Photoadduct between Tris(1,4,5,8-tetraazaphenanthrene)ruthenium(II) and Guanosine Monophosphate—a Model for a New Mode of Covalent Binding of Metal Complexes to DNA

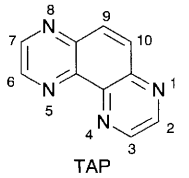
Luc Jacquet,[a] John M. Kelly*[a] and Andrée Kirsch-De Mesmaeker*[b]†

[a] Chemistry Department, Trinity College, Dublin 2, Ireland
[b] Chimie Organique Physique, CP160/08, Université Libre de Bruxelles, B-1050 Bruxelles, Belgium

Visible light irradiation of $[Ru(TAP)_3]^{2+}$ (TAP = 1,4,5,8 -tetraazaphenanthrene) in aqueous solutions containing 5′-guanosine-monophosphate (GMP) and subsequent treatment with 1 mol dm^{-3} HCl yields $Ru(TAP)_2(2\text{-}TAP\text{-}G)^{2+}$, in which the guanine moiety is bound *via* N-2 to C-2 of one of the TAP ligands.

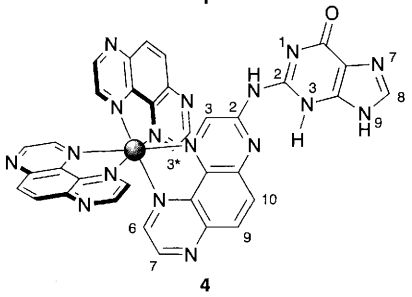
The formation of metal complex–DNA adducts is expected to have a significant effect on the biological function of DNA as has been extensively demonstrated with $Pt(NH_3)_2Cl_2$ and related compounds, which are widely used as antitumour drugs.[1,2] However, there have been few reports of light-induced adduct formation between metal complexes and DNA. One example is that of the complexation of $[Rh(phen)_2Cl_2]^+$ to guanine on irradiation,[3] the mechanism involving loss of the chloride ligand and subsequent coordination of the metal directly to a nucleobase. Here we present structural evidence for a new kind of photochemical adduct between a metal complex and nucleotide in which the binding of the metal complex $[Ru(TAP)_3]^{2+}$ proceeds through covalent binding of the guanine to one of the tetraazaphenanthrene (TAP) ligands **1**. The formation of this compound is proposed to be a consequence of initial electron transfer from the nucleobase (guanine) to the metal complex excited state.

Despite the extensive study of polypyridyl complexes as photophysical probes for DNA and as photosensitisers for strand cleavage in DNA,[4–7] it is only in the case of $[Ru(TAP)_3]^{2+}$ and related compounds that there is evidence for adduct formation.[8] Unlike complexes such as $[Ru(phen)_3]^{2+}$, the excited state of $[Ru(TAP)_3]^{2+}$ is sufficiently oxidising to abstract an electron from guanine in DNA.[6,9,10] The resulting radical cation of guanine has been proposed as responsible for the more efficient induction of single-strand breaks in DNA photosensitised by $[Ru(TAP)_3]^{2+}$,[6,8b,11] compared to $[Ru(phen)_3]^{2+}$, $[Ru(bpy)_3]^{2+}$ and related complexes which proceed by different mechanisms.[12] The evidence for adduct formation between $[Ru(TAP)_3]^{2+}$ and DNA comes from electrophoresis of the photoproducts formed with oligonucleotides,[8] and, more recently UV–VIS spectroscopic measurements with calf thymus DNA .[8c] The similar spectroscopic changes found with DNA and with 5′-guanosine-monophosphate (GMP) **2** in slightly acidic deoxygenated solution suggest that the adduct is formed between the metal complex and guanine.



TAP

1



4

A solution (40 ml) of $[Ru(TAP)_3Cl_2]$ (1.4×10^{-3} mol dm^{-3}) and Na_2GMP (2×10^{-2} mol dm^{-3}) was adjusted to pH 4.5 and illuminated for 3 d in a Pyrex photoreactor, under continuous stirring and Ar bubbling, using a 125 W medium-pressure mercury lamp. The evolution of the photoreaction was followed simultaneously by absorption spectroscopy and analytical HPLC. As found previously for dilute solutions,[8c] the maximum of the broad visible band of the photolyte shifted progressively from 407 to 393 nm upon irradiation. In HPLC the dominant species observed showed an absorption maximum at *ca.* 390 nm. The photoadduct was separated from excess GMP and ruthenium-containing side-products‡ by cation exchange chromatography. As attempts to isolate the zwitterionic nucleotide photoproduct **3** from the resulting salt-containing eluant solution were unsuccessful, this salt solution of the photoproduct was heated for 1 h at 60 °C in 1 mol dm^{-3} HCl to form the nucleobase derivative **4**, which was isolated as its PF_6 salt (non-optimised overall yield 25%). The spectrum of the photoadduct was recorded at pH 5.5 after isolation [λ_{max} = 390, 440 (sh) nm. This had features similar to the spectrum obtained upon HPLC analysis of the photolyte, showing an important hyperchromic and hypsochromic shift (17 nm) of the maximum compared to $[Ru(TAP)_3]^{2+}$

The electrospray mass spectra (ESMS) of the adduct PF_6 salt of **4** after purification showed strong peaks at *m/z* = 398.5 (100%) and *m/z* = 942.5 (4%), corresponding respectively to 0.5 $(M - 2H - 2PF_6)^{2+}$, and $(M - 2H - PF_6)^+$, where M = $[Ru(TAP)_3(PF_6)_2$ + guanine]. In each case the theoretical isotopic profile is observed. These data prove clearly that the guanine moiety has added to the complex without loss of a TAP ligand. In agreement with this, the IR spectra showed bands characteristic of both the coordinated TAP and guanine.

Although the adduct **4** is rather insoluble in most usual NMR solvents, its solubility in $(CD_3)_2SO$ or in a 60 : 40 CD_3CN–D_2O mixture is sufficient to allow the 600 MHz ^1H, ^1H–^1H and ^1H–^{13}C COSY spectra to be recorded; the concentration, however, was below that required for ^{13}C spectra. Fig. 1 shows the spectrum in $(CD_3)_2SO$. The absence of protons below δ 7.5 confirms the successful removal of the ribose moieties and the rearomatisation of the substituted TAP (consistent with the loss of 2H detected by ESMS). The absence of one of the 2-protons in this modified ligand allows the identification of the position of connection of the guanine as β to the complexing N of the pyrazinic ring. The asymmetry induced by the substitution of one of the TAP protons by the guanine group causes shifts of the remaining TAP proton signals compared to $[Ru(TAP)_3]^{2+}$; principal changes are: (i) the ^1H ($\Delta\delta$ − 0.6) and its correlated ^{13}C signal ($\Delta\delta$ − 12) of the C–H in position 3 (α to the substituted position), and (ii) the ^1H ($\Delta\delta$ − 0.2) and ^{13}C ($\Delta\delta$ − 2) signals of the C–H in the 7 and 10 positions. There is also a strong ring current effect on the H3 proton (labelled* in **4**) of one of the other TAP ligands, which points towards the 2-TAP-G ligand. The 5 ppm downfield shift of the NH signal in the 2-TAP-G ligand compared to the NH_2 signal of the guanine (at

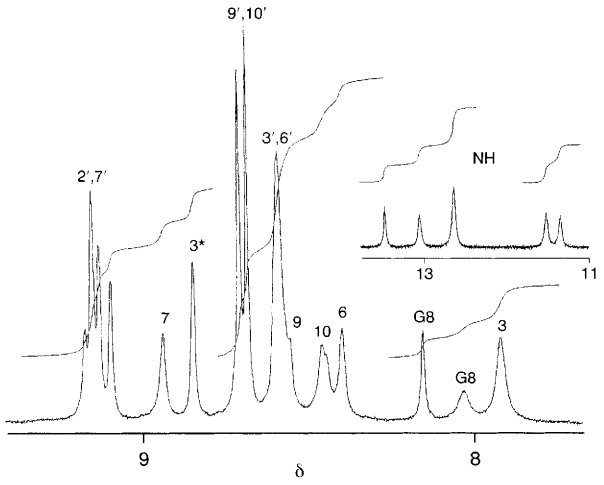


Fig. 1 600 MHz ^{1}H NMR spectrum of adduct **4** in $(CD_3)_2SO$ (numbered: 2–7, G8 in TAP-G; 2′–7′ in non-modified TPA; 3* see text). *Insert*: spectra of the exchangeable protons.

δ 6.5), is in agreement with the connection of the guanine to the TAP through this NH_2.

The broadening of the TAP peaks close to the point of connection and the splitting of the guanine H-8 proton into two *ca.* 50% contributions (δ 8.03 and 8.15, assigned by correlation with their ^{13}C signal)§ suggests the presence of a dynamic process in the photoadduct, most probably caused by a slow rotation of the guanine moiety about the linking N atom. A similar proposal has been made for the adduct between nitrobenzo[α]pyrene and GMP (where the moieties are also linked through the guanine N-2).[13] In that case the energy barrier was found to be high enough to cause the splitting of the GMP proton signals at low temperature and their broadening beyond recognition as the temperature is raised. The behaviour of **4** indicates a higher energy barrier than that found in the nitrobenzo[α]pyrene adduct, probably because of (i) the possibility of an H-bond between TAP N1 and the N1 or N3 of guanine, and (ii) the enhancement of the sp^2 character of the N bridging the TAP and guanine group owing to the strong electron-acceptor character of the $[Ru(TAP)_3]^{2+}$ moiety.

In conclusion, the above NMR and ESMS studies indicate that photolysis of $[Ru(TAP)_3]^{2+}$ in the presence of GMP yields a complex in which the guanine is covalently linked to the heterocyclic ligand by the N2 of the guanine. The reaction is proposed to proceed by initial oxidation of the guanine by the metal complex excited state [eqn. (1)], subsequent proton transfer [eqn. (2)] (both these processes have been verified by laser flash photolysis[10]) and a subsequent coupling of the radicals so formed [eqn. (3)]. The product must then re-aromatise by loss of two hydrogen atoms to give the final product [eqn. (4)]

$$[Ru(TAP)_3]^{2+*} + GMP^{2-} \rightarrow \{[Ru(TAP)_2(TAP^{\cdot -})]^{+}, GMP^{\cdot -}\} \quad (1)$$

$$\{[Ru(TAP)_2(TAP^{\cdot -})]^{+}, GMP^{\cdot -}\} \rightarrow \{[Ru(TAP)_2(TAPH^{\cdot})]^{2+}, G(-H)MP^{\cdot 2-}\} \quad (2)$$

$$\{[Ru(TAP)_2(TAPH^{\cdot})]^{2+}, G(-H)MP^{\cdot 2-}\} \rightarrow [Ru(TAP)_2(2\text{-}TAPH_2\text{-}GMP)] \quad (3)$$

$$[Ru(TAP)_2(2\text{-}TAPH_2\text{-}GMP)] \rightarrow [Ru(TAP)_2(2\text{-}TAP\text{-}GMP)] \quad (4)$$

Experiments are in progress to isolate the nucleotide adducts formed in DNA and to determine the structure of adducts formed with other purine nucleotides.

The authors thank the EU for a Human Capital and Mobility grant, and Professor R. J. H. Davies, Professor T. B. H. McMurry and Dr J.-P. Lecomte for helpful discussions, Dr John O'Brien and Claude Marschal for the NMR measurements and Drs Alain van Dorsselaer and A. Dupont for electrospray mass spectroscopy. The Communauté Française de Belgique is also gratefully acknowledged for its financial support (ARC 91/96-149).

Received, 21st December 1994; Com. 4/07782B

Footnotes

† Director of Research at the National Fund for Scientific Research, Belgium.

‡ At the GMP concentration used, only *ca.* 70% of the excited states are quenched and there is therefore competition with the photodechelation of the complexes.[14] The resulting mixture of $Ru(TAP)_2$ compounds is readily separated on a Sephadex SP C-25 column (10 × 150 mm) using NaCl (0.03 mol dm^{-3}) as eluent.

§ The exchangeable protons (verified by addition of D_2O) are also split at 25 °C, [11.34 (0.5H), 11.51 (0.5H), 12.62 (2 × 0.5H), 13.04 (0.5H), 13.45 (0.5H)] (insert on Fig. 1). When the temperature is raised to 100 °C all these signals broaden further.

References

1 W. L. Sundquist and S. J. Lippard, *Coord. Chem. Rev.*, 1990, **100**, 293.
2 B. K. Keppler, *Metal Complexes in Cancer Chemotherapy*, Verlag Chemie, Weinheim, 1993.
3 R. E. Mahnken, M. Bina, R. M. Diebel, K. Luebke and H. Morrison, *Photochem. Photobiol.*, 1989, **49**, 519; R. E. Mahnken, M. A. Billadeau, E. P. Nikonowicz, and H. Morrison, *J. Am. Chem. Soc.*, 1992, **114**, 9253.
4 N. J. Turro, J. K. Barton and D. A. Tomalia, *Acc. Chem. Res.*, 1991, **24**, 332.
5 C. Hiort, P. Lincoln and B. Nordén, *J. Am. Chem. Soc.*, 1993, **115**, 344.
6 A. B. Tossi and J. M. Kelly, *Photochem. Photobiol.*, 1989, **49**, 545.
7 S. Satyanarayana, J. C. Dabrowiak and J. B. Chaires, *Biochemistry* 1993, **32**, 2573
8 (*a*) J. M. Kelly, A. B. Tossi, D. J. McConnell, C. OhUigin, C. Hélène and T. LeDoan, in *Free Radicals, Metal Ions and Biopolymers*, ed. P. C. Beaumont, D. Deeble, B. Parsons and C. Rice-Evans, Richelieu Press, London, 1989, pp. 143–156; (*b*) J. M. Kelly, M. M. Feeney, A. B. Tossi, J.-P. Lecomte and A. Kirsch-De Mesmaeker, *Anti-cancer Drug Design*, 1990, **5**, 69; (*c*) M. M. Feeney, J. M. Kelly, A. B. Tossi, A. Kirsch-De Mesmaeker and J.-P. Lecomte, *J. Photochem. Photobiol. B: Biol.*, 1994, **23**, 69.
9 A. Kirsch-De Mesmaeker, G. Orellana, J. K. Barton and N. J. Turro, *Photochem. Photobiol.*, 1990, **52**, 461.
10 J.-P. Lecomte, A. Kirsch-De Mesmaeker, J. M. Kelly, A. B. Tossi and H. Görner, *Photochem. Photobiol.*, 1992, **55**, 681.
11 J. M. Kelly, D. J. McConnell, C. OhUigin, A. B. Tossi, A. Kirsch-De Mesmaeker, A. Masschelein and J. Nasielski, *J. Chem. Soc., Chem. Commun.*, 1987, 1821.
12 J. M. Kelly, A. B. Tossi, D. J. McConnell and C. OhUigin, *Nucl. Acids. Res.*, 1985, **13**, 6017; M. B. Fleisher, K. C. Waterman, N. J. Turro and J. K. Barton, *Inorg. Chem.*, 1986, **25**, 3549; A. Aboul-Enein and D. Schulte-Frohlinde, *Photochem. Photobiol.*, 1988, **48**, 27.
13 F. E. Evans, J. Deck, D. Herreno-Saenz and P. P. Fu, *Mag. Res. Chem.*, 1993, **31**, 531.
14 A. Kirsch-De Mesmaeker, D. Maetens and R. Nasielski-Hinkens, *J. Electroanal. Chem.*, 1985, **182**, 123; A. Masschelein, L. Jacquet, A. Kirsch-De Mesmaeker and J. Nasielski, *Inorg. Chem.*, 1990, **29**, 855.