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Photooxidation of Guanine by a Ruthenium Dipyridophenazine Complex Intercalated in a Double-Stranded Polynucleotide Monitored Directly by Picosecond Visible and Infrared Transient Absorption Spectroscopy

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Abstract: Transient species formed by photoexcitation (400 nm) of $[Ru(dppz)(tap)_2]^{2+}$ (**1**) (dppz = dipyrido[3,2-*a*:2′,3′-*c*]phenazine; tap = 1,4,5,8-tetraazaphenanthrene) in aqueous solution and when intercalated into a double-stranded synthetic polynucleotide, $[poly(dG\text{-}dC)]_2$, have been observed on a picosecond timescale by both visible transient absorption (allowing monitoring of the metal complex intermediates) and transient infrared (IR) absorption spectroscopy (allowing direct study of the DNA nucleobases). By contrast with its behavior when free in aqueous solution, excitation of **1** when bound to $[poly(dG\text{-}dC)]_2$ causes a strong increase in absorbance at 515 nm due to formation of the reduced complex $[Ru(dppz)(tap)_2]^{+}$ (rate constant $= (2.0 \pm 0.2) \times 10^9\ s^{-1}$). The subsequent reformation of **1** proceeds with a rate constant of $(1.1 \pm 0.2) \times 10^8\ s^{-1}$. When the process is carried out in D_2O, the rates of formation and removal of $[Ru(dppz)(tap)_2]^{+}$ are reduced (rate constants $(1.5 \pm 0.3) \times 10^9$ and $(0.7 \pm 0.2) \times 10^8\ s^{-1}$ respectively) consistent with proton-coupled electron transfer processes. Picosecond transient IR measurements in the 1540–1720 cm^{-1} region in D_2O solution confirm that the reduction of **1** intercalated into $[poly(dG\text{-}dC)]_2$ is accompanied by bleaching of IR ground-state bands of guanine (1690 cm^{-1}) and cytosine (1656 cm^{-1}), each with similar rate constants.

Keywords: electron transfer · guanine · photooxidation · reaction mechanisms · ruthenium

Introduction

The study of electron-transfer reactions in DNA is an important problem both from a fundamental materials science perspective[1–10] and because of its biological consequences.[11–15] As such electron transfers are fast chemical processes, they are usually initiated by short laser-pulse excitation of sensitizers such as phenothiazonium dyes,[16,17] anthraquinones[18,19] or metal complexes.[20–24] The study of compounds intercalated into DNA is especially appropriate for theoretical treatment as the relative orientation of the electron donor and acceptor is well defined.

Ruthenium–polypyridyl complexes, in particular, are excellent sensitizers for probing various properties of DNA, as their photophysical and electrochemical properties can be varied readily in a controlled fashion. For example, it has been known for some years that complexes containing the 1,4,5,8-tetraazaphenanthrene (tap), 1,4,5,8,9,12-hexaazatriphenylene (hat) or 2,2′-bipyrazine (bpz) can photooxidise

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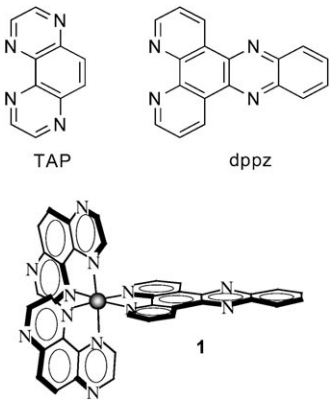
guanine either in nucleotides (GMP or dGMP) or in the double-stranded polynucleotides.[25–30] With complexes such as $[Ru(bpy)_{3-n}(tap)_n]^{2+}$ ($n=2$ or 3) or $[Ru(hat)_2(phen)]^{2+}$, direct evidence for electron transfer from GMP was obtained by nanosecond laser flash photolysis.[27] However, analogous experiments with DNA revealed a complex situation with evidence that several processes were occurring, including some with rates faster than the resolution of the nanosecond equipment.[31]

A major problem with $[Ru(bpy)_{3-n}(tap)_n]^{2+}$ complexes is that their precise mode of binding is uncertain although, as with analogous complexes such as $[Ru(phen)_3]^{2+}$,[32,33] it is likely that they are groove-bound with some partial intercalation between the base-pairs. However, complexes of dipyrido[3,2-*a*:2′,3′-*c*]phenazine such as $[Ru(dppz)(phen)_2]^{2+}$ are known to have the dppz ligand fully intercalated[34–38] and such species are therefore more appropriate for detailed kinetic studies. As the excited state of $[Ru(dppz)(phen)_2]^{2+}$ is insufficiently oxidizing to produce the guanine radical cation, we have studied $[Ru(dppz)(tap)_2]^{2+}$ (**1**), which has



similar redox chemistry to that of $[Ru(phen)(tap)_2]^{2+}$, but unlike that species complex **1** binds to DNA in a well-defined intercalating fashion.[39] Preliminary picosecond studies have indicated that the kinetic behavior of transient species formed from $[Ru(dppz)(tap)_2]^{2+}$ bound to DNA is markedly different from that of the complex free in solution.[40,41] Here we present the results of transient studies with a double-stranded synthetic polynucleotide, $[poly(dG\text{-}dC)]_2$, monitored by both visible and IR methods. These produce clear direct evidence for oxidation of guanine by the intercalated metal-complex excited state. Rates of both the forward and back electron-transfer reactions are shown to be significantly slower in deuterated water; this is attributed tentatively to proton coupling of the electron-transfer process. The results may be compared with the oxidation of guanine by direct photoionisation using 200 nm excitation.[42]

Results

Visible transient absorption (TA): It has been shown previously that the reduction of the excited state of $[Ru(dppz)(tap)_2]^{2+}$ **1** by sufficiently strong reducing agents such as guanosine-5′-monophosphate (GMP) can be readily monitored by observing the TA spectra in the 450–650 nm region, as the reduced species of **1** absorbs strongly at approximately 500 nm.[39–41] However, nanosecond (time resolution >50 ns) experiments with $[poly(dG\text{-}dC)]_2$ did not reveal any evidence for the reduced species even though the emission of **1** is almost completely quenched. We have therefore carried out picosecond time domain experiments on **1** in solution and bound to $[poly(dG\text{-}dC)]_2$.

Transient absorption spectra of a solution of **1** (8.5×10^{-5} M) in 10 mM aqueous phosphate buffer were recorded between 1 and 1500 ps after excitation with a 200 fs pulse of 400 nm light (Figure 1, top). These show a strong bleaching between 450 and 500 nm and a region of weaker positive absorption above 500 nm. It is clear that a transient species is produced that has a lifetime greater than 10 ns, consistent with the previous emission lifetime studies, which showed that the lowest excited state (that is, Ru–tap metal-to-ligand charge transfer (MLCT)) has a lifetime of 820 ns in aerated aqueous solution.[39] The assignment of this MLCT state ($[Ru^{III}(dppz)(tap)(tap^{\bullet-})]^{2+*}$) has been confirmed previously by transient resonance Raman spectroscopy.[41] This behav-

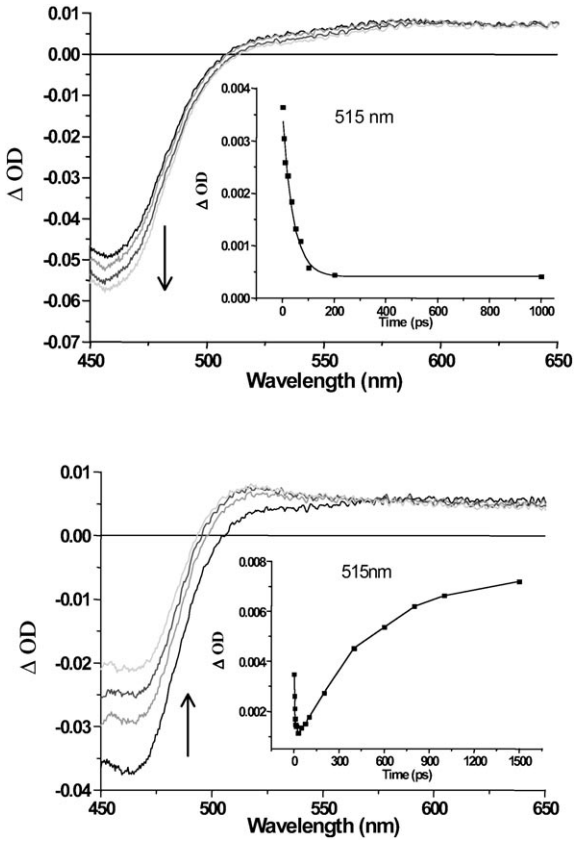


Figure 1. Picosecond TA spectra of $[Ru(dppz)(tap)_2]Cl_2$ (**1**.Cl_2) (8.5×10^{-5} M) in 10 mM aqueous phosphate buffer in the absence (top) and in the presence (bottom) of $[poly(dG\text{-}dC)]_2$ (1.7×10^{-3} M). For clarity, only traces at 10, 20, 70 and 1000 ps (from top to bottom) in the top part of the graphic and at 100, 600, 1000 and 1500 ps (from bottom to top) in bottom part of the graphic are shown. Insets: kinetics at 515 nm.

ior contrasts with that of $[Ru(dppz)(phen)_2]^{2+}$, in which two excited states are formed which have lifetimes of approximately 3 ps and 250 ps in aqueous solution.[34,44,45] In the case of **1**, there is also evidence for a short-lived species. Thus monitoring at 515 nm reveals a partial decrease in the TA (Figure 1, top, inset) giving a first-order decay rate constant of $(2.5\pm0.3)\times10^{10}\,s^{-1}$ and corresponding lifetime of 41 ps; similar rate constants are obtained at other wavelengths, whether in the transient bleaching or absorption regions. The same decay rate constant is found in buffered D_2O. The process is probably due to the conversion of an initially formed excited state to the long-lived triplet Ru–tap MLCT state.

A very different behavior was found when the TA spectra of **1** bound to $[poly(dG\text{-}dC)]_2$ were recorded (Figure 1, bottom). These experiments were carried out at a nucleotide/Ru binding ratio of 20:1 to ensure that most complexes were bound and to minimize aggregation effects. The dominant feature is strong absorption in the 500–550 nm region, which grows exponentially with a lifetime τ of 506 ps (rate constant $=(2.0\pm0.2)\times10^{9}\,s^{-1}$). This is consistent with the formation of the reduced complex $[Ru(dppz)(tap)_2]^{+}$, probably as a result of electron transfer from guanine. Additionally, a very rapid reaction ($\tau=4$ ps; rate constant $=(2.5\pm0.2)\times10^{11}\,s^{-1}$) occurs that could be an interstate process similar to that suggested for the complex alone in aqueous solution.

From the experimental results in Figure 1 (bottom), the reduced product of **1** appears to be stable at 1500 ps. However, as mentioned above, in earlier nanosecond flash photolysis experiments[39] we were unable to find evidence for electron-transfer products when **1** was bound to $[poly(dG\text{-}dC)]_2$. This implies that the lifetime of the transient must be less than 50 ns. Transient absorption studies with the femtosecond equipment were therefore performed using a longer delay line, to allow measurements to be made to 5 ns. These data (Figure 2, top) provide evidence for partial decay of the 515 nm-absorbing species. Assuming that the species decays to zero absorbance, a decay rate constant of $(1.1\pm0.2)\times10^{8}\,s^{-1}$ ($\tau=8850$ ps) is derived for this process.

TA of **1** intercalated in $[poly(dG\text{-}dC)]_2$ in buffered D_2O was used to determine whether the photoreduction process is affected by the deuteration of the solvent (and hence isotopic substitution of exchangeable protons in the DNA bases). Both the forward (ET) and back electron-transfer reactions (BET) were found to be slower ($k_{ET}=(1.5\pm0.3)\times10^{9}\,s^{-1}$; $k_{BET}=(0.7\pm0.2)\times10^{8}\,s^{-1}$) than in H_2O, yielding isotope effects k_H/k_D of approximately 1.3 for the forward reaction and 1.6 for the reverse process. However, large errors in these values mean that further work is required to determine the kinetic isotope effect precisely.

Picosecond transient IR absorption measurements: The 1540–1720 cm^{-1} region of the FTIR spectrum of $[poly(dG\text{-}dC)]_2$ contains strong absorptions that can be assigned primarily to the carbonyl stretches of guanine (at 1690 cm^{-1}) and cytosine (at 1656 cm^{-1}). Monitoring transient IR spectra

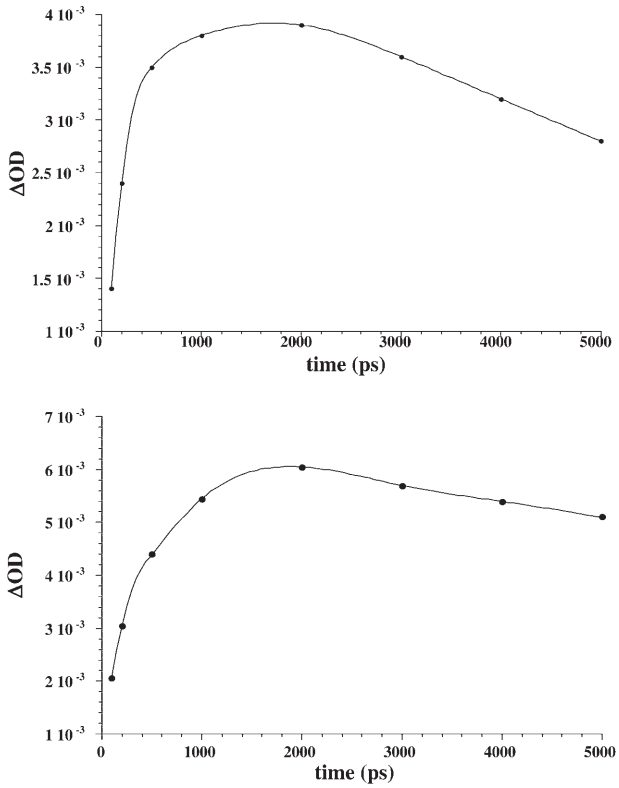


Figure 2. Transient absorption measured at 515 nm for $[Ru(dppz)(tap)_2]Cl_2$ (8.5×10^{-5} M) in the presence of $[poly(dG\text{-}dC)]_2$ (1.7×10^{-3} M) in 10 mM aqueous phosphate buffer prepared with H_2O (top) and D_2O (bottom).

(TRIR) in this region therefore provides a means of probing the Watson–Crick H bonds of the duplex, and we have already exploited this fact to monitor transient IR changes following direct UV excitation ($\lambda_{exc}=267$ nm) or photoionisation of polynucleotides.[42,48] In this work we employ the transient IR absorption technique to study spectroscopic changes induced in the polynucleotide as a consequence of the excitation of $[Ru(dppz)(tap)_2]^{2+}$ ($\lambda_{exc}=400$ nm); note that the polynucleotide itself does not absorb at this wavelength.

These measurements were made on samples containing **1** (8×10^{-4} M) and $[poly(dG\text{-}dC)]_2$ (1.7×10^{-2} M) in buffered (10 mM phosphate) D_2O (H_2O could not be used as it absorbs too strongly in this region of the IR). Under these conditions each ruthenium complex is intercalated between two GC base-pairs and is hence in close contact with guanine bases. Transient picosecond-TRIR spectra were recorded at time delays ranging from 2 to 2000 ps after the excitation of the sample with 400 nm irradiation (Figure 3, top). In each spectrum there is clear evidence for the bleaching of both the 1656 and 1690 cm^{-1} bands, indicating that the excitation of the ruthenium complex has induced changes in the base-pairing of the polynucleotide. Thus even within the time resolution of our apparatus, strong bleaching of both the predominantly G- and C-localized vibrations is observed. There is also simultaneous production of TA bands

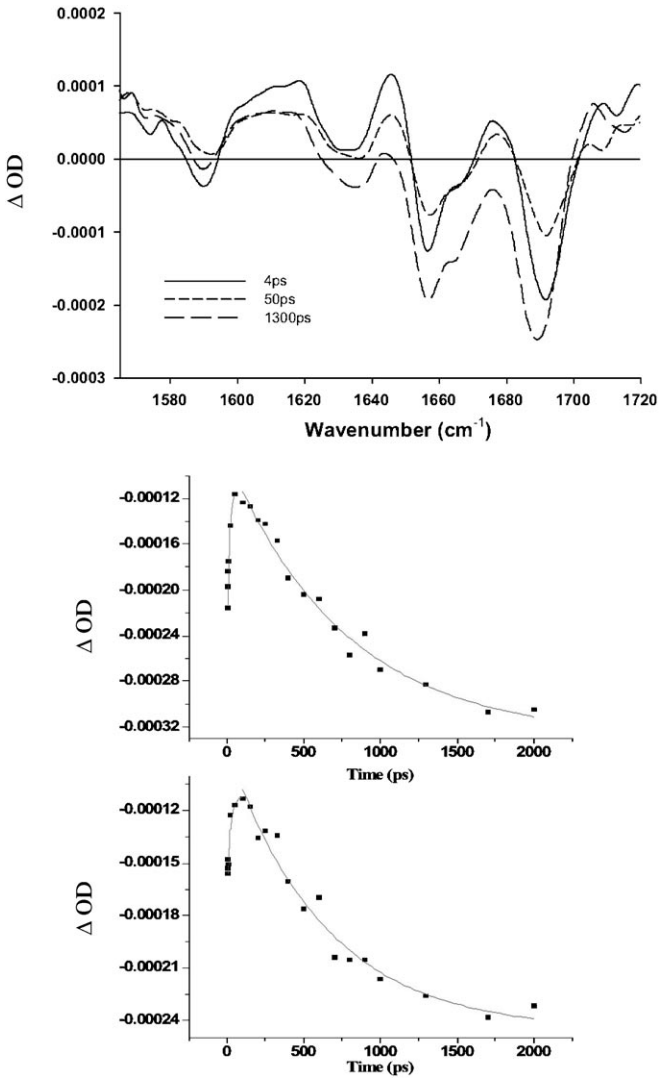


Figure 3. Top: ps-TRIR spectra of $[poly(dG\text{-}dC)]_2$ (1.7×10^{-2} M) and $[Ru(dppz)(tap)_2]^{2+}$ (8×10^{-4} M) at selected times (4, 50 and 1300 ps) following a 400 nm laser excitation. Middle: kinetic trace at G depletion (1690 cm^{-1}). Bottom: kinetic trace at C depletion (1656 cm^{-1}).

between 1590 and 1630 cm^{-1} and at 1645 cm^{-1}. Subsequently a rapid partial recovery of the bleaching and a parallel decay of the TA bands ($\tau=10$ ps, $k=(1.0\pm0.2)\times10^{11}$ s^{-1}) are observed. Similar processes have been monitored with direct UV excitation of $[poly(dG\text{-}dC)]_2$ itself.[48] One explanation is that the polynucleotide has become vibrationally excited due to a rapid rise in temperature coming from the release of energy from the initially excited state of **1**. However, unlike the case of 267 nm excitation, recovery of the bleaching is incomplete and is followed by a slower process of further bleaching at both 1690 cm^{-1} (G band; $\tau=708$ ps, $k=(1.4\pm0.2)\times10^{9}$ s^{-1}; Figure 3, middle) and 1656 cm^{-1} (C band; $\tau=654$ ps, $k=(1.5\pm0.2)\times10^{9}$ s^{-1}; Figure 3, bottom). This process we ascribe to guanine oxidation by the excited state of **1**; in agreement with this, the rate constant observed is the same within error as that determined for the formation of $[Ru(dppz)(tap)_2]^{+}$ in D_2O by visible TA spectroscopy (vide supra). The bleaching of all the bands does not recover on the timescale studied (up to 2000 ps). Interestingly, the broad TA band observed in the 1590–1630 cm^{-1} region and already present after 50 ps does not decay appreciably on the timescale of the experiment. Therefore it is clear that this band is characteristic of the DNA before and after the proposed oxidation process. A grow-in of a very weak band at 1700 cm^{-1} after 50 ps is also observed. A similar band has recently been reported for oxidized guanine formed by photoionisation of 5′-dGMP and $[poly(dG\text{-}dC)]_2$.[42]

Discussion

Complexes of the type $[Ru(dppz)(L)_2]^{2+}$ are known to bind strongly to DNA. We may assume that the binding geometry of $[Ru(dppz)(tap)_2]^{2+}$ (**1**) is similar to that found for $[Ru(dppz)(phen)_2]^{2+}$, which has been studied very extensively,[36–38] and in which it is known that the dppz ligand is intercalated between the base-pairs of DNA with the other ligand (phen) present in the groove. It is also expected that the binding of the excited state of **1** and of its corresponding reduced species $[Ru(dppz)(tap)_2]^{+}$ will be similar, as the promoted or donated electron is expected to be located on one of the tap ligands.[39]

Excitation of complex **1** at 400 nm is expected to populate initially the dppz centered π–π*, the Ru–tap MLCT and possibly the Ru–dppz MLCT singlet excited states. It is expected that, as for other ruthenium complexes, intersystem crossing will take place in less than a picosecond.[49] The transient UV/visible absorption data, however, reveal that there is a rapid (relatively small-amplitude) process for **1** alone in aqueous solution ($\tau=41$ ps). Elucidation of the nature of this short-lived species will require a detailed investigation, especially of solvent effects, which is beyond the scope of this study. One possibility is that the process involves an interstate transition; for example, from the π–π* (dppz) or the Ru–dppz MLCT to the most stable excited state. The excited state is known to be the triplet Ru–tap MLCT state, which can be represented formally as $[Ru^{III}(dppz)(tap)(tap^{\bullet-})]^{2+}$*, and is long-lived (820 ns) in water.[39] While it is known that interligand electron transfers (ILETs) are very fast in homoleptic complexes such as $[Ru(bpy)_3]^{2+}$,[50] it is likely that in heteroleptic complexes the rate of such processes will depend on the specific nature of the ligand. This may be the case particularly for the dppz ligand, in which the possible occupancy of the phenazine- or phenanthroline-localized orbitals can lead to remarkable photophysical behavior.[34–35,44,51] The other processes such as vibrational–solvent relaxation, solvation or protonation which also occur in this time region would need to be considered in a detailed study. The shorter transient (approximately 4 ps) observed when the complex is bound to $[poly(dG\text{-}dC)]_2$ suggests that it is indeed very sensitive to the medium.

However, a striking difference for the intercalated complex is the strong rise in the visible TA between 550–550 nm

with a lifetime of 506 ps. This is entirely consistent with the prediction that the reduction of the complex would give $[Ru^{II}(dppz)(tap)(tap^{\bullet-})]^{+}$.[39] The oxidized guanine, whether as its radical cation ($G^{\bullet+}$), or the deprotonated guanine radical ($G^{\bullet}$) absorbs only weakly above 500 nm and therefore will not contribute appreciably to the signal.

Interestingly, this electron-transfer process is relatively slow compared with that of other intercalating systems in which the electron-transfer rate is much faster (for example, for thionine the reciprocal rate constant is 200 fs).[16] In these cases the electron transfer occurs between the nucleobase and the dye, which are in close contact and strongly coupled to the polynucleotide base stack. For $[Ru^{III}(dppz)(tap)(tap^{\bullet-})]^{2+}$* the electron from guanine eventually effects a reduction of the ruthenium, which is expected to be located at the wall of the groove, hence giving rise to a slower process.

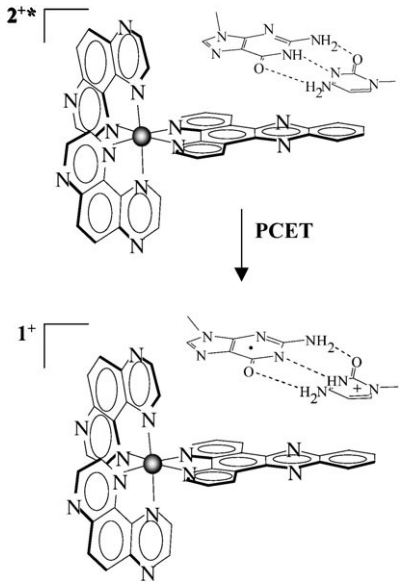
However, the observation of an appreciable solvent isotope effect indicates that the oxidation process might not be a simple electron transfer [Eq. (1)].

$$[Ru^{III}(dppz)(tap)(tap^{\bullet-})]^{2+}* + \text{“G}\equiv\text{C”} \rightarrow [Ru^{II}(dppz)(tap)(tap^{\bullet-})]^{1+} + \text{“G}^{\bullet+}\equiv\text{C”} \quad (1)$$

The observed isotope effects ($k_H/k_D = 1.3$ for the excited-state forward electron transfer and $k_H/k_D = 1.6$ for the back electron transfer) are consistent with our observation of processes involving simultaneous transfer of a proton or deuteron (in D_2O all exchangeable hydrogen atoms are replaced by deuterium), that is, proton-coupled electron transfer (PCET).[52–58] A similar isotopic effect has already been observed for **1*** in the presence of 5′-GMP,[39] in which, in contrast to the present case, the proton is presumably lost to the solvent. As **1** here is bound between the GC base-pairs, we propose that the most likely process involves a transfer of the imino proton from the guanine to yield the protonated cytosine [Eq. (2); Scheme 1].

$$[Ru^{III}(dppz)(tap)(tap^{\bullet-})]^{2+}* + \text{“G}\equiv\text{C”} \rightarrow [Ru^{II}(dppz)(tap)(tap^{\bullet-})]^{+}\text{“G}^{\bullet}\equiv\text{CH}^{+}\text{”} \quad (2)$$

As suggested in our earlier study of the reaction of **1*** and 5′-GMP,[39] the energetics associated with reaction given in Equation (1) are expected to be less favorable than those for that given in Equation (2), even if the oxidation potential of guanine in the double-stranded $[poly(dG\text{-}dC)]_2$ is expected to be somewhat lower than that of the free nucleotide.[59,60] Moreover, if we assume that the pK_a of the guanine radical in $[poly(dG\text{-}dC)]_2$ is similar to those of the free nucleotide ($pK_a = 3.9$) and of protonated cytosine ($pK_a = 5.5$), one would expect simultaneous proton transfer upon oxidation of the guanine nucleobase. One may also speculate that the protonation occurs at the level of the reduced metallic entity. Indeed, even though the pK_a of $[Ru^{II}(dppz)(tap)(tap^{\bullet-})]^{+}$ has not been determined, a rough estimate based on the pK_a of the reduced parent complex $[Ru^{II}(tap)_2(tap^{\bullet-})]^{+}$ ($pK_a = 7.5$)[61] indicates that the proton may also be transferred to the reduced tap ligand. We are currently unable to distinguish between this mechanism and that proposed above.



Scheme 1. Possible mechanism of the proton-coupled electron transfer (PCET) between $[poly(dG\text{-}dC)]_2$ and excited $[Ru(dppz)(tap)_2]^{2+}$: upon guanine photooxidation, the proton is simultaneously transferred to the adjacent cytosine.

The picosecond TRIR study in D_2O allows us to probe the effect of the proposed oxidation of guanine (and indeed of its associated cytosine). The IR spectrum of $[poly(dG\text{-}dC)]_2$ shows two major bands in the region of interest, which have been previously assigned primarily to guanine C=O (1690 cm^{-1}) and cytosine C=O (1656 cm^{-1}) respectively.[42,48] For both bands strong bleaching is noted after the excitation pulse. There is a subsequent partial recovery, which is then followed by re-bleaching. The rate constant determined for this second bleaching process is the same within error as that observed by transient visible spectroscopy and may therefore be attributed to oxidation of the guanine. It is striking that the rate constant for the bleaching observed at the 1656 cm^{-1} band, which is substantially cytosine-based, has a similar value, indicating that the guanine oxidation induces changes in the cytosine simultaneously. It would also be expected that the cytosine vibrations would be affected by the simple formation of the guanine radical cation [Eq. (1)], especially as the bases are quite strongly coupled.[47] However, taking into account the entire set of data presented here, we believe that the results are more consistent with proton-coupled electron transfer [Eq. (2)]. Such a process is also predicted from recent theoretical studies for the oxidation of a GC-containing polynucleotide.[62,63]

Interestingly, our results indicate that the deprotonation rate of the guanine radical cation within DNA is two orders of magnitude faster than that recently measured by using indirect pulse radiolysis methods through the formation of the powerful oxidizing agent $SO_4^{\bullet-}$.[64] The fact that in our work

we observe changes on ultrafast timescales and that these match the direct photoionisation (see reference [48]) of G indicate that the chemistry is occurring faster than that reported by Kobayashi and Tagawa. Our kinetics also appear to be influenced by deuteration, suggesting involvement of a proton reaction. Clearly, further work is required to establish the precise nature of these differences.

The very rapid (<2 ps) change in the vibrational spectrum of the guanine–cytosine base-pair caused by visible light excitation of the intercalated complex is also interesting. The nature of this effect will require further study, but provisionally we assign it to thermal population of vibrational excited states of the nucleobases by the extremely rapid radiationless deactivation of the intercalated metal complex. We plan to carry out further experiments to see whether this is a general phenomenon.

Conclusion

Above we present direct evidence that excitation of $[Ru(dppz)(tap)_2]^{2+}$, when intercalated into the double-stranded polynucleotide $[poly(dG\text{-}dC)]_2$, causes both reduction of the metal complex and simultaneous changes in guanine and cytosine IR bands. The rates of this oxidation/reduction process and the subsequent reverse process are both slower when D_2O is used as solvent. The derived isotope effect supports assignment of the processes as proton-coupled electron transfer. Tentatively it is proposed that the process involves the formation of the guanine radical with transfer of a proton to cytosine within the Watson–Crick base-pair.

Experimental Section

Materials: $[Ru(dppz)(tap)_2]^{2+}Cl_2^{2-}$ (**1**·Cl_2), (tap = 1,4,5,8-tetraazaphenanthrene; dppz = dipyrido-[3,2-*a*:2′,3′-*c*]phenazine) was synthesized and purified as previously described.[39] Solutions were prepared either in aqueous phosphate buffer (10 mM, pH 6.9) or phosphate (10 mM, pH 6.9)-buffered D_2O (99.9 atom % D). $[Poly(dG\text{-}dC)]_2$ (Pharmacia–Amersham/Sigma) was used as received. Polynucleotide concentration was determined spectrophotometrically ($\varepsilon_{254\,nm} = 8400\,M^{-1}\,cm^{-1}$ for $[poly(dG\text{-}dC)]_2$).[65]

Femtosecond transient visible absorption spectroscopy: The equipment for femtosecond TA is described in detail elsewhere.[43a] Briefly, this system comprises a Spectra Physics/Positive Light Ti-sapphire regenerative amplifier system producing 200 fs pulses at 800 nm and 0.6 kHz repetition rate. The pulse train was beam-split in the ratio 1:3, with 25% converted to the second harmonic (400 nm) to pump the sample and the other 75% generating a white-light continuum probe from a 10 mm pathlength fused-silica water flow cell. This white-light beam was split to give a probe and reference beam, the latter bypassing the sample. The pump beam (pulse energy typically 6 μJ) traveled along a delay line and later passed through the 5 mm path length Suprasil sample-containing cuvette, intersecting the probe beam at a small angle. Beyond the cell a bandpass filter was inserted in the probe and reference beams to exclude residual pump light. The spatial overlap of pump and probe beams was optimized daily to ensure a good signal-to-noise ratio. The signals from the sample and reference beams were detected on two diode array detectors. This set-up provided a 400 nm pump and probed the 450–650 nm region. Pump and probe polarizations were set at 54.7° to each other to remove orientational effects. In most experiments the temporal range was 1–1500 ps; adjustments were later made to the delay line which yielded an extended range of 1–5000 ps. Typically solutions (300 μL) with a nucleotide/Ru binding ratio of 20:1 were prepared and stirred continuously during each experiment. UV/Vis spectra taken before and after each run showed sample decomposition to be negligible.

Picosecond transient IR absorption spectroscopy: The picosecond TRIR experiments were carried out on the PIRATE (picosecond infrared absorption and transient excitation) apparatus as previously described.[43b] Briefly, part of the output from a 1 kHz, 800 nm, 150 fs, 2 mJ Ti-sapphire oscillator/regenerative amplifier (Spectra Physics Tsunami/Spitfire) was used to pump a white-light continuum seeded β-BaB_2O_4 (BBO) optical parametric amplifier (OPA). The signal and idler produced by this OPA were difference frequency mixed in a type I $AgGaS_2$ crystal to generate tunable mid-IR pulses ($\approx 150\,cm^{-1}$ FWHM, 1 μJ), which were split to give probe and reference pulses. Second harmonic generation of the residual 800 nm light provided 400 nm pump pulses. Both the pump and probe pulses were focused to a diameter of 200–300 μm in the sample. Changes in IR absorption at various pump–probe time delays were recorded by normalizing the outputs from a pair of 64-element MCT IR linear array detectors on a shot-by-shot basis. Samples (typically 50 μL) were contained in 56 μm cells (Harrick Scientific Corp.) fitted with CaF_2 windows. The samples were rastered to prevent decomposition.

Data processing: For the visible TA, each experimental run comprised five sets of data, with the order of time delays randomly varied, averaged over 20 s at each delay position. The raw data from the reference and probe signals for each pixel were imported into Excel and Δ(abs) was calculated using Equation (3) for all wavelengths.

$$\Delta(\text{abs}) = \log(I_{\text{ref}}/I_{\text{probe,pumped}}) - \log(I_{\text{ref}}/I_{\text{probe,unpumped}}) \quad (3)$$

Kinetic data were averaged over a range of 10 pixels (≈ 5 nm). Δ(abs) versus time delay curves were fitted for single exponential decays to the function $\Delta(\text{abs}) = A_1\exp(-\text{time}/\tau) + \Delta(\text{abs})^{\infty}$.

The data from the picosecond IR absorption experiments were processed at different single pixels corresponding to the most relevant bands in the IR spectra, and fitted to single or multi-exponential functions using Origin 6.0/7.0 software.

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[1] L. W. Roberts, G. B. Schuster, *Photochem. Photobiol.* **2004**, *80*, 456.
[2] A. Okamoto, K. Tanaka, I. Saito, *J. Am. Chem. Soc.* **2004**, *126*, 9458.
[3] Y. Ito, E. Fukusaki, *J. Mol. Catal. B* **2004**, *28*, 155.
[4] H. Yan, S. H. Park, G. Finkelstein, J. H. Reif, T. H. LaBean, *Science* **2003**, *301*, 1882.
[5] T. Carell, C. Behrens, J. Gierlich, *Org. Biomol. Chem.* **2003**, *1*, 2221.
[6] J. Reynisson, S. Steenken, *Phys. Chem. Chem. Phys.* **2002**, *4*, 5346.
[7] M. K. Beissenhirtz, I. Willner, *Org. Biomol. Chem.* **2006**, *4*, 3392.
[8] D. Porath, A. Bezryadin, S. de Vries, C. Dekker, *Nature* **2000**, *403*, 635.
[9] L. Wang, Y. Li, *Chem. Commun.* **2006**, 2557.
[10] S. O. Kelley, J. K. Barton, *Science* **1999**, *283*, 375.
[11] S. Boiteux, L. Gellon, N. Guibourt, *Free Radical Biol. Med.* **2002**, *32*, 1244.
[12] K. A. Friedman, A. Heller, *J. Phys. Chem. B* **2001**, *105*, 11859.
[13] H. E. Poulsen, H. Prieme, S. Loft, *Eur. J. Cancer Prev.* **1998**, *7*, 9.
[14] H. Wiseman, B. Halliwell, *Biochem. J.* **1996**, *313*, 17.

[15] Y. Chen, M. Wang, C. Mao, *Angew. Chem.* **2004**, *116*, 3638; *Angew. Chem. Int. Ed.* **2004**, *43*, 3554.
[16] G. D. Reid, D. J. Whittaker, M. A. Day, D. A. Turton, V. Kayser, J. M. Kelly, G. S. Beddard, *J. Am. Chem. Soc.* **2002**, *124*, 5518.
[17] C. Wagner, H.-A. Wagenknecht, *Chem. Eur. J.* **2005**, *11*, 1871.
[18] M. Fernandez-Saiz, P. T. Henderson, W. D. Wilson, G. B. Schuster, *Photochem. Photobiol.* **1999**, *70*, 847.
[19] P. T. Henderson, B. Armitage, G. B. Schuster, *Biochemistry* **1998**, *37*, 2991.
[20] J. Brunner, J. K. Barton, *Biochemistry* **2006**, *45*, 12295.
[21] D. Garcia-Fresnadillo, O. Lentzen, I. Ortmans, E. Defrancq, A. Kirsch-De Mesmaeker, *Dalton Trans.* **2005**, 852.
[22] B. Elias, A. Kirsch-De Mesmaeker, *Coord. Chem. Rev.* **2006**, *250*, 1627.
[23] E. Gicquel, N. Paillous, P. Vicendo, *Photochem. Photobiol.* **2000**, *72*, 583.
[24] L. Jacquet, R. J. H. Davies, A. Kirsch-De Mesmaeker, J. M. Kelly, *J. Am. Chem. Soc.* **1997**, *119*, 11763.
[25] A. Del Guerzo, A. Kirsch-De Mesmaeker, *Inorg. Chem.* **2002**, *41*, 938.
[26] C. Moucheron, A. Kirsch-De Mesmaeker, *J. Phys. Org. Chem.* **1998**, *11*, 577.
[27] J. P. Lecomte, A. Kirsch-De Mesmaeker, M. M. Feeney, J. M. Kelly, *Inorg. Chem.* **1995**, *34*, 6481.
[28] I. Ortmans, P. Didier, A. Kirsch-De Mesmaeker, *Inorg. Chem.* **1995**, *34*, 3695.
[29] C. Moucheron, A. Kirsch-De Mesmaeker, J. M. Kelly, *J. Photochem. Photobiol. B* **1997**, *40*, 91.
[30] L. Jacquet, J. M. Kelly, A. Kirsch-De Mesmaeker, *J. Chem. Soc. Chem. Commun.* **1995**, 913.
[31] J. M. Kelly, M. M. Feeney, A. B. Tossi, J. P. Lecomte, A. Kirsch-De Mesmaeker, *Anti-Cancer Drug Des.* **1990**, *5*, 69.
[32] D. Z. M. Coggan, I. S. Haworth, P. J. Bates, A. Robinson, A. Rodger, *Inorg. Chem.* **1999**, *38*, 4486.
[33] S. Satyanarayana, J. C. Dabrowiak, J. B. Chaires, *Biochemistry* **1992**, *31*, 9319.
[34] B. Onfelt, P. Lincoln, B. Norden, J. S. Baskin, A. H. Zewail, *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 5708.
[35] C. G. Coates, L. Jacquet, J. J. McGarvey, S. E. J. Bell, A. H. R. Al-Obaidi, J. M. Kelly, *J. Am. Chem. Soc.* **1997**, *119*, 7130.
[36] C. M. Dupureur, J. K. Barton, *J. Am. Chem. Soc.* **1994**, *116*, 10286.
[37] C. Hiort, P. Lincoln, B. Norden, *J. Am. Chem. Soc.* **1993**, *115*, 3448.
[38] R. M. Hartshorn, J. K. Barton, *J. Am. Chem. Soc.* **1992**, *114*, 5919.
[39] I. Ortmans, B. Elias, J. M. Kelly, C. Moucheron, A. Kirsch-De Mesmaeker, *Dalton Trans.* **2004**, 668.
[40] J. M. Kelly, C. M. Creely, M. M. Feeney, S. Hudson, W. J. Blau, B. Elias, A. Kirsch-De Mesmaeker, P. Matousek, M. Towrie, A. W. Parker, *Central Laser Facility Annual Report 2001*, CCLRC, Chilton, **2001**, p. 111.
[41] C. G. Coates, P. Callaghan, J. J. McGarvey, J. M. Kelly, L. Jacquet, A. Kirsch-De Mesmaeker, *J. Mol. Struct.* **2001**, *598*, 15.
[42] M. K. Kuimova, A. J. Cowan, P. Matousek, A. W. Parker, X. Z. Sun, M. Towrie, M. W. George, *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 2150.
[43] a) M. Towrie, A. W. Parker, W. Shaikh, P. Matousek, *Meas. Sci. Technol.* **1998**, *9*, 816; b) M. Towrie, D. C. Grills, J. Dyer, J. A. Weinstein, P. Matousek, R. Barton, P. D. Bailey, N. Subramaniam, W. M. Kwok, C. S. Ma, D. Phillips, A. W. Parker, M. W. George, *Appl. Spectrosc.* **2003**, *57*, 367.
[44] C. G. Coates, J. Olofsson, M. Coletti, J. J. McGarvey, B. Onfelt, P. Lincoln, B. Norden, E. Tuite, P. Matousek, A. W. Parker, *J. Phys. Chem. B* **2001**, *105*, 12653.
[45] E. J. C. Olson, D. Hu, A. Hormann, A. M. Jonkman, M. R. Arkin, E. D. A. Stemp, J. K. Barton, P. F. Barbara, *J. Am. Chem. Soc.* **1997**, *119*, 11458.
[46] M. Banyay, R. Sarkar, A. Gräslund, *Biophys. Chem.* **2003**, *104*, 477.
[47] A. T. Krummel, M. T. Zanni, *J. Phys. Chem. B* **2006**, *110*, 13991.
[48] M. K. Kuimova, J. Dyer, M. W. George, D. C. Grills, J. M. Kelly, P. Matousek, A. W. Parker, X. Z. Sun, M. Towrie, A. M. Whelan, *Chem. Commun.* **2005**, 1182.
[49] a) J. K. McCusker, *Acc. Chem. Res.* **2003**, *36*, 87; b) A. Vlcek, *Coord. Chem. Rev.* **2000**, *200*, 933.
[50] S. Wallin, J. Davidsson, J. Modin, L. Hammarstrom, *J. Phys. Chem. A* **2005**, *109*, 4697.
[51] a) J. Dyer, W. J. Blau, C. G. Coates, C. M. Creely, J. D. Gavey, M. W. George, D. C. Grills, S. Hudson, J. M. Kelly, P. Matousek, J. J. McGarvey, J. McMaster, A. W. Parker, M. Towrie, J. A. Weinstein, *Photochem. Photobiol. Sci.* **2003**, *2*, 542; b) J. Dyer, C. M. Creely, J. C. Penedo, D. C. Grills, S. Hudson, P. Matousek, A. W. Parker, M. Towrie, J. M. Kelly, M. W. George, *Photochem. Photobiol. Sci.* **2007**, *6*, 741.
[52] M. Sjodin, S. Styring, H. Wolpher, Y. H. Xu, L. C. Sun, L. Hammarstrom, *J. Am. Chem. Soc.* **2005**, *127*, 3855.
[53] a) D. O'Connor, V. Shafirovich, N. E. Geacintov, *J. Phys. Chem.* **1994**, *98*, 9831; b) V. Shafirovich, S. H. Courtney, N. Ya, N. E. Geacintov, *J. Am. Chem. Soc.* **1995**, *117*, 4920; c) V. Shafirovich, A. Dourandin, N. P. Luneva, N. E. Geacintov, *J. Phys. Chem. B* **2000**, *104*, 137; d) V. Shafirovich, A. Dourandin, N. E. Geacintov, *J. Phys. Chem. B* **2001**, *105*, 8431; e) V. Shafirovich, N. E. Geacintov, *Top. Curr. Chem.* **2004**, *237*, 129.
[54] S. C. Weatherly, I. V. Yang, P. A. Armistead, H. H. Thorp, *J. Phys. Chem. B* **2003**, *107*, 372.
[55] N. Iordanova, S. Hammes-Schiffer, *J. Am. Chem. Soc.* **2002**, *124*, 4848.
[56] B. T. Farrer, H. H. Thorp, *Inorg. Chem.* **1999**, *38*, 2497.
[57] R. I. Cukier, *J. Phys. Chem.* **1994**, *98*, 2377.
[58] E. L. Lebeau, R. A. Binstead, T. J. Meyer, *J. Am. Chem. Soc.* **2001**, *123*, 10535.
[59] S. Steenken, S. V. Jovanovic, *J. Am. Chem. Soc.* **1997**, *119*, 617.
[60] S. Schumm, M. Prévost, D. Garcia-Fresnadillo, O. Lentzen, C. Moucheron, A. Kirsch-De Mesmaeker, *J. Phys. Chem. B* **2002**, *106*, 2763.
[61] J.-P. Lecomte, A. Kirsch-De Mesmaeker, J. M. Kelly, A. Tossi, H. Görner, *Photochem. Photobiol.* **1992**, *55*, 681.
[62] F. L. Gervasio, A. Laio, M. Iannuzzi, M. Parrinello, *Chem. Eur. J.* **2004**, *10*, 4846.
[63] L. Sun, Y. Bu, *J. Phys. Chem. B* **2005**, *109*, 593.
[64] K. Kobayashi, S. Tagawa, *J. Am. Chem. Soc.* **2003**, *125*, 10213.
[65] R. D. Wells, J. E. Larson, R. C. Grant, B. E. Shortle, C. R. Cantor, *J. Mol. Biol.* **1970**, *54*, 465.

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