Time-resolved studies of guanine photo-oxidation by a DNA-bound Ru(II) complex in solution and crystal states

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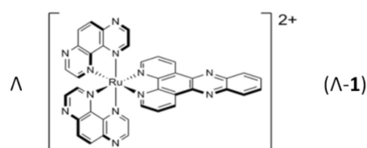
1. Introduction

Photo-sensitised oxidation of guanine is an important route to DNA damage with possible applications in phototherapeutics. In solution, an understanding of the process requires reliable knowledge of where and how the sensitiser is bound in DNA, a non-trivial matter due to the array of binding sites in a typical sequence. Dipyridophenazine (dppz) metal complexes are valuable photosensitisers as they are known to intercalate into DNA and their photophysical properties can be tuned either by changing the central metal atom or the ancillary ligands. Thus, using transient absorption and time-resolved infra-red measurements, we have shown that both $[Ru(TAP)_2(dppz)]^{2+}$ (Λ-**1**; TAP = 1,4,5,8-tetraazaphenanthrene)[1] and $[Cr(phen)_2(dppz)]^{3+}$ (phen = 1,10-phenanthroline)[2] can oxidise DNA, whereas $[Ru(phen)_2(dppz)]^{2+}$ does not. It has also been demonstrated for the first time that while both enantiomers intercalate into DNA the rates and efficiency of the electron transfer (ET) reaction is different.[1a] A general aim of our research, as illustrated below, is to understand the excited-state behaviour by (i) using insights from the structures revealed by X-ray crystallography studies of this class of compound[3] to predict the binding and ET in solution and (ii) to perform experiments in the crystal state where the binding geometry of all interacting species is precisely known.[3]



2. Experiments in solution

One of the most revealing results from our crystallographic studies is for Λ-$[Ru(phen)_2(dppz)]^{2+}$, which intercalates at the 5′-TA-3′ site in $\{CCGG\underline{TA}CCGG\}_2$ (oligodeoxynucleotide (ODN) **B**) but not the 5′-AT-3′ site in $\{CCGG\underline{AT}CCGG\}_2$ (ODN **A**).[3b] The binding selectivity observed in the crystal offers an opportunity to investigate whether this could also be identified in solution for Λ-$[Ru(TAP)_2(dppz)]^{2+}$ and, if so, how ET might be affected by binding at a 5′-TA-3′ site.[1b] In order to achieve this, the electron transfer between bound photo-excited Λ-**1** and guanine was studied on ULTRA with 400 nm excitation, using a combination of ps/ns transient absorption (to monitor reduction of the complex) and TRIR (to monitor oxidation of the DNA).

The TA spectra of Λ-**1** (400 μM) in the presence of ODN **A** (500 μM) show bleaching of the ground-state at 460 nm and rapid formation of the strongly oxidising ^{3}MLCT* state at 600 nm (data not shown). In the presence of **B** a new feature grows in at 515 nm, assigned to the reduction of the complex following ET from guanine to the complex. This species decays on the nanosecond timescale (17 ± 3 ns) corresponding to the reversible back ET from the $TAP^{\bullet-}$ ligand to $G^{\bullet+}$. Contrasting behaviour is observed in **B**; there is no significant formation of the reduced species, while the excited state decays with a lifetime of 120 ns, much slower than in ODN **A**, but still faster than the complex bound a G-deficient sequence such as poly$\{dA\text{-}dT\}_2$.

TRIR experiments were then performed in order to observe the corresponding behaviour in the DNA nucleobases region (*ca.* 1500 cm^{-1} – 1750 cm^{-1}). In the presence of ODN **B**, there is initial bleaching of C and G carbonyl bands at 1650 and 1680 cm^{-1}, respectively (see Fig. 1a). Most notably, a transient band is identified at 1700 cm^{-1}, previously attributed to the guanine radical cation.[4] Again, contrasting behaviour was observed with ODN **B** (Fig. 1b). The profiles of the bleaches differ substantially, and are similar to those of adenine and thymine. Also there is no formation of the G radical cation, confirming that photo-oxidation is very inefficient in this system.

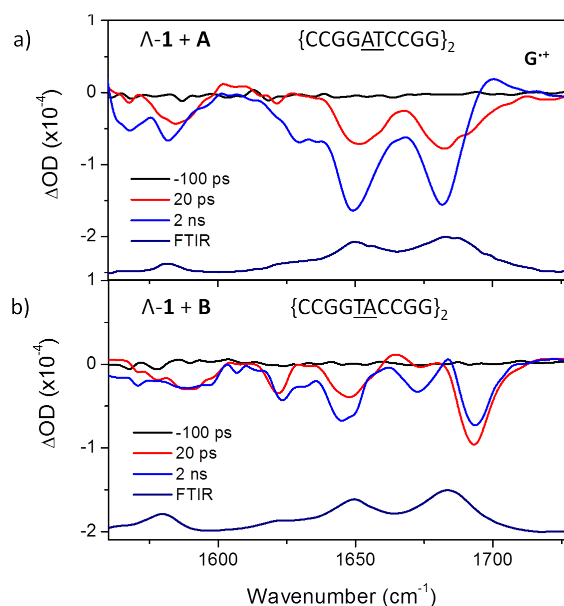


Figure 1. TRIR spectra of Λ-**1** in the presence of (a) ODN **A** (b) ODN **B**. DNA region shown (adapted from Keane *et al*[1b]).

These results are consistent with the predictions from the crystal structure, in that the complex can only bind to a GC-containing site in ODN **A**, while it can bind to the central 5 -AT-3′ step in ODN **B**. The latter results in IR bleaching signals indicative of thymine and adenine, and although formation of the electron transfer products was not observed, a slower long-range ET may be responsible for the 120 ns lifetime observed. These results showed that (1) the binding preference for 5 -TA-3′ vs 5 -AT-3′ is a phenomenon in solution as well as in the crystal (2) the efficient generation of the reduced and oxidised products requires the complex to be bound at a G-containing site, (c) the TRIR spectra offers a way to distinguish between binding at TA- and GC-rich sites, which would be difficult to do using other techniques.

However, in the case of ODN **B**, we are unable to confirm which of the possible G-containing steps the complex is bound at, and whether it was the terminal $C_1C_2;G_9G_{10}$ step as in the crystal structure. Indeed other experiments with different sequences have shown that the behaviour of Λ-**1** in solution can be very sensitive to small changes in sequence,[1a,c] and that it can be difficult to unambiguously verify whether the predictions of the crystal structure are valid under different conditions. Therefore there is a need, in order to more accurately define the site of ET in a given system, to study it in conditions where the geometry is accurately known.

3. TRIR experiments in the crystal state

To this end we turned our attention to the challenge of conducting TRIR measurements on crystal samples where the precise geometry of the intercalator is known. Crystals of Λ-**1** bound to $\{TCGGCGCCGA\}_2$ were chosen as the focus of this study[3a] as these crystals have been shown to be stable to reversible dehydration and hydration at room temperature.[5] The time-resolved experiment was performed on D_2O-exchanged micron-sized crystal fragments which allow transmission of infrared light. These crystals were placed between CaF_2 plates in a standard Harrick cell and excited at 400 nm using a 1 kHz laser under continuous raster. The samples were imaged before and after the experiment using an optical microscope to ensure that the integrity of the crystals was maintained.

Using this approach highly structured TRIR spectra were obtained upon excitation. At lower wavenumbers (1250-1545 cm^{-1}) the spectra are dominated by bleach (1275 cm^{-1}) and transient (1456 cm^{-1}) bands associated with the metal complex, see Fig. 2a. At higher wavenumbers features associated with the DNA bases are observed. The four strong distinct bleaches can be assigned to the ring vibrations of adenine and guanine (at 1620 cm^{-1} and 1580 cm^{-1} resp.) and the carbonyl stretches of cytosine and guanine (1645 cm^{-1} 1680 cm^{-1} resp.). The appearance of these bands may be attributed to the perturbation of the ground-state vibrations by the change in the charge distribution of the proximal excited state complex (Stark effect). This effect is highly localised (typically < 6 Å) and therefore is a useful reporter of the excited state environment in a DNA sequence.

Importantly the presence of these bands is also in excellent agreement with the position of the complex as resolved by X-ray diffraction methods, see Fig. 2b.[3a] Interestingly, while the FTIR spectra for the crystals and the solution are similar, a notable difference is the absence of the adenine bleach in the latter. This suggests that the binding site may be different in the crystal and solution.

Next the dynamics of the electron transfer were considered. The profile of the spectra is found to change as a function of time. Importantly, between 20 ps and 2 ns an additional transient band is found to grow in at 1709 cm^{-1}. This band is assigned to the formation of the guanine radical cation.[4] Kinetic analysis of this band yields a rate constant for the forward electron transfer of 1/500 ps^{-1}. The disappearance of this band is found to occur on the same timescale as the repopulation of the metal complex ground state (recovery of the bleach at 1275 cm^{-1}) with a rate constant of 1/10 ns^{-1}.

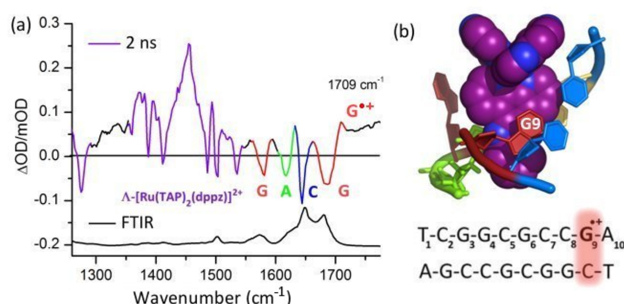


Figure 2. (a) TRIR spectra after 2 ns following 400 nm (120 fs, 1 μJ, 1 kHz) excitation of Λ-**1** in the presence of $\{TCGGCGCCGA\}_2$. (b) The terminal intercalation site showing the complex intercalated, through the dppz group, into the G_9A_{10}:T_1C_2 step and the proposed site of photo-oxidation (adapted from Hall *et al*[6]).

X-ray crystallography reveals three possible sites for guanine oxidation in this system. These are the G_3 and G_4 bases, which are bound by TAP ligand wedged into the G_3G_4:C_7C_8 step and the G_9 base which is stacked onto the central pyrazine ring of the intercalated dppz ligand at the terminal T_1C_2:G_9A_{10} step (Fig. 2b). By considering the proximity of these bases to the central metal of the excited state we are able to propose the G_9 base as the site of reversible photo-oxidation in this system.

Conclusions

The ultrafast dynamics of photosensitised electron transfer in DNA systems has been profiled in both the solution and solid state. In the case of solution studies the use of complementary TA and TRIR provides an excellent tool to follow the excited state of the metal complex and the DNA photoproducts. Here we have demonstrated how, informed by crystallography, these techniques can be used to resolve the mechanism of guanine photo-oxidation by a DNA-bound Ru(II) complex in solution and crystal states.

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