

Dipyridophenazine Complexes of Cobalt(III): DNA Photocleavage and Photobiology

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The UV-visible spectroscopy and photochemistry of $[\text{Co}(\text{en})_2(\text{DPPZ})](\text{ClO}_4)_3$ (DPPZ = dipyrido[3,2-*a*:2',3'-*c*]-phenazine) in the presence of plasmid DNA and the nucleoside 2'-deoxyguanosine have been investigated. Evidence for the intercalation of the complex with DNA and photoinduced DNA strand breakage is found. The structurally related complexes $[\text{Co}(\text{en})_2(\text{DPPN})]\text{Cl}_3$ and $[\text{Co}(\text{en})_2(\text{DPPA})]\text{Cl}_2$, where DPPN = benzo[*i*]dipyrido[3,2-*a*:2',3'-*c*]phenazine and DPPA = dipyrido[3,2-*a*:2',3'-*c*]phenazine-11-carboxylic acid, have also been synthesized and characterized. In vitro cytotoxicity studies and photocytotoxicity studies of the complexes using the C6 rat glioma cell line are reported and indicate significant increases in toxicity following irradiation.

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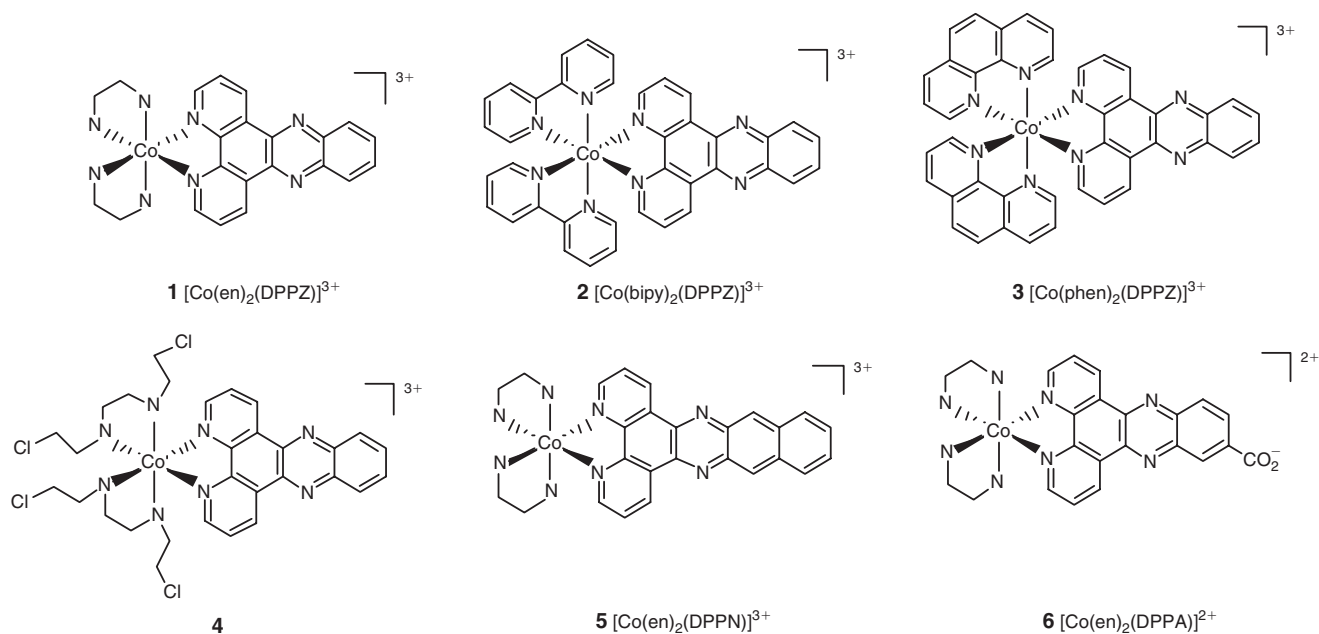
Introduction

The irradiation of metal complexes that contain polyimine ligands in the presence of DNA can lead to interesting and potentially useful chemistry. Most notable is the propensity of these complexes to break DNA strands upon photoirradiation. The induced strand breaks are typically single-strand breaks^[1–5] with some evidence of double-strand breakage, although complexes that are selective for double-strand breakage have been reported.^[6,7] Several mechanisms of DNA strand breakage have been proposed to account for this behaviour of polyimine complexes and these rely on the oxidative degradation of the DNA strands by the abstraction of hydrogen atoms, although the species responsible for the oxidative degradation is not clear in all cases.^[1,6–9]

We have previously described the synthesis and characterization of the complex $[\text{Co}(\text{en})_2(\text{DPPZ})](\text{ClO}_4)_3$ (**1**, Scheme 1; where DPPZ is dipyrido[3,2-*a*:2',3'-*c*]phenazine),^[10] which is a structural analogue of known DNA intercalating agents including $[\text{Ru}(\text{phen})_2(\text{DPPZ})]^{2+}$, $[\text{Ru}(\text{bipy})_2(\text{DPPZ})]^{2+}$, $[\text{RuO}(\text{DPPZ})(\text{tpy})]^{2+}$, and $[\text{Re}(\text{DPPZ})(\text{py})(\text{CO})_3]^+$.^[4,11–15] The intercalative binding in these systems is facilitated by the presence of the large, planar DPPZ chromophore and the presence of a dicationic charge, which contributes substantially to the DNA affinity of these molecules. Structurally related cobalt(III) complexes of DPPZ have also been described and, of particular relevance to this work, the complexes $[\text{Co}(\text{bipy})_2(\text{DPPZ})]^{3+}$ and $[\text{Co}(\text{phen})_2(\text{DPPZ})]^{3+}$ (**2** and **3**

respectively, Scheme 1) have been shown to cause DNA strand breakage when photoirradiated in the presence of DNA.^[1,2] The photochemistry of rhodium(III) complexes of 1,10-phenanthroline has also been thoroughly examined and it was found that Rh–DNA base adducts are formed in a photochemical reaction involving these complexes and DNA.^[16]

Molecules that cause light-induced DNA damage may be useful as photoactivated cytotoxic drugs. A compound such as $[\text{Co}(\text{en})_2(\text{DPPZ})](\text{ClO}_4)_3$ and its analogues could have applications in this regard, although for this to be the case it would need to be established that: (a) $[\text{Co}(\text{en})_2(\text{DPPZ})](\text{ClO}_4)_3$ interacted with DNA and caused strand breaks on irradiation, and (b) the cytotoxicity of such compounds was markedly enhanced in the presence of light. Another potential advantage of the complex $[\text{Co}(\text{en})_2(\text{DPPZ})](\text{ClO}_4)_3$ is that the dipyridophenazine chromophore can be readily modified to modulate the solubility, cellular uptake, and electronic properties of derived analogues. The cobalt(II) oxidation state is frequently implicated in the photochemistry of cobalt complexes. If this were to occur for these analogues then the potential exists to replace the coordinated ethylenediamine ligands with more potent cytotoxins, such as nitrogen mustards. (An exemplar is shown as **4** in Scheme 1.) The cytotoxicity of the mustard ligands will be masked on complexation to the kinetically inert cobalt(III) but their release as cytotoxins could occur on reduction to the labile cobalt(II) state.



Scheme 1. Structures of the cobalt(III) complex cations $[\text{Co}(\text{en})_2(\text{DPPZ})]^{3+}$, $[\text{Co}(\text{en})_2(\text{DPPN})]^{3+}$, and $[\text{Co}(\text{en})_2(\text{DPPA})]^{2+}$, and related systems.

In this report we describe the results of our investigations of the photochemistry of the complex $[\text{Co}(\text{en})_2(\text{DPPZ})](\text{ClO}_4)_3$ in the presence of plasmid DNA and the nucleoside 2'-deoxyguanosine. We also describe the synthesis and characterization of the related complexes $[\text{Co}(\text{en})_2(\text{DPPN})]\text{Cl}_3$ and $[\text{Co}(\text{en})_2(\text{DPPA})]\text{Cl}_2$ (**5** and **6** respectively, in Scheme 1; where DPPN and DPPA are benzo[*i*]dipyrido-[3,2-*a*:2',3'-*c*]phenazine and dipyrido[3,2-*a*:2',3'-*c*]phenazine-11-carboxylic acid, respectively). Aspects of the photobiology of $[\text{Co}(\text{en})_2(\text{DPPZ})](\text{ClO}_4)_3$ and these other compounds are also examined.

Results and Discussion

The UV-visible spectrum of $[\text{Co}(\text{en})_2(\text{DPPZ})]^{3+}$ is shown in Fig. 1*a*. The spectrum is dominated by the transitions of the dipyridophenazine chromophore. Intense absorption bands due to charge-transfer transitions are observed in the UV region of the spectrum. At lower energies, $\pi-\pi^*$ transitions of the dipyridophenazine are apparent^[2,11,12,17] with absorbances at wavelengths of 380 and 362 nm, and a poorly resolved transition centred around 344 nm. Absorption maxima occur at wavelengths of 344 ($\epsilon_{\text{max}} 1.04 \times 10^4$), 362 ($\epsilon_{\text{max}} 1.24 \times 10^4$), and 380 nm ($\epsilon_{\text{max}} 1.2 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$). Absorption bands due to the ligand-field transitions are lower in energy than the dipyridophenazine $\pi-\pi^*$ transitions and appear as a single broad absorption band with λ_{max} at 464 nm. The absorption of this band is too low in intensity to be evident in the spectrum shown in Fig. 1*a*. Deconvolution of this band gives two peaks of λ_{max} 437 and 470 nm, assigned to the two spin-allowed transitions, $^1A_{1g} \rightarrow ^1T_{2g}$ and $^1A_{1g} \rightarrow ^1T_{1g}$, respectively.

The similarity of both the energies and absorbances of the dipyridophenazine $\pi-\pi^*$ transitions in $[\text{Co}(\text{en})_2(\text{DPPZ})]^{3+}$ with those of other complexes containing dipyridophenazine

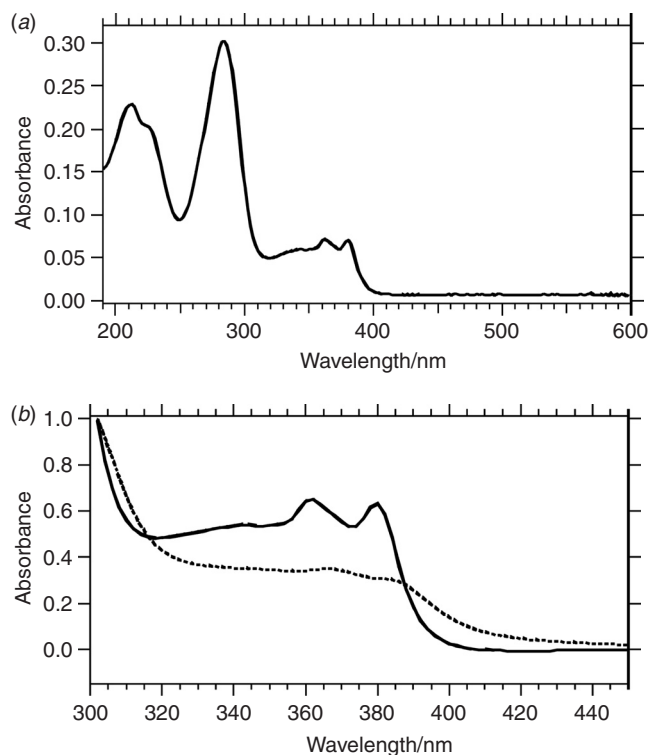


Fig. 1. (a) UV-vis spectrum of $[\text{Co}(\text{en})_2(\text{DPPZ})](\text{ClO}_4)_3 \cdot 0.5\text{H}_2\text{O}$, $5.19 \times 10^{-6} \text{ M}$, in water; (b) UV-vis spectra of $[\text{Co}(\text{en})_2(\text{DPPZ})](\text{ClO}_4)_3 \cdot 0.5\text{H}_2\text{O}$, $50 \times 10^{-6} \text{ M}$, in the presence (---) and absence (—) of $645 \times 10^{-3} \text{ M}$ (base pairs) calf thymus DNA.

indicate that the transitions are affected very little by the identity of the complexing metal. This observation is consistent with the lowest energy π^* excited state being localized on the non-complexing phenazine moiety of dipyridophenazine as observed for other complexes containing dipyridophenazine.^[11]

In the presence of DNA, a dramatic change in the spectrum of the complex is apparent (Fig. 1b), indicative of an interaction between the cobalt complex and the DNA. Hypochromism of the π - π^* bands, along with a red-shift of the original bands to lower energies (the transition at 362 nm is red-shifted to 366 nm while that centred at 380 nm appears as a shoulder at around 384 nm) is observed.

Hypochromism accompanied by a shift to the red in the absorption bands of the π - π^* transition of polyimine metal complexes are typical spectroscopic changes corresponding to the orbital interactions associated with the DNA intercalation reaction of polyimine ligands.^[1,2,4,17-19] This change in the absorption spectrum is similar to that observed for polyimine complexes that have subsequently been proven unequivocally to intercalate.^[11,15,20] These spectroscopic observations, along with the structural similarities of $[\text{Co}(\text{en})_2(\text{DPPZ})]^{3+}$ to other established DPPZ-based intercalators, are consistent with this complex acting as an intercalator.

Evidence of the intercalative binding of the complexes $[\text{Ru}(\text{phen})_3]^{2+}$, $[\text{Zn}(\text{phen})\text{Cl}_2]$, $[\text{Zn}(\text{phen})_2]^{2+}$, and $[\text{Zn}(\text{phen})_3]^{2+}$ has been reported using a gel procedure involving the migration of supercoiled plasmid DNA through an agarose gel matrix containing various concentrations of the complex.^[19,20] DNA unwinding experiments of this type with supercoiled plasmid DNA were attempted using the complex $[\text{Co}(\text{en})_2(\text{DPPZ})]^{3+}$. However, it was found that the presence of the complex in the gel led to the complete dispersion of the DNA bands upon electrophoresis so that these experiments provided no further insight into the DNA binding mode of $[\text{Co}(\text{en})_2(\text{DPPZ})]^{3+}$. It is possible that the application of a potential difference across the gel, and the resultant migration of the triply charged complex (which was incorporated into the matrix of the gel before its setting), led to a collapse of the gel structure and, hence, dispersion of the plasmid DNA through the gel.

Irradiation of a solution of $[\text{Co}(\text{en})_2(\text{DPPZ})]^{3+}$ at the wavelength of the π - π^* transition of the dipyrrophenazine ligand (372 nm) in the presence of the plasmid pBS(II) Stratagene for varying amounts of time and at different concentrations followed by analysis of the gels (Figs 2 and 3) revealed an extremely efficient photoinduced DNA-strand breakage. The extent of strand breakage increased with irradiation time and complex concentration. Irradiation for just two minutes was sufficient to single-strand nick over half the supercoiled plasmid (form I) to produce a relaxed circular (form II) plasmid. Irradiation for longer times resulted in the formation of linear (form III) DNA. At a complex concentration of 20×10^{-6} M, sufficient single- and double-strand breakage was obtained within 5 min of irradiation to convert all the supercoiled plasmid from form I into either the relaxed circular form (form II) or linear form (form III). No relaxation of the supercoiled DNA was observed either in the absence of the cobalt complex upon irradiation or in the presence of the complex in the dark.

However, although the results of our gel experiments indicate that the complex $[\text{Co}(\text{en})_2(\text{DPPZ})]^{3+}$ causes DNA-strand breaks, they do not provide evidence for the

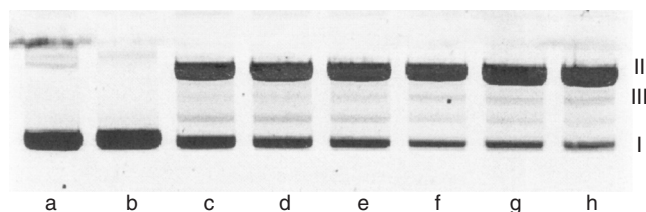


Fig. 2. Gel resulting from the electrophoresis of an irradiated solution containing $[\text{Co}(\text{en})_2(\text{DPPZ})]^{3+}$, 10×10^{-6} M, and pBluescript (II) KS+ plasmid DNA, 40×10^{-6} M (base pairs), where the irradiation time was: for b, 0 min; c, 2 min; d, 5 min; e, 10 min; f, 15 min; g, 20 min; h, 30 min. Solution a was a control, with only 40×10^{-6} M (base pairs) pBS(II) Stratagene plasmid DNA that had been irradiated for 30 min.

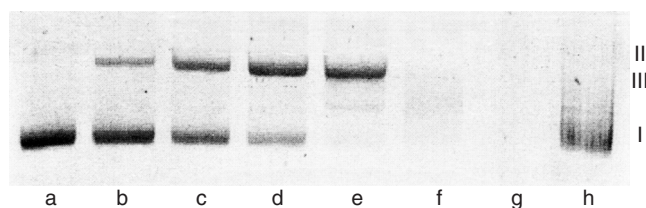


Fig. 3. Gel resulting from the electrophoresis of solutions containing pBluescript (II) KS+ plasmid DNA, 40×10^{-6} M (base pairs), and $[\text{Co}(\text{en})_2(\text{DPPZ})]^{3+}$ at the following concentrations: a, 0×10^{-6} M; b, 2×10^{-6} M; c, 5×10^{-6} M; d, 10×10^{-6} M; e, 20×10^{-6} M; f, 40×10^{-6} M; g, 80×10^{-6} M; h, 80×10^{-6} M. Each solution was irradiated for 5 min with the exception of h, which remained unirradiated.

nature of the strand-breakage mechanism, or information on the way in which the complex interacts with the DNA bases themselves. To probe the latter issue (which may contribute to the strand-breaking activity of these compounds) the photochemistry of the interaction of $[\text{Co}(\text{en})_2(\text{DPPZ})]^{3+}$ with 2'-deoxyguanosine was examined.

Initial investigations of the photochemistry of $[\text{Co}(\text{en})_2(\text{DPPZ})]^{3+}$ were carried out using UV-visible spectroscopy (see Fig. 4). Irradiation of $[\text{Co}(\text{en})_2(\text{DPPZ})]^{3+}$ in the absence of 2'-deoxyguanosine led to only a slight decrease in the absorbance of all peaks in the UV-visible spectrum. Irradiation of a solution of the complex $[\text{Co}(\text{en})_2(\text{DPPZ})]^{3+}$ at 372 nm (π - π^* band) in aqueous solution, buffered at pH 7 with phosphate buffer, in the presence of 2'-deoxyguanosine led to a change in the colour of the solution from yellow to orange with the concomitant production of a small amount of a light orange solid. The UV-visible spectra following irradiation of $[\text{Co}(\text{en})_2(\text{DPPZ})]^{3+}$ in aerobic and anaerobic conditions showed a greater degree of photoreaction under anaerobic conditions. Hence, the photoreaction is inhibited in the presence of oxygen. A distinct shift in the wavelength of the ligand-field band of the complex is observed from λ_{max} of 464 nm in the discrete complex to lower energies (λ_{max} around 512 nm) upon photoreaction, indicating a change in the coordination sphere of the complex. In addition to this shift, the intensity of all bands due to the ligand dipyrrophenazine are decreased significantly and the dominating species present in solution absorbing in the 200–410 nm

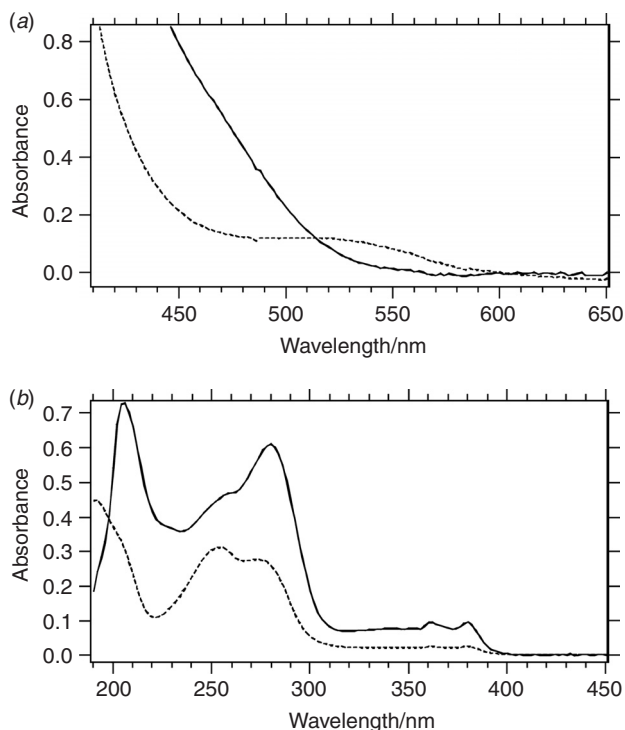


Fig. 4. Changes in UV-vis spectra in different regions of the spectrum following irradiation of $[\text{Co}(\text{en})_2(\text{DPPZ})](\text{ClO}_4)_3$ for 5 h at $\lambda > 350$ nm under anaerobic conditions: control solution (—); irradiated solution (---). (a) $[\text{Co}(\text{en})_2(\text{DPPZ})](\text{ClO}_4)_3$, 3.0×10^{-3} M, 2'-deoxyguanosine, 9.3×10^{-3} M, pH 7; (b) $[\text{Co}(\text{en})_2(\text{DPPZ})](\text{ClO}_4)_3$, 6.3×10^{-6} M, 2'-deoxyguanosine, 1.9×10^{-5} M, pH 7.

region is 2'-deoxyguanosine, indicative of either the degradation of the dipyridophenazine ligand or its precipitation from solution.

A possible explanation to account for these observations is that the photoinduced reaction between $[\text{Co}(\text{en})_2(\text{DPPZ})]^{3+}$ and 2'-deoxyguanosine proceeds by the reductive quenching by 2'-deoxyguanosine of the $[\text{Co}(\text{en})_2(\text{DPPZ})]^{3+}$ excited state formed upon irradiation. A similar mechanism has been proposed to account for the photoreaction between a polyimine complex of the Rh^{III} to form the Rh^{II} complex.^[16,21–23] In these studies, reductive quenching of the excited state formed upon irradiation of the complex $[\text{Rh}(\text{phen})_2\text{Cl}_2]^+$ by 2'-deoxyguanosine was observed. The mechanism follows a chain reaction and, ultimately, the photochemical reaction results in the formation of a rhodium–DNA base adduct.^[22,23] Adducts of these types were also isolated from the photoreaction of the complexes with the DNA helix itself.^[16] For $[\text{Rh}(\text{phen})_2\text{Cl}_2]^+$, the excited-state reduction potential was estimated as $^*E_{\text{red}}$ 1.18 V vs normal hydrogen electrode (NHE), which is sufficiently positive to oxidize 2'-deoxyguanosine (E_{ox} guanosine 1.03 V vs NHE^[24]). Using the same method,^[25] we estimate the excited-state reduction potential of the cobalt(III) complex $[\text{Co}(\text{en})_2(\text{DPPZ})]^{3+}$ to be $^*E_{\text{red}} > 2.4$ V based upon the $\text{Co}^{\text{III/II}}$ peak potential for $[\text{Co}(\text{en})_2(\text{DPPZ})]^{3+}$.^[26] That is, the excited-state cobalt complex $[\text{Co}(\text{en})_2(\text{DPPZ})]^{3+}$ is a sufficiently strong oxidizing agent to be reductively quenched by 2'-deoxyguanosine.

Table 1. LD₁₀ and LD₅₀ cytotoxicity concentrations determined for the complexes $[\text{Co}(\text{en})_2(\text{DPPZ})]^{3+}$, $[\text{Co}(\text{en})_2(\text{DPPN})]^{3+}$, and $[\text{Co}(\text{en})_2(\text{DPPA})]^{2+}$ in vitro (C6 rat glioma cell line)

Complex	LD ₁₀ [$\times 10^{-3}$ M]	LD ₅₀ [$\times 10^{-3}$ M]
$[\text{Co}(\text{en})_2(\text{DPPZ})]^{3+}$	2.80	17.0
$[\text{Co}(\text{en})_2(\text{DPPN})]^{3+}$	<2.86	<2.86
$[\text{Co}(\text{en})_2(\text{DPPA})]^{2+}$	14.2	116

This mechanism would explain the change in the coordination sphere of the cobalt atom upon photoirradiation. A one electron reduction of the cobalt(III) centre of $[\text{Co}(\text{en})_2(\text{DPPZ})]^{3+}$ generates the corresponding cobalt(II) complex. As cobalt(III) is extremely labile, the dissociation of the ligand ethylenediamine from this ion is rapid^[27–29] and likely to occur before re-oxidation of the metal centre. Likewise, loss of the dipyridophenazine ligand may also occur before re-oxidation of the metal centre.^[29] Hence, the kinetic lability of the d^7 cobalt(II) ion produced upon the reductive quenching of $[\text{Co}(\text{en})_2(\text{DPPZ})]^{3+}$ by 2'-deoxyguanosine results in a scrambling of the ligands according to the amounts and ligating equilibria of the possible ligands within solution. The observation that the reaction is hindered by oxygen infers that oxygen is also able to react with one of the initial irradiation products. Oxygen is known to rapidly oxidize cobalt(II) to cobalt(III) and it may be this back oxidation that inhibits the formation of irradiation products in the presence of oxygen. The quenching of the excited state of the cobalt(III) complex by oxygen would also result in a lower photochemical yield. A similar mechanism could also play a role in the strand cleavage of the plasmid DNA by $[\text{Co}(\text{en})_2(\text{DPPZ})]^{3+}$, since production of the radical cation of guanine, $\text{G}^{\cdot+}$, leads to complex chemistry in which cleavage of the sugar phosphate backbone may be one of the final outcomes.^[30]

The efficient light-activated DNA nuclease activity displayed by $[\text{Co}(\text{en})_2(\text{DPPZ})]^{3+}$, along with the observed photoinduced reaction with 2'-deoxyguanosine, prompted us to examine the toxicity of this complex, as well as the related complexes $[\text{Co}(\text{en})_2(\text{DPPN})]^{3+}$ and $[\text{Co}(\text{en})_2(\text{DPPA})]^{2+}$, against a tumour cell line both in the presence and absence of light. The extension and/or functionalization of the DPPZ ligand to DPPN and DPPA may be expected to alter the cellular uptake of the complexes while preserving the DNA affinity and photoactivity displayed by the DPPZ analogue.

The complexes were screened in vitro for cytotoxicity/phototoxicity using C6 rat glioma cells. The LD₅₀ and LD₁₀ concentrations (concentration of drug required to kill 50 and 10% of cells, respectively) were determined for the complexes in the absence of light and the results are tabulated in Table 1. All three complexes were determined to be cytotoxic in the absence of light, resulting in a complex-dependent LD₁₀ range of $2.80\text{--}14.20 \times 10^{-6}$ M. The cytotoxicity of these compounds in the absence of light may not be conducive to their usefulness as radiation-activated cytotoxins.

The phototoxicity experiments were performed using a concentration of complex sufficient to kill only 10% of cells in the absence of light (the LD₁₀) using a combination of

Table 2. Phototoxicity measurements on the complexes [Co(en)₂(DPPZ)]³⁺, [Co(en)₂(DPPN)]³⁺, and [Co(en)₂(DPPA)]²⁺ in vitro (C6 rat glioma cell line)

Cell survival (plus/minus standard error) is expressed as a percentage of the control (at 0 min and no light exposure)

Complex	Cell survival		
	20 min	30 min	60 min
[Co(en) ₂ (DPPZ)] ³⁺	78.3 ± 1.2	75.4 ± 1.5	73.9 ± 2.1
[Co(en) ₂ (DPPN)] ³⁺	74.3 ± 2.1	73.8 ± 2.6	73.3 ± 1.5
[Co(en) ₂ (DPPA)] ²⁺	76.2 ± 1.7	78.4 ± 1.2	74.1 ± 0.9

broad-band filters to irradiate in the range of about 360–400 nm. The results (shown in Table 2) were encouraging, as there was a reduction in cell survival relative to the LD₁₀ complex control (in the absence of light) for all the complexes after a light exposure period of 1 h. Little structure-activity variation was observed across the set of complexes, and for all of them a plateau was reached after 20 min of exposure, at which time a radiation-selective cell kill of approximately 25% was achieved. The plateauing of the phototoxicity of these compounds may be a consequence of their reduction and consequent decomposition in the cellular environment, and this may be responsible for the limited effects of light.

The complexes [Co(en)₂(DPPZ)]³⁺, [Co(en)₂(DPPN)]³⁺, and [Co(en)₂(DPPA)]³⁺ are not ideal as radiation-activated cytotoxins due to their inherent high toxicity and limited increase in toxicity following irradiation. However, they may be a useful platform for elaboration to systems containing cytotoxic groups (for example, mustards) which are released following irradiation. In addition, the photobiology of compounds in which these complexes are tethered to porphyrins would be worthy of exploration, since porphyrins generally display an enhanced affinity for tumour cells and are themselves widely used in photodynamic therapy.^[31]

Experimental

Materials

Solvents and reagents used during preparation of the compounds were of either laboratory or analytical reagent (LR, AR) grade. Calf thymus DNA (Sigma) and 2'-deoxyguanosine (Aldrich) were used as received. Titrisol phosphate buffer at pH 7 was purchased from Merck and contained 0.026 M KH₂PO₄ and 0.041 M NaHPO₄. Agarose was of molecular biology grade. All reagents used were of AR grade and solutions were prepared using ultrapure water from a Millipore system.

Instrumentation

Photolysis experiments were carried out using either an OSRAM Mercury 100 W high-pressure arc equipped with a 372 nm narrow bandpass filter focussed into the solution at a distance of 10 cm from the arc, or a portable ROFIN Polylight PL6 equipped with a broad band filter for $\lambda > 350$ nm. UV-vis spectra of aqueous solutions were measured using a Hewlett–Packard 8452A diode array spectrophotometer or a Perkin–Elmer Lambda2 spectrophotometer. ¹H NMR spectra were recorded in aqueous solutions by using either a Varian UnityPlus 400 spectrometer or an Inova 400, as convenient, with D₂O as solvent unless otherwise specified. Chemical shifts (δ , positive downfield) are given in ppm, and sodium trimethylsilylpropanesulfonate was used as the standard for ¹H NMR spectroscopy in D₂O. ¹H chemical-shift data are not given for

exchangeable (amine) protons because of the variation in position and intensity of these signals between samples.

Synthesis

Preparation of [Co(en)₂(DPPZ)](ClO₄)₃

[Co(en)₂(DPPZ)](ClO₄)₃ was synthesized according to the literature procedure.^[10]

Preparation of [Co(en)₂(dione)]Cl₃

The perchlorate salt of [Co(en)₂(dione)]³⁺ was synthesized according to the literature method^[10] and was converted into the chloride salt using cation-exchange chromatography. The solid perchlorate salt (typically approximately 1.0 g) was dissolved in water (1 L) and loaded onto a 30 by 5 cm² Dowex cation-exchange column. The column was washed with water (1 L) and 1 M HCl (1 L) and then eluted with 4 M HCl. The yellow band was collected and evaporated to dryness to yield the chloride salt. ¹H NMR spectroscopy indicated this material to be a mixture of [Co(en)₂(dione)]Cl₃ (70%) and [Co(en)₂(5-NO₂-phen)]Cl₃ (30%). This material was used without further purification in the steps below.

Preparation of [Co(en)₂(DPPN)]Cl₃·4.5H₂O

The material resulting from the preparation of [Co(en)₂(dione)]Cl₃ (0.59 g) was dissolved in deaerated (N₂ flushed) water (20 mL) containing concentrated HCl (0.13 mL). A solution of 2,3-diaminonaphthalene (0.10 g, 6.4 × 10⁻⁴ mol) in EtOH (10 mL) was flushed with N₂ gas for 5 min, and then added to the warm solution of the cobalt complexes under a nitrogen atmosphere. The yellow solution immediately turned dark red/black. Upon being heated to reflux, the solution lightened in colour. The solution was subject to reflux for 10 min, and then cooled gradually to -20°C under N₂ whereupon an orange precipitate formed. The precipitate was collected under a nitrogen atmosphere, washed with ice-cold ethanol (2 × 2 mL), and dried at the pump. The product was recrystallized twice from the minimum amount of warm 0.1 M HCl and dried under vacuum to yield [Co(en)₂(DPPN)]Cl₃·4.5H₂O (0.20 g) as an orange microcrystalline solid (Found: C 44.8, H 4.9, Cl 14.8, N 15.9. C₂₆H₂₈Cl₃CoN₈·4.5H₂O requires C 44.7, H 5.3, Cl 15.2, N 16.0%). $\lambda_{\text{max}}/\text{nm}$ ($\epsilon/\text{L mol}^{-1} \text{cm}^{-1}$) 460sh (88.1). δ_{H} (D₂O) 3.03 (4H, br, en CH₂), 3.30 (4H, br, en CH₂), 7.60 (2H, dd, DPPN CH), 8.20 (2H, dd, DPPN CH), 8.53 (2H, dd, DPPN CH), 8.95 (2H, s, DPPN CH), 9.29 (2H, d, DPPN CH), 10.00 (2H, d, DPPN CH).

Preparation of [Co(en)₂(DPPA)]Cl₂·3.5H₂O

3,4-Diaminobenzoic acid (0.24 g, 1.6 × 10⁻³ mol) was suspended in ethanol (10 mL) and the mixture degassed by purging with N₂. The suspension was added to a warm deoxygenated solution (N₂ gas) of the material resulting from the preparation of [Co(en)₂(dione)]Cl₃ (1.28 g) and concentrated HCl (0.13 mL) in water (20 mL). The yellow solution immediately turned dark red. The solution was subject to reflux for 10 min, during which time it became turbid. Upon cooling a brown solid precipitated. This was filtered off under a nitrogen atmosphere and washed with ice-cold water (2 × 2 mL). The product was recrystallized twice from the minimum amount (200 mL) of warm 0.02 M HCl and dried under vacuum to yield [Co(en)₂(DPPA)]Cl₂·3.5H₂O (0.60 g) as light brown needles (Found: C 43.2, H 5.2, Cl 11.1, N 17.6. C₂₃H₂₅Cl₂CoN₈O₂·3.5H₂O requires C 43.3, H 5.1, Cl 11.1, N 17.6%). δ_{H} (D₂O) 3.05 (4H, br, en CH₂), 3.30 (4H, br, en CH₂), 7.55 (1H, d, DPPA CH), 7.66 (1H, s, DPPA CH), 7.98 (1H, d, DPPA CH), 8.55 (2H, m, DPPA CH), 9.36 (2H, dd, DPPA CH), 9.83 (2H, dd, DPPA CH).

Plasmid Isolation

The plasmid pBluescript (II) KS+ was grown overnight in *Escherichia coli* cells and was isolated using a Qiagen Maxi-prep kit. The plasmid was dissolved in 10 × 10⁻³ M Tris buffer at pH 8 to a concentration of 320 ng μL^{-1} .

DNA Binding and Photo-Cleavage Experiments

An aqueous 500×10^{-6} M stock solution of $[\text{Co}(\text{en})_2(\text{DPPZ})](\text{ClO}_4)_3$ was prepared and stored at -20°C . This was diluted into buffer (1×10^{-3} M Tris and 10×10^{-3} M NaCl, pH 7.0) immediately before use in spectroscopic studies and cleavage experiments. Spectra of the complex in the presence of calf thymus DNA were recorded at a complex concentration of 50×10^{-6} M and a DNA concentration of 645×10^{-6} M with respect to base pairs.

DNA photo-cleavage experiments were performed using the supercoiled DNA plasmid, pBluescript (II) KS+. The concentration of plasmid was maintained at 40×10^{-6} M (base pairs) and either the concentration of the complex (0 – 100×10^{-6} M) or the irradiation time (0 – 60 min) was varied. The DNA was treated with the metal complex at the appropriate concentration in buffer (1×10^{-3} M Tris and 10×10^{-3} M NaCl, pH 7.0) and kept at 0°C in the dark while not being irradiated. The solutions were irradiated for a known time. Following the addition of $1 \mu\text{L}$ of loading buffer (60% glycerol, 0.02% bromophenol blue) to each of the $10 \mu\text{L}$ samples, they were loaded into an agarose gel [1% agarose in TAE buffer (40×10^{-3} M TRIS-acetate, 1×10^{-3} M EDTA, pH 8.1)]. The samples were subjected to electrophoresis at 120 V for 40 min in the dark in TAE buffer. The gel was stained with ethidium bromide ($0.75 \mu\text{g mL}^{-1}$) for 30 min, and then destained in water for 30 min to remove excess ethidium bromide. The resultant DNA bands were viewed with a transilluminator.

Reaction with 2'-Deoxyguanosine

A 3 mL aliquot of a solution containing 3.6×10^{-3} M $[\text{Co}(\text{en})_2(\text{DPPZ})](\text{ClO}_4)_3$ and 10×10^{-3} M 2'-deoxyguanosine in either phosphate buffer at pH 7 or water or 1.6×10^{-3} M $[\text{Co}(\text{en})_2(\text{DPPZ})](\text{ClO}_4)_3$ in phosphate buffer at pH 7 was placed in a glass cuvette and degassed by purging the solution with N_2 gas for 10 min. The cuvette was capped and sealed using Parafilm, and then irradiated for 5 h. A control solution was prepared similarly from the same stock solution and kept in the dark for the duration of the irradiation. During this time the irradiated solution changed from yellow to orange and a light orange solid formed in the presence of 2'-deoxyguanosine; in the absence of 2'-deoxyguanosine the solution became turbid upon irradiation. No change was observed in the control solutions.

Cytotoxicity Studies

The C6 rat glioma cell line was obtained from the American Type Culture Collection (ATCC; Rockville, MD) and was maintained in Roswell Park Memorial Institute 1640 (RPMI 1640) medium (GIBCO BRL Invitrogen, Melbourne) and 10% fetal bovine serum (FBS; GIBCO BRL Invitrogen, Melbourne) in a humidified incubator at 37°C in 5% CO_2 .

C6 cells were cultured and harvested into 25 cm^2 Nunclon tissue culture flasks (Medos Co., Melbourne) and wrapped in aluminium foil. A 2 mL cell suspension volume containing $100 \text{ cells mL}^{-1}$ was added to each flask. Six hours were allowed for cell attachment to the flask at 37°C in a 5% CO_2 environment.

Initially the cytotoxicity of each of the dipyridophenazine complexes was tested over the range 0 – $400 \mu\text{g mL}^{-1}$. After the addition of a complex in near darkness, the flasks were re-incubated at 37°C with 5% CO_2 for a further 16–18 h. The medium containing the complex was removed and the adhered cells were washed twice in near darkness using fresh, complex-free RPMI 1640 medium. The flasks were incubated for one week post-washing after which the adhered cell colonies were fixed with a solution of 70% methanol/30% glacial acetic acid over three 15 min periods. The colonies were then stained over 1 h with 1% crystal violet (Sigma Chemical Co.) and the stained colonies counted over a white fluorescent light box. Experiments were performed in triplicate with each complex.

The phototoxicity experiments were performed using a concentration of complex that resulted in a 10% reduction (LD_{10}) in survival from the control flask in the cytotoxicity experiments. C6 cells were seeded as described previously and after the 6 h attachment phase, an amount of complex corresponding to the LD_{10} concentration was added after which there was additional 16–18 h incubation. The cells were then washed

with fresh medium in a near darkness environment and then exposed to filtered light using a combination of broadband filters (Rosco Supergel filters #45 and #50, Lightmoves, Melbourne) allowing light irradiation mainly in the 350–450 nm range. The filters were placed directly on the light box and a 5 mm sheet of Perspex was placed on the filters to prevent damage to the filters during the experiments. The light box was switched on approximately 30 min before irradiation to allow for a consistent output of filtered light (which can change as the filters warm up). The light source was a cool white light source (NEC, Melbourne). The flasks were wrapped in aluminium foil again, incubated for a further week, and processed for counting as described previously.

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