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Study of picosecond processes of an intercalated dipyridophenazine Cr(III) complex bound to defined sequence DNAs using transient absorption and time-resolved infrared methods†

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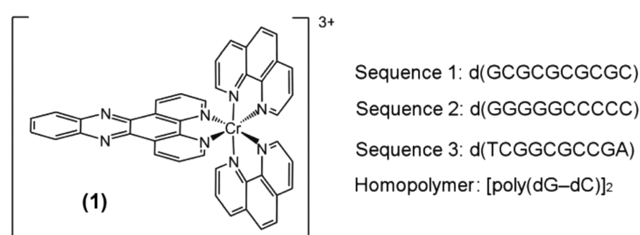
Picosecond transient absorption (TA) and time-resolved infrared (TRIR) measurements of *rac*-$[Cr(phen)_2(dppz)]^{3+}$ (1) intercalated into double-stranded guanine-containing DNA reveal that the excited state is very rapidly quenched. As no evidence was found for the transient electron transfer products, it is proposed that the back electron transfer reaction must be even faster (<3 ps).

It is well known that the fluorescence or phosphorescence of many intercalated organic molecules and metal complexes is quenched when the molecules bind to DNA and especially in the presence of guanine bases.[1] The quenching mechanism is usually assumed to proceed *via* electron transfer from the guanine to the photosensitiser, but definitive evidence is not always available. However in the case of metal complexes such as $[Ru(TAP)_2(dppz)]^{2+}$ [2] or various $Re(CO)_3$dppz complexes[3] bound to synthetic polynucleotides the forward and back electron transfer processes have been monitored by TA and TRIR. The dynamics of these systems are expected to be influenced by factors such as the spin state of the excited species (*e.g.* singlet *versus* triplet), the nature of the excited state (in the case of metal complexes whether ligand-centred, metal-centred or charge transfer in nature) or possibly by the precise geometry of the binding site or the thermodynamics of the electron transfer process.

In contrast to ruthenium and rhenium dppz complexes their Cr(III) counterparts have received relatively little attention, although $[Cr(phen)_2(dppz)]^{3+}$ (**1**) has been shown to bind to DNA by intercalation.[4] The binding modes are likely to be similar to those revealed by recent crystal structures of $[Ru(phen)_2(dppz)]^{2+}$ or $[Ru(TAP)_2(dppz)]^{2+}$ bound to short defined-sequence DNA.[5] Chromium polypyridyls are also known to have a strongly oxidising long-lived excited state making them potentially powerful photooxidative agents.[6] In solution the excited state, which is a doublet metal-centred (^{2}MC) species, has a lifetime of (>50 μs) and is readily monitored both by its phosphorescence and by transient absorption. As with other Cr(III) polypyridyls,[7] the excited state of **1** is quenched by 5′-GMP *via* a dynamic process.[8] In contrast when bound to DNA the quenching is dominated by a static process, which indicates that the process occurs on a sub-nanosecond time scale.[8] In both cases, while the quenching is fully consistent with electron transfer to the excited state of the metal complex from the guanine, no evidence of this could be found in our nanosecond laser flash photolysis experiments.[8] This suggests that the back reaction must also be rapid.

To explore this matter further we have carried out picosecond experiments with $[Cr(phen)_2(dppz)]^{3+}$ bound to defined sequence short double-stranded guanine-containing DNA molecules (Scheme 1) and to the synthetic polynucleotide $[poly(dG–dC)]_2$. In order to monitor both the metal complex and the DNA nucleobases on a picosecond timescale, we have used transient visible absorption (allowing us to monitor the



Scheme 1 $[Cr(phen)_2dppz]^{3+}$ complex (**1**) together with the series of self complementary sequences used in this study.

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† Electronic supplementary information (ESI) available: For experimental details and additional steady-state and transient data for d(GGGGGCCCCC); d(TCGGCGCCGA), as well as to $[poly(dG–dC)]_2$ systems. See DOI: 10.1039/c4dt01989j

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metal complex and/or products) and time-resolved infrared spectroscopy (where both the characteristic vibrations of the metal complex ligands and the nucleobase vibrations can be followed[9]). We have previously taken this approach when studying selected ruthenium[2] and rhenium complexes.[3]

Titrations of *rac*-[Cr(phen)$_2$(dppz)](Cl)$_3$ with each of the four GC systems resulted in significant hypochromism (35%) in the absorbance of the dppz $\pi \rightarrow \pi^*$ bands (*ca.* 360 nm), see Fig. 1(a). This change is accompanied by the appearance of characteristic isosbestic points near 388 nm and increased absorption at longer wavelengths. In aqueous solution the chromium complex displays strong room-temperature emission at 730 nm due to metal-centred phosphorescence. Fig. 1(b) shows that excitation of the complex at the isosbestic point in the presence of increasing concentration of d(GCGCGCGCGC) resulted in quenching of the emission (the same phenomenon was observed at λ_{ex} = 400 nm). We can see that this emission is fully quenched in the presence of 20 nucleobases, which is the equivalent of a single duplex formed from the d(GCGCGCGCGC) sequence. This was also found for the other GC-rich sequences (see Fig. S1–S5, ESI†). The emission quenching combined with the strong hypochromism in the UV-visible spectrum indicates that the complex possesses a strong affinity for these systems.

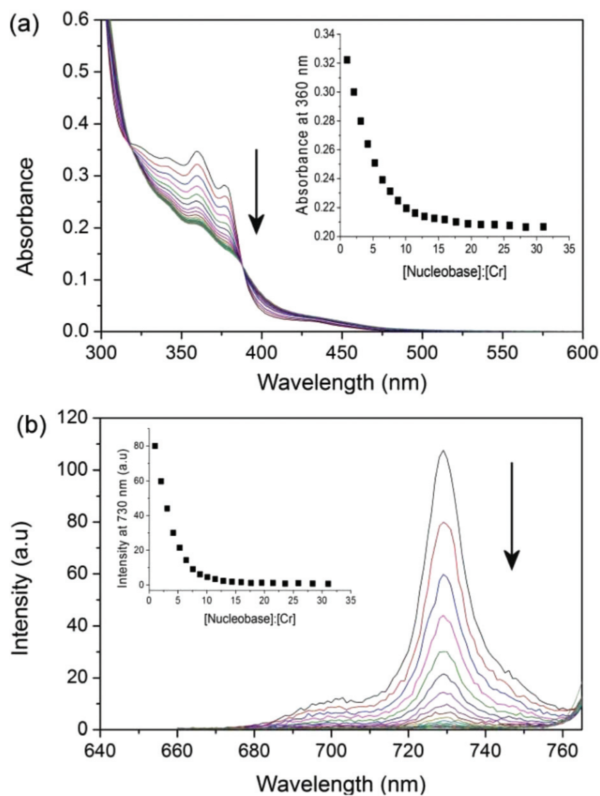

Fig. 1 (a) UV-visible absorption spectra of *rac*-[Cr(phen)$_2$dppz]$^{3+}$ (24 μM in 50 mM phosphate buffer solution, pH = 7.4) in the presence of increasing concentrations of d(GCGCGCGCGC). Inset: Change in absorbance at 360 nm. (b) The corresponding phosphorescence spectra (λ_{ex} = 388 nm). Inset: Change in the emission intensity at 730 nm.

Excitation with a 400 nm (150 fs) pulse of *rac*-[Cr(phen)$_2$dppz]$^{3+}$ (**1**) bound to each of the GC-containing DNAs in D_2O solution yields a strong transient absorption with maximum absorption at about 500 nm, see Fig. 2, S6–S7 and Table S1, ESI.† This spectrum is closely similar to that previously recorded for the free complex on the nanosecond timescale,[8] which suggests that by 2 ps the complex is already in its ^{2}MC state. However the kinetics of the complex in the presence of DNA are quite different to that of the complex in free solution. When bound to DNA there is a very rapid decay of the transient absorption, dominated by a species with a lifetime of *ca.* 3 ps, whereas in free solution the excited state has a lifetime in excess of >50 μs. This is consistent with very rapid electron transfer from the guanine to the excited state. This rapid decay is, however, not accompanied by the growth of any new species having a characteristic strong absorption band. As well as the very rapid decay, another slower-decaying smaller-amplitude transient is observed in each case (This minor transient species appears to have its absorption maximum at slightly longer wavelength). This could possibly originate from complex **1** bound in a different binding site or perhaps to a transient product with an absorption spectrum similar to that of the excited state. Distinguishing these alternatives may be possible using oligonucleotides with different sequences.

To investigate these phenomena more thoroughly we have also recorded the time-resolved infrared spectra of *rac*-[Cr(phen)$_2$dppz]$^{3+}$ bound to each DNA sequence. As shown in Fig. 3, the TRIR spectrum recorded from **1** bound to

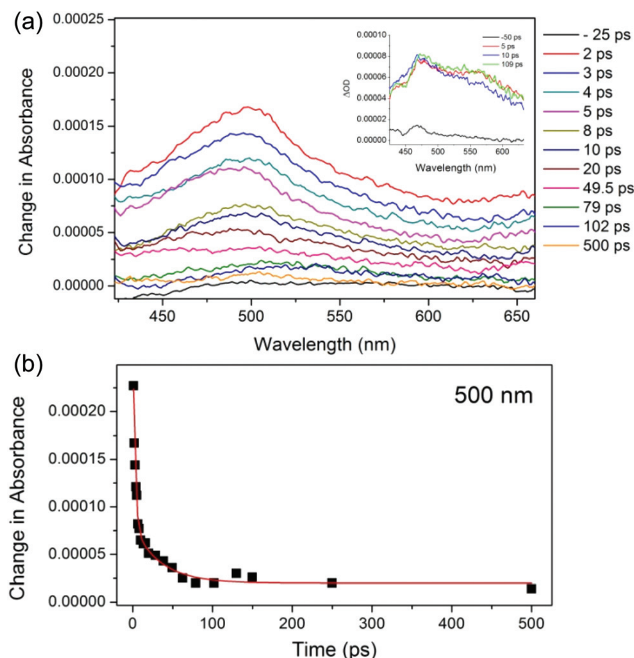

Fig. 2 (a) ps-TA of 800 μM *rac*-[Cr(phen)$_2$dppz]$^{3+}$ in presence of 2 mM d(GCGCGCGCGC) in 50 mM phosphate buffer solution, pH = 7.4. Inset: ps-TA for 400 nm excitation of *rac*-[Cr(phen)$_2$dppz]$^{3+}$ in D_2O. (b) Biexponential fit for the loss of absorbance at 500 nm. Excitation at 400 nm, 1 μJ.

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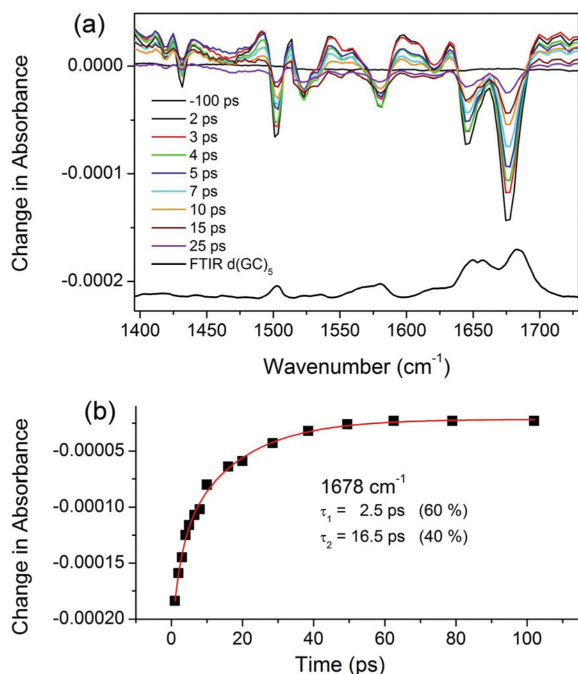

Fig. 3 (a) ps-TRIR of 400 μM *rac*-$[Cr(phen)_2dppz]^{3+}$ in presence of 2 mM d(GCGCGCGCGC) in 50 mM phosphate buffer solution, pH = 7.4. (b) Biexponential fit for the recovery of the guanine bleach at 1678 cm^{-1}. Excitation at 400 nm, 1 μJ.

d(GCGCGCGCGC) after laser excitation shows a number of transient absorption and bleaching (due to depletion of the ground state) bands. Those in the region below 1610 cm^{-1} are predominantly due to the phenanthroline and phenazine ligand components of the Chromium complex, (see Fig. S8, ESI†). The signals decay with rates similar to those found by transient absorption and no significant shift in the position of the bands was observed during the decay process. This is consistent with both the short and longer decay processes involving the decay of excited states, possibly at different sites on the duplex. Above 1610 cm^{-1} the TRIR spectrum is dominated by two strong bleaches at *ca.* 1644 and 1678 cm^{-1}, which are due primarily to cytosine and guanine absorptions in the DNA (Fig. 3 and Fig. S9–S11, ESI†). These bands recover rapidly with kinetics similar to those observed both for the TA and for the TRIR of the metal complex bands, see ESI Table S2.†[10]

The appearance of bleached bands in the region of DNA IR absorption indicates that the base-pair vibrations are affected by the excitation of **1**. This change may be caused by perturbation of the binding site by the changed electron density of the excited state. It is also noteworthy that the bleaching pattern is rather similar to that found on direct excitation of $[poly(dG–dC)]_2$.[11] In that case the initial process observed was proposed to be due to vibrationally excited DNA and it is possible that such a species might be present here to some extent. Bleaching was also previously observed upon excitation of $[Ru(TAP)_2dppz]^{2+}$ bound to $[poly(dG–dC)]_2$.[2] However the subsequent changes in the absorbance were quite different, as the guanine underwent oxidation.

It has previously been reported in a number of studies of both direct (UV) and photosensitised photo-oxidation of DNA that one-electron oxidised guanine has a characteristic absorption at about 1700 cm^{-1}.[12] However no clear evidence for this band was observable for any of the systems studied here (Fig. 3, S9–11†) This is therefore consistent with any electron transfer products formed by the initial electron transfer being very short-lived. Very short-lived electron transfer products (760 fs) have previously been observed for thionine bound to $[poly(dG–dC)]_2$.[1c] Such rapid back electron transfer may be a consequence of the favourable thermodynamics due to the exothermicity of the forward electron transfer process (estimated for this Cr-dppz complex to be *ca.* 22 kJ mol^{-1} assuming the oxidation potential of guanine to be 1.29 V).[8]

Conclusions

The concurrent use of high sensitivity TA and TRIR not only allows the excited state dynamics of *rac*-$[Cr(phen)_2(dppz)]^{3+}$ (**1**) in the presence of G-containing DNA to be profiled, but also enables the monitoring of the ultrafast processes induced in the DNA. In contrast to the free complex ps-TA results reveal rapid deactivation of the excited state of *rac*-$[Cr(phen)_2(dppz)]^{3+}$ (**1**) when bound, while ps-TRIR reveals bleaching of the ground state GC bands of DNA which recover on the ps timescale. Neither technique reveals evidence for electron transfer products, consistent with the back reaction being extremely rapid (<3 ps).

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