Ruthenium Polypyridyl Complexes; Their Interaction with DNA and Their Role as Sensitisers for its Photocleavage

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The excited state of the 1,4,5,8-tetra-azaphenanthrene complex, $[Ru(TAP)_3]^{2+}$, which unlike $[Ru(phen)_3]^{2+}$ (phen = 1,10-phenanthroline) or $[Ru(bipy)_3]^{2+}$ (bipy = 2,2′-bipyridyl) is strongly quenched upon binding to poly[d(G–C)], is found to be much more effective than either $[Ru(phen)_3]^{2+}$ or $[Ru(bipy)_3]^{2+}$ in causing cleavage of the DNA backbone.

The use of photophysical measurements and of selective photochemistry of metal complexes when bound to DNA promises to be a very useful approach to the study of nucleic acids. Recently, there has been considerable interest in characterising the binding of ruthenium polypyridyl complexes with DNA.[1—6] Surprisingly, it has been found that complexes such as $[Ru(phen)_3]^{2+}$† cause unwinding and stabilisation of the DNA double helix which is consistent with intercalation of one ligand between its base pairs.[2,3] Unlike that found with ethidium bromide and other typical intercalators, however, the binding of $[Ru(phen)_3]^{2+}$ is highly salt dependent.[3,5] A high degree of stereoselectivity of binding has also been reported for this and the analogous 4,7-diphenyl-1,10-phenanthroline complex.[6] Excited state emission from $[Ru(phen)_3]^{2+}$ and $[Ru(bipy)_3]^{2+}$† is enhanced upon binding to DNA and to the synthetic polynucleotides poly[d(G–C)] or poly[d(A–T)].[3,4] These complexes are also known to sensitise photocleavage of DNA by visible light[3] although with low quantum efficiency.[7]

We report here that the closely related 1,4,5,8-tetra-azaphenanthrene complex $[Ru(TAP)_3]^{2+}$,[8] which also appears to intercalate,[9] shows quite different photophysical and photochemical behaviour from $[Ru(phen)_3]^{2+}$ or $[Ru(bipy)_3]^{2+}$

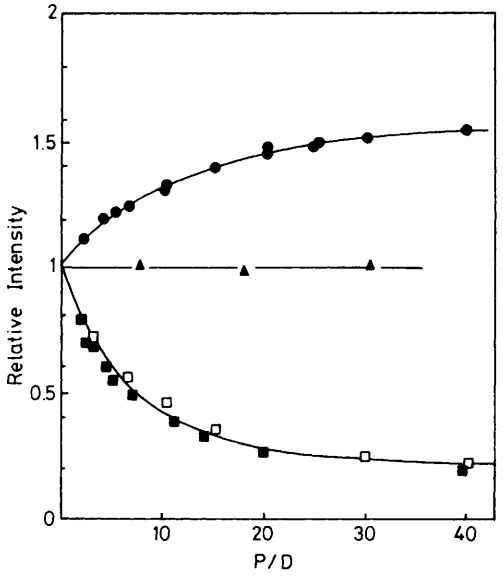

Figure 1. The relative emission intensity of $[Ru(TAP)_3]^{2+}$ at 600 nm as a function of the DNA phosphate to $[Ru(TAP)_3]^{2+}$ concentration ratio (P/D). $[Ru(TAP)_3]^{2+}$ concentration, 2×10^{-6} M in air saturated phosphate buffer (3 mM) at 22 °C with (●) poly[d(A–T)], (■) poly[d(G–C)], (□) calf thymus DNA, and (▲) poly[d(A–T)]–poly[d(G–C)] (1 : 1). (λ_{exc} 450 nm).

† phen = 1,10-phenanthroline; bipy = 2,2′-bipyridyl; TAP = 1,4,5,8-tetra-azaphenanthrene; poly[d(G–C)] = poly(deoxyguanylate–deoxycytidylate); poly[d(A–T)] = poly(deoxyadenylate–deoxythymidylate).

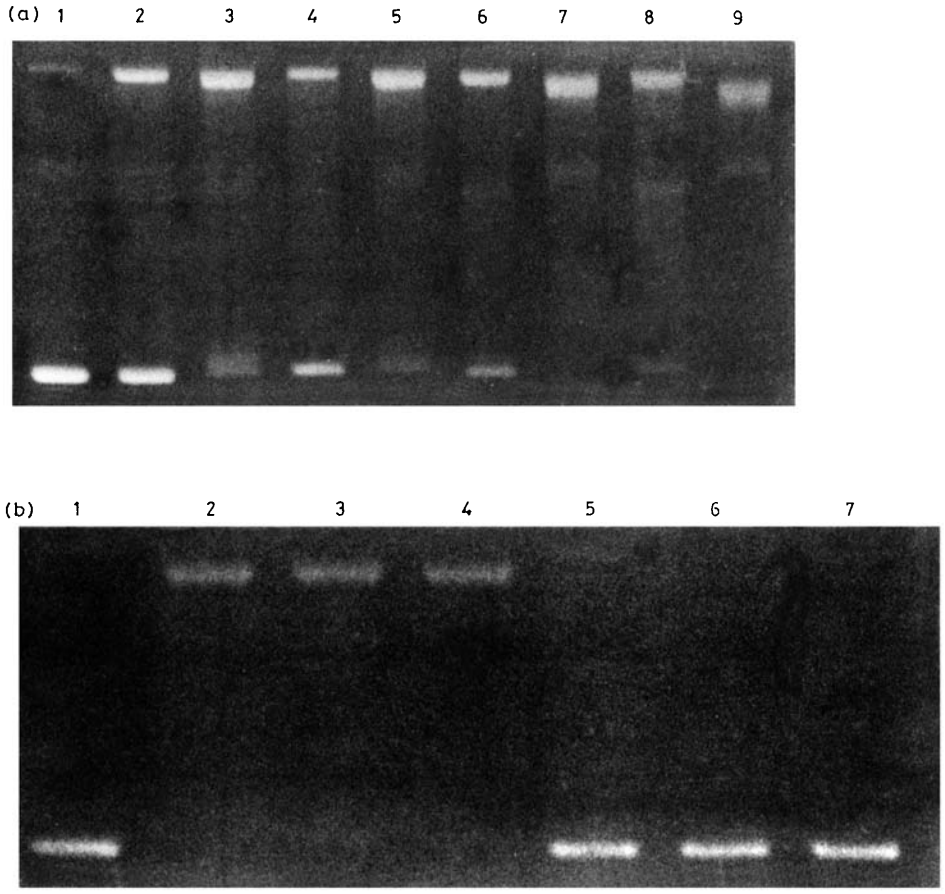

Figure 2. Gel electrophorograms of ruthenium polypyridyl compound-sensitised cleavage of pBR322 ccc-DNA. (a) Comparison of the efficiency of $[Ru(TAP)_3]^{2+}$ (**1**) and $[Ru(phen)_3]^{2+}$ (**2**). 20 μl samples containing $[RuL_3]^{2+}$ (10^{-5} M) and DNA (10^{-4} M) in tris (1 mM)–EDTA (0.1 mM) buffer [tris = $(CH_2OH)_3NMe$, EDTA = ethylenediaminetetra-acetic acid] were irradiated with 488 nm Ar ion laser (200 mW) radiation. Lane 1, before irradiation; irradiation for 5s, 10s, 20s, and 40s of (**1**) samples, lanes 3,5,7,9, respectively, and of (**2**), lanes 2,4,6,8, respectively.

(b) The effect of magnesium ions on reaction efficiency of $[Ru(TAP)_3]^{2+}$ (**1**), $[Ru(phen)_3]^{2+}$ (**2**) and $[Ru(bipy)_3]^{2+}$ (**3**). Lane 1, before 488 nm (200 mW) radiation; lanes 2—4, 20s irradiation in the presence of (**2**), (**3**), or (**1**), respectively; lanes 5—7, irradiation in the presence of 20 mM Mg^{2+} and (**2**), (**3**), or (**1**), respectively.

when bound to DNA.‡ Figure 1 contrasts the marked quenching of $[Ru(TAP)_3]^{2+}$ emission in the presence of poly[d(G–C)] and calf thymus DNA, with the enhancement of luminescence intensity for poly[d(A–T)]. Lifetime measurements confirm these observations.§ The reduced lifetime when bound to G–C residues may be attributed to photo-oxidation of the guanine [E^0 1.53 V *vs.* standard hydrogen electrode (s.h.e.)][10] by the strongly oxidising $[Ru(TAP)_3]^{2+}$ excited stated (E^{0*} *ca.* 1.62 V *vs.* s.h.e.).[11]¶ A similar explanation has been postulated for analogous behaviour with certain organic dyes.[10] However, dye laser flash photolysis (λ_{exc} 450 nm; τ *ca.* 15 ns) of solutions of $[Ru(TAP)_3]^{2+}$ in the presence of poly[d(G–C)], has as yet failed to reveal any $[Ru(TAP)_3]^{+}$ (λ_{max} 490 nm).[12] This probably indicates that the reverse electron transfer is very rapid (<50 ns).

The effectiveness of $[Ru(TAP)_3]^{2+}$ as a sensitiser for the photocleavage [using 436 nm (Hg lamp) or 488 nm (Ar ion laser)] of the phosphodiester backbone of DNA was investigated by monitoring the conversion of the covalently-closed-circular (ccc) form of pBR322 plasmid DNA to its open circular (oc) form. Figure 2a shows the gel electrophoresis trace for samples of $[Ru(phen)_3]^{2+}$ and $[Ru(TAP)_3]^{2+}$ exposed to identical doses of 488 nm light. It may be observed that for similar radiation doses the conversion of the ccc form into the oc form is much less for the sample containing $[Ru(phen)_3]^{2+}$ than for that containing $[Ru(TAP)_3]^{2+}$. Conversion of the ccc form into the oc form indicates that single strand cleavage has taken place at one location or more.

Possible roles for the complex's excited state are direct reaction with DNA or the production of singlet oxygen or some other reactive intermediate. It has been possible to demonstrate that strand scission requires the $[RuL_3]^{2+}$ complex to be bound to the DNA by studying the effect of Mg^{2+}, which is known at low concentrations to remove the $[RuL_3]^{2+}$ from the DNA.[3,5] It may be observed (Figure 2b) that conversion of ccc-DNA into oc-DNA is suppressed by the

‡ All these studies were carried out in phosphate buffer (3 mM), the ionic strength of which did not significantly inhibit the binding of these complexes to DNA.[3]

§ The lifetime of $[Ru(TAP)_3]^{2+*}$, in aerated phosphate buffer (3 mM), is 220 ns. Preliminary studies indicate that in the presence of poly[d(A–T)], one component of the decay has a lifetime of 600 ns, whereas in the presence of poly[d(G–C)] decay components with lifetime of 38 ns and <3 ns are detected.

¶ E^0 values taken in MeCN (*c.f.* $[Ru(bipy)_3]^{2+*/+}$ 1.01 V). Cyclic voltammetry measurements of $[Ru(TAP)_3]^{2+}$ in water were not possible because of irreversible reduction.

presence of 20 mM-Mg^{2+}. Singlet oxygen produced in bulk solution is thus incapable of effecting DNA strand breaks,[13] indicating a direct reaction of the bound complex with the DNA. It may be argued, at least for $[Ru(TAP)_3]^{2+}$, that this direct reaction is linked to the observed quenching of the excited state at G–C centres. In this case, selective cleavage of the DNA backbone at readily-oxidised guanine might be expected. Using ^{32}P-labelled DNA[14] we found that all three ruthenium complexes sensitise cleavage and the formation of alkali-labile sites, predominantly at guanine. The lower efficiency of $[Ru(phen)_3]^{2+}$ and $[Ru(bipy)_3]^{2+}$ in cleaving the plasmid DNA may be due either to a less efficient photo-redox process or to another (as yet unknown) mechanism.

In conclusion, the obervations reported here indicate that by appropriate choice of ligands it is possible to tune the photophysical and photochemical properties of the complexes in such a way as to cause markedly different types of excited state interaction with DNA. This holds promise that photochemical methods can be developed to cause the selective modification of DNA, which may have multiple and useful applications in areas such as DNA sequencing, DNA deactivation, and phototherapy.

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