes such as thionine (ref. 16).

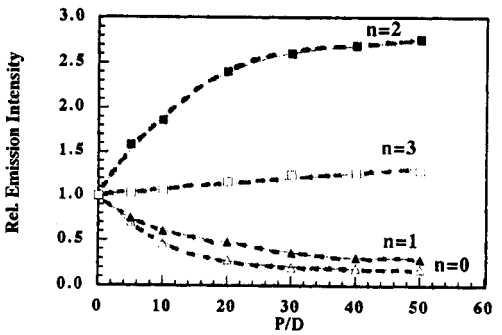



Fig. 1. Effect of DNA concentration on emission intensity of $Ru(bpy)_n(TAP)_{3-n}^{2+}$

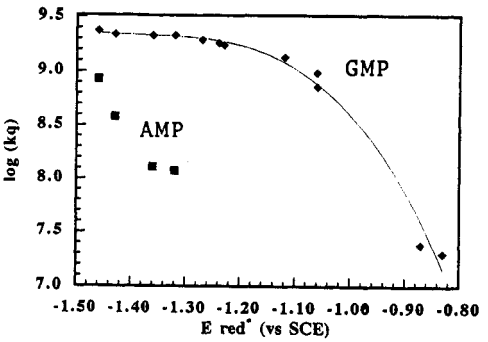



Fig. 2. Variation of $\log(k_q)$ with excited state reduction potential $E^o(Ru^{2+*}, Ru^+)$.

Studies with mononucleotides show analogous behaviour. In these cases dynamic quenching occurs, and linear Stern-Volmer plots are observed, allowing the determination of the quenching rate constant ($k_q$) (ref. 6,18). Figure 2 shows a plot of $\log(k_q)$ versus $E^o(Ru^{2+*}/Ru^+)$ for those nucleotides where quenching is observed. (No excited state deactivation is found with TMP or CMP). The $k_q$ value is observed to reach a maximum value of $2.2\pm 0.2 \times 10^9$ dm$^3$ mol$^{-1}$ s$^{-1}$ and to drop off for the less oxidising excited states, as expected for reductive quenching of the excited state by the purine nucleotide (equation 1). For the scheme in equation 1 the quenching rate constant $k_q$ is given by equation 2. The data in Fig. 2 have been modelled using the Marcus equation (ref 6), leading to a value for the oxidation potential of guanine in 5'-GMP of 0.92 V vs SCE (1.16 V vs NHE).

$$RuL_3^{2+*} + Q \underset{k_{-d}}{\overset{k_d}{\rightleftharpoons}} \{RuL_3^{2+} \cdots Q\} \xrightarrow{k_{el}} \{RuL_3^{+} \cdots Q^{+}\} \qquad (1)$$

$$k_q = k_d/(1+k_{-d}/k_{el}) \qquad (2)$$

Direct evidence for electron transfer has been obtained from laser flash photolysis studies (ref. 6,13, 18) as both the reduced ruthenium complex and oxidised GMP species can be observed. At pH = 7, the reduced ruthenium complex is expected to be protonated ($pK_a$ = 7.5 for $Ru(TAP)_3^+$) (equation 3) and the guanine moiety in the oxidised GMP to be deprotonated ($pK_a$ = 3.9) (19).

$$Ru(TAP)_2(TAP^{\bullet-})^+ + H^+ \rightarrow Ru(TAP)_2(TAPH^{\bullet})^{2+} \qquad (3)$$

$$G^{\bullet+}MP \rightarrow (G\text{-}H)MP^{\bullet} + H^{+} \quad (4)$$

## PHOTOCHEMICAL REACTIONS

An interesting feature of polypyridyl-type ruthenium complexes is that they can show a considerable range of photochemical reactions, acting as both oxidising and reducing agents and as efficient sensitisers for singlet oxygen (20). It is probable therefore that they can be used as DNA-modification agents and by suitable derivatisation (e.g. by attachment to an oligonucleotide) it should be possible to target such reactions to specific sites on DNA. Two particular classes of reaction which are of interest are the induction of strand breaks in the DNA and the formation of covalent adducts between the metal complex and the DNA.

*Photocleavage* It has been known for some years that ruthenium complexes such as $Ru(phen)_3^{2+}$ or $Ru(bpy)_3^{2+}$ can photosensitise strand breaks in DNA (ref. 9, 21-23). The reaction proceeds with relatively low quantum yields (1 - 7 x $10^{-6}$) and has both an oxygen-dependent and independent component (ref. 23). In aerated solution these sensitisers also cause damage to DNA which induces DNA strand scission upon treatment with organic bases - this damage occurs preferentially at guanine and is consistent with singlet oxygen attack. Monitoring of direct strand breaks is commonly performed with plasmid DNA, where the conversion of the covalently closed-circular form to the open circular form gives a measure of the number of strand breaks induced. DNA labelled with $^{32}P$ at either its 3'- or 5'- end is usually used to monitor the base-specificity of both direct and alkali-induced strand cleavage.

These techniques have been employed to monitor the reactivity of the series of complexes containing HAT and TAP ligands. Fig. 3 illustrates the relative effectiveness of the series $Ru(bpy)_n(TAP)_{3-n}^{2+}$ (n = 0-3) (ref. 6,13). It may be noted that those complexes, which have been shown by the photophysical measurements to photo-oxidise DNA, are much more efficient at causing the conversion of plasmid DNA to its open circular form. This suggests that the guanine radical cation is playing a role (in a fashion possibly similar to that proposed for DNA bases oxidised by ionizing radiation or high power laser excitation.) (ref. 24). The generality of this reaction has recently been extended by the comparison of the intercalating complexes $Ru(bpy)_2(dppz)^{2+}$ and the more strongly photo-oxidising $Ru(bpz)_2(dppz)^{2+}$ (ref 25).

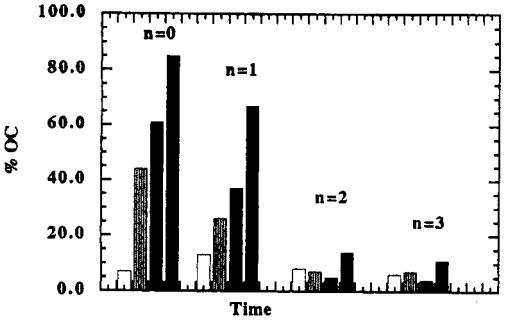



Fig. 3 Relative rates of creation of strand breaks induced in plasmid DNA by $Ru(bpy)_n(TAP)_{3-n}^{2+}$ (n=0-3) . Each sample irradiated (436 nm) for 0, 0.5, 1 and 3 min.

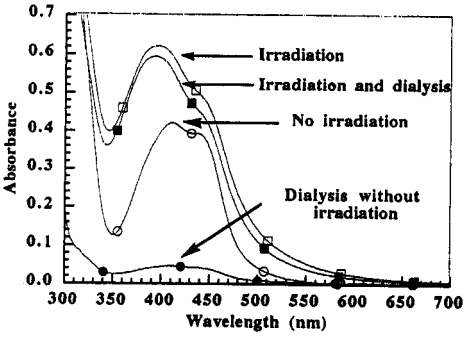



Fig. 4 Photoaddition of $Ru(TAP)_3^{2+}$ to DNA. Sample photolysed for 120 min with visible light, followed by dialysis, compared to unphotolysed sample.

*Photoadduct formation* In order to investigate these cleavage reactions in more detail short strands of DNA were $^{32}P$ 5'-end-labelled and sensitised by $Ru(TAP)_3^{2+}$. Surprisingly it was found that the dominant process was not strand scission, which would have led to shorter fragments with consequent higher electrophoretic mobility, but rather to production of a broad band of lower mobility than the starting DNA material (ref 13,26). This is consistent with the formation of an adduct between the metal complex and the DNA. Similar behaviour is found for $Ru(bpy)_n(L)_{3-n}^{2+}$ (n=0 or 1; L = TAP or HAT) but not for $Ru(bpy)_3^{2+}$ or $Ru(bpy)_2(L)^{2+}$.

If an adduct is indeed being formed it should be possible to monitor this process also by UV/visible spectrophotometry. Furthermore if the complex is covalently bound to the DNA then this can be verified by dialysis of the photolysed sample under conditions where the parent metal complex, but not the DNA, would pass through the dialysis membrane. Figure 4 shows the result of a typical experiment, which confirms that the metal complex is now bound to the DNA (ref 6,26). The spectrum of the adduct is quite different from that found when the complex is photolysed in the absence of DNA, where it is known that the complex undergoes photosubstitution and the loss of one of the chelating ligands (ref 27). The spectral bands of these products are markedly shifted to longer wavelengths, whereas in the adduct the bands are found at somewhat shorter wavelengths. This suggests that the metal is still coordinated to the three chelating ligands.

*Molecular structure of photoadducts with 5'-guanosine monophosphate* . Photolysis of $Ru(TAP)_3^{2+}$ (0.5 - 1 x $10^{-4}$ M) in the presence of GMP (1 x $10^{-2}$ M) can be readily monitored by changes in the UV/visible aborption spectrum. Although spectral changes depend strongly on solution aeration and vary with pH (ref 26), it was found that the product formed by photolysis with GMP at pH5 in deaerated solution had a spectrum similar to that found with DNA. Monitoring by HPLC showed that the maximum yield of the product occurred at pH 6. (By contrast to the behaviour with GMP the absorption spectrum of the photoproduct with DNA is not affected by solution aeration or pH in the range 5-9, presumably indicating that side reactions of the reduced ruthenium complex and the oxidised guanine moiety are suppressed when diffusion is restricted).

The reaction was scaled up [e.g. $Ru(TAP)_3^{2+}$ (0.5 - 1.2 x $10^{-3}$ M) in the presence of GMP (1 x $10^{-2}$ M)] so as to isolate the adduct. It was found that, by working in deaerated solutions at pH in the range 4-6, side reactions could be minimised, although even under these conditions some photodechelation was observed. The GMP-adduct was precipitated as the $PF_6$ salt, purified by ion exchange and HPLC chromatography and characterised by $^1H$ and $^{13}C$ NMR and electro-spray mass spectrometry. Alternatively the sugar and phosphate moieties can be removed by treatment with hot 1 M hydrochloric acid and the guanine-adduct isolated (ref. 28). The products show an unusual form of binding of a metal complex to DNA with the formation of a covalent bond between the N2 of guanine and the C2 position of the TAP ligand (Fig.5). In most other nucleic acid-metal complex compounds, such as those formed by the reaction with the anti-tumour drug cis-platin (ref. 29) or photochemically from $Rh(phen)_2Cl_2^+$ (ref. 30) there is direct coordination of the nucleo-base to the metal centre. Broadened peaks in the NMR indicate the presence of two conformer-tautomers as shown. The formation of these adducts can be ascribed to the combination of the $Ru(TAP)_2(TAPH)^{2+}$ and guanine radical $G(-H)^{\bullet}$ formed by the initial electron transfer (equations 2 and 3), and subsequent rearomatisation.

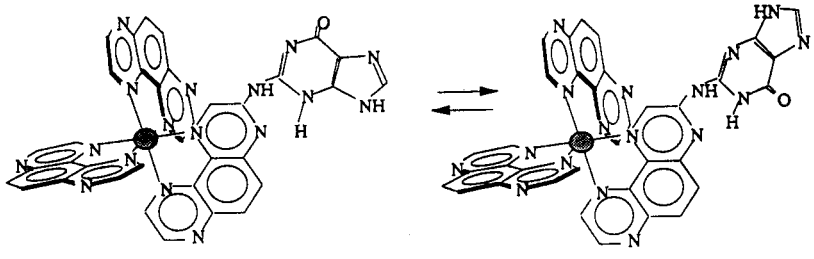


Fig. 5. Molecular structure of adduct formed from $Ru(TAP)_3^{2+}$ and GMP , subsequently treated with HCl to remove the ribose-phosphate (ref. 28).

$Ru(HAT)_2bpy^{2+}$ also forms photo-adducts with GMP. This complex has several advantages as a DNA probe as it interacts more strongly with DNA and is also less susceptible to photodechelation. The adduct isolated by HPLC was shown by NMR to consist of two geometrical isomers, in both of which a covalent bond has been formed between the N2 of GMP and the C β to the coordinated N in the HAT ligand (ref. 31). The formation of these two geometrical isomers is a consequence of the lower symmetry of the complex. Adducts are also formed between $Ru(bpz)_3^{2+}$ and GMP (ref. 31). In this case HPLC indicate the presence of two adducts with different retention times and somwhat different absorption spectra. It is probable that these two adducts are a consequence of the bond beween the N2 of the guanine and the C3 or C5 positions of the 2,2'-bipyrazine.

Fig. 6. Geometrical isomers of the photoadduct formed between $Ru(HAT)_2(bpy)^{2+}$ and 5'-GMP. (The ribose-phosphate is not shown.)

Work is currently in progress to isolate the adducts formed between $Ru(HAT)_2(bpy)^{2+}$ and calf thymus DNA. HPLC studies show that treatment of a DNA sample containing adducts with DNase1, S1-endonuclease and phosphodiesterases produces a complex with properties identical to those of the $Ru(HAT)_2(bpy)^{2+}$-dGMP complex and it is hoped to obtain sufficient quantitities for NMR characterisation in the near future (ref. 32).

## Conclusions

Ruthenium polypyridyl complexes are finding applications in molecular biology both for monitoring the presence of DNA and as probes for its structure. It is clear from the data so far obtained that the photochemical reactions of this class of metal complex, especially those with strongly oxidising excited states, could also have considerable potential in enabling the modification of the chemical and biological processes in DNA. The formation of stable covalent adducts between molecules such as $Ru(TAP)_3^{2+}$ and nucleic acids, coupled with the known anti-tumour activity of other metal complexes, offers the possibility of exploiting these compounds as phototherapeutic agents.

## ACKNOWLEDGEMENTS

The authors thank the European Union for support under the Human capital and Mobility programme (Grants No ERBCHB1CT 941117 and CHRX-CT92-0016).

## REFERENCES

1. A. Kirsch-De Mesmaeker, J-P. Lecomte and J.M. Kelly. *Topics Curr. Chem.* **177**, 25 (1996).
2. A.M. Pyle and J.K Barton. *Progr. Inorg. Chem.*. **38**, 413 (1990).
3. B. Norden, P. Lincoln, B. Akerman and E.Tuite. In *Metal Ions in Biological Systems* (A. Sigel and H. Sigel, ed.) pp. 177-252, Decker, New York (1996).
4. E.D.A. Stemp and J.K. Barton. In *Metal Ions in Biological Systems* (A. Sigel and H. Sigel, ed.) pp. 177-252, Decker, New York (1996).
5 S. Satyanarayana, J.C. Dabrowiak and J.B. Chaires. *Biochemistry* **32**, 2573 (1993)
6. J-P. Lecomte, A. Kirsch-De Mesmaeker, M. M. Feeney and J. M. Kelly. *Inorg. Chem.* **34**, 6481 (1995),
7. F. de Buyl, A. Kirsch-De Mesmaeker, A.B. Tossi and J. M. Kelly. *J. Photochem. Photobiol. A: Chem.*. **60**, 27 (1991)
8. J.P. Lecomte, A. Kirsch-De Mesmaeker and G. Orellana. *J. Phys. Chem.* **98**, 5382 (1994).
9. A.B. Tossi and J. M. Kelly, *Photochem. Photobiol.*, **49**, 545 (1989).
10. A.E. Friedman, J-C. Chambron, J.-P. Sauvage, N.J. Turro and J.K. Barton *J.Am.Chem.Soc.* **112**, 4960 (1990)
11. C. Hiort, P. Lincoln and B. Norden, *J.Am.Chem.Soc.* **115**, 3448 (1993)

12. J. M. Kelly, D.J. McConnell, C. OhUigin, A.B. Tossi, A. Kirsch-De Mesmaeker, A. Masschelein and J. Nasielski, *J.Chem. Soc., Chem. Comm.*, 1821 (1987)
13. J. M. Kelly, M.M. Feeney, A.B. Tossi, J.P. Lecomte and A. Kirsch-De Mesmaeker, *Anti-Cancer Drug Design* **5,** 69 (1990).
14. L. Kittler, G. Löber, F. Gollmick and H. Berg. *J. Electroanal. Chem.*. **116,** 503 (1980).
15. J.M. Kelly, W.J.M. van der Putten and D.J. McConnell. *Photochem. Photobiol.* **45,** 167 (1987).
16. E. Tuite, J. M. Kelly, G. S. Beddard and E. Reid. *Chem. Phys. Lett.* **226,** 517 (1994)
17. A. Kirsch-De Mesmaeker, G. Orellana, J.K. Barton and N.J. Turro. *Photochem. Photobiol.* **52,** 461 (1990).
18. J.P. Lecomte, A. Kirsch-De Mesmaeker, J. M. Kelly, A.B. Tossi and H. Görner. *Photochem. Photobiol.* **55,** 681 (1992).
19. L.P. Candeias and S. Steenken. *J.Am..Chem.Soc.* **11,** 1094 (1989).
20. L. Tan Sien Hee, L. Jacquet and A. Kirsch-De Mesmaeker, *J. Photochem. Photobiol. A: Chem.* **81,** 169 (1994)
21. J. M. Kelly, A.B. Tossi, D.J. McConnell and C. OhUigin. *Nucl. Acids. Res.* **13,** 6017 (1985).
22. M.B. Fleisher, K.C. Waterman, N.J. Turro and J.K. Barton. *Inorg, Chem.* **25** , 3549 (1986)
23. A. Aboul-Enein and D. Schulte-Frohlinde. *Photochem. Photobiol.* **48,** 27 (1988).
24. H. Görner. *J. Photochem. Photobiol. B: Biol*, **26,** 115 (1994)
25. C. Sentagne, J.C. Chambron, J.P. Sauvage and N. Paillous, *J. Photochem. Photobiol. B: Biol* . **26,** 165 (1994)
26. M.M. Feeney, J.M. Kelly, A. Kirsch-De Mesmaeker, J.-P. Lecomte and A.B. Tossi. *J. Photochem. Photobiol. B: Biol*, **23,** 69 (1994)
27. J-P. Lecomte, A. Kirsch-De Mesmaeker, and J. M. Kelly.*Bull. Soc.Chim Belge,* **103,** 193 (1994).
28. L. Jacquet, J. M. Kelly, and A. Kirsch-De Mesmaeker. *J.Chem. Soc., Chem. Comm.*, 913 (1995)
29. J. Reedijk. *J.Chem. Soc., Chem. Comm.*, 801 (1996)
30. R.E. Mahnken, M.A. Billadeau, E.P. Nikonowicz and H. Morrison. *J.Am. Chem. Soc.,* **1992,** *114*, 9253.
31. L. Jacquet, J.M. Kelly and A. Kirsch-De Mesmaeker, manuscript in preparation
32. R.J.H. Davies, L. Jacquet and J.M. Kelly, unpublished work.