Time-resolved studies reveal that DNA photo-oxidation by a Ru(II) polypyridyl complex is highly sensitive to the presence of modified bases

Contact keanepa@tcd.ie, c.j.cardin@rdg.ac.uk, susan.quinn@ucd.ie, jmkelly@tcd.ie

P. M. Keane, J. P. Hall, S. P. Gurung, C. J. Cardin
Dept. of Chemistry, University of Reading
RG6 6AD, UK

F. E. Poynton, T. Gunnlaugsson, J. M. Kelly
School of Chemistry, Trinity College Dublin
Dublin 2, Ireland

I. V. Sazanovich, I. P. Clark, M. Towrie
Central Laser Facility, Research Complex at Harwell, STFC
OX11 0QX UK

F. R. Baptista, S. J. Devereux, S. J. Quinn
School of Chemistry, University College Dublin
Belfield, Dublin 4, Ireland

Introduction

Inosine is a naturally occurring DNA nucleobase that has several biological roles including RNA editing and the promotion of genetic biodiversity.[1,2 and refs therein] Inosine can substitute for guanine in double-stranded DNA forming an IC base-pair. While IC and GC present a similar profile in the major groove of DNA, the IC base-pair is structurally comparable to AT when approached from the minor groove (Fig. 1). This presents the intriguing possibility that a DNA-binding drug molecule may recognise an inosine-modified site as either GC or AT, depending on which DNA groove it binds in. Moreover, it suggests that this property could be exploited to determine where and how the drug binds - a key factor in determining its biological activity.

A further useful property of inosine is the fact that its oxidation potential is ca. 200 mV higher than guanine. As a result, it can be used as a control to study molecules that photo-oxidise guanine.[1-3] An important class of photoactive DNA-binding compounds are Ru(II) polypyridyl complexes, which exhibit tunable photochemical properties and show promise as phototherapeutic agents. One well studied example in our team is $[Ru(TAP)_2(dppz)]^{2+}$ (**1**, see Fig. 1), which intercalates into DNA and can photo-oxidise guanine by one-electron transfer.[3] We previously reported time-resolved infrared (TRIR) studies of Λ-**1** bound to $\{TCGGCGCCGA\}_2$ (**G9**) in the crystal state. These studies suggest that, in the crystal form at least, G_9 is the primary target for photo-oxidation as the complex intercalates at the $T_1C_2;G_9A_{10}$ step.[4] In order to determine whether comparable behaviour occurs in solution, transient absorption (TrA) and TRIR experiments were performed with **G9** and the inosine-substituted sequence **I9**. We expected a decrease in yield and lengthening of lifetime if binding occurred at $T_1C_2;I_9A_{10}$, as this site does not contain guanine.

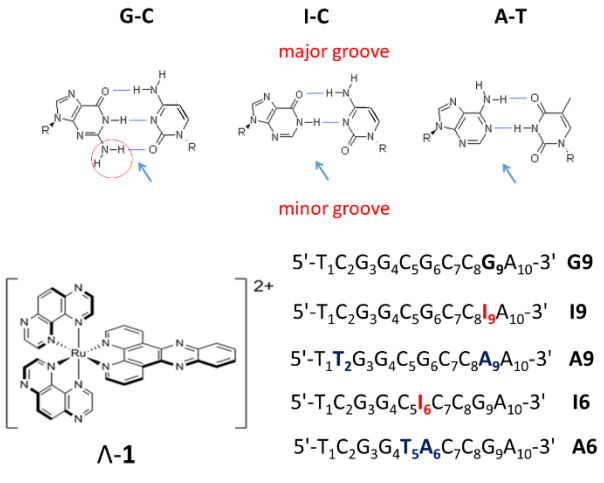

Figure 1. Ru(II) complex and DNA sequences in this study.

Results

Ps/ns TrA/TRIR experiments were performed on ULTRA for Λ-**1** in the presence of **G9**, **I9**, and the AT-substituted sequence **A9**. TrA is very sensitive to electronic changes in the metal complex, as the reduced species generated after guanine photo-oxidation has a strong absorption at 515 nm. The grow-in of this band, revealing the rate of electron transfer (ET), is plotted in Fig. 2. Interestingly, for both **I9** and **A9**, a higher yield of reduced species is observed, although the rates of ET are similar with all three sequences (ca. 500 ps). However, when the rate of reverse ET is monitored on the ns timescale, these were found to be similar for **I9** and **A9** (8 ns), and significantly faster than **G9** (17 ns).

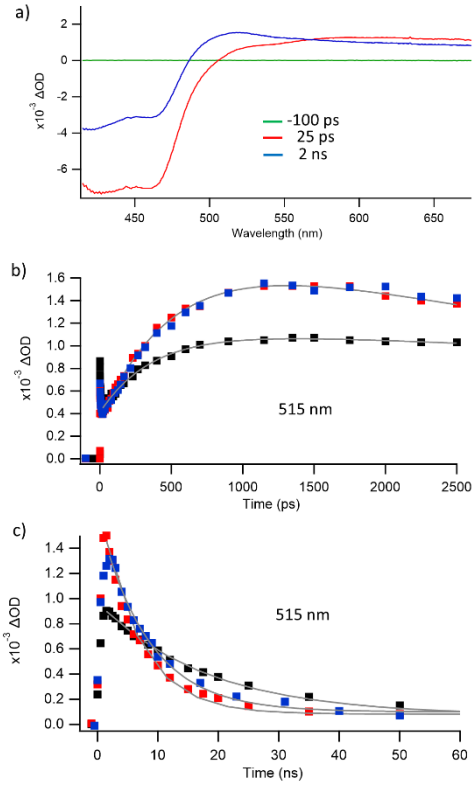

Figure 2. (a) Ps-TrA spectra of Λ-**1** bound to **I9** at selected delays (λ_{exc} = 400 nm) (b) Kinetics for forward ET for Λ-**1** in presence of **G9** (black), **I9** (red) and **A9** (blue) (c) Kinetics for reverse ET (λ_{exc} = 355 nm). From Keane et al.[1] Copyright (2017) Wiley-VCH.

TRIR is an excellent reporter on DNA as the nucleobase functional groups (especially carbonyls) are relatively strongly absorbing in the 1600-1700 cm^{-1} region. The characteristic features of DNA photo-oxidised by this class of compound are

(a) bleaching of the C and G carbonyl bands at 1650 cm^{-1} and 1680 cm^{-1}, respectively, and (b) formation of a transient feature at 1700 cm^{-1}, assigned as the guanine radical cation. These features are evident in the TRIR spectra of Λ-**1** with **G9** and **I9** (Fig. 3). However, there is a greater extent of oxidation with **I9**, as demonstrated by the more intense bleaching and a stronger band at 1700 cm^{-1} (similar behaviour was recorded with **A9**).

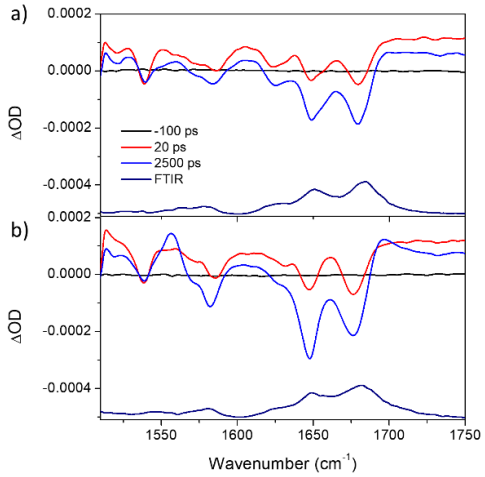

Figure 3. Ps-TRIR spectra of Λ-**1** in the presence of (a) **G9** (b) **I9** at selected delays (λ_{exc} = 400 nm). See Keane et al.[1]

The transient studies showed that Λ-**1** interacted similarly with **I9** and **A9**. This was confirmed by steady-state binding titrations, where stronger binding was observed with **I9** and **A9** compared to **G9**.[1] While the **I9**/**A9** sequences represent replacement of one base in a binding site, substitution of the C_5G_6;C_5G_6 central step creates a completely modified site. Therefore, in order to see if Λ-**1** would interact similarly with a CI;CI and TA;TA step, we also performed transient spectroscopic studies on Λ-**1** in the presence of **I6** and **A6** (Fig. 4). We have previously observed, in both solution[5] and crystal structures,[6] that the Λ (though not Δ) enantiomer binds preferentially to TA;TA steps. Binding to these steps is readily identifiable by inefficient ET, and consistent with this, ps-TrA spectra revealed a very low yield of ET with **I6** and **A6** (data not shown). Exponential fits to the ns-TrA spectra at 600 nm give an excited state decay of ca. 80 ns for both **I6** and **A6**, significantly slower than that with **G9** (17 ns). TRIR spectra have prominent bleaches at short delay times, believed to be indicative of the interaction of the photo-excited complex with closely neighbouring nucleobases. However, there is (a) no evolution of bleaches over the following 2 ns and (b) no guanine radical cation band at 1700 cm^{-1}, implying that very little guanine photo-oxidation takes place.

Discussion

The data demonstrates that for both the 6- and 9-substituted systems, Λ-**1** recognises the IC and AT base-pairs similarly. This offers strong evidence that this class of compound intercalates from the minor groove in solution, as previously observed in crystal structures.[3b,6] An initially surprising observation is that the removal of guanine-9 actually increases the yield of ET. It is suggested that in this case binding is favoured at the inosine site due to the removal of the 2-amino group (see Fig. 1), reducing hindrance with the ancillary TAP ligands. The proposed binding site is not the terminal step but the neighbouring C_2G_3;C_8I_9 step, where the complex would be close to the 5′-G of the GG doublet, a known 'hotspot' for DNA damage (Fig. 5) (consistent with this model, substitution of either or both of these guanines with inosine results in a considerable decrease in the yield of ET compared to **G9**).[2] By contrast with substitution of guanine-9, replacement of guanine-6 has the opposite effect, with a drastic decrease in the extent of ET. The 80 ns decay with Λ-**1** - **I6**/**A6** lifetime is characteristic of binding at a site (Fig. 5) that does not contain guanine, but is still close enough to cause some excited state quenching (the lifetime of free Λ-**1** is approx. 1000 ns).

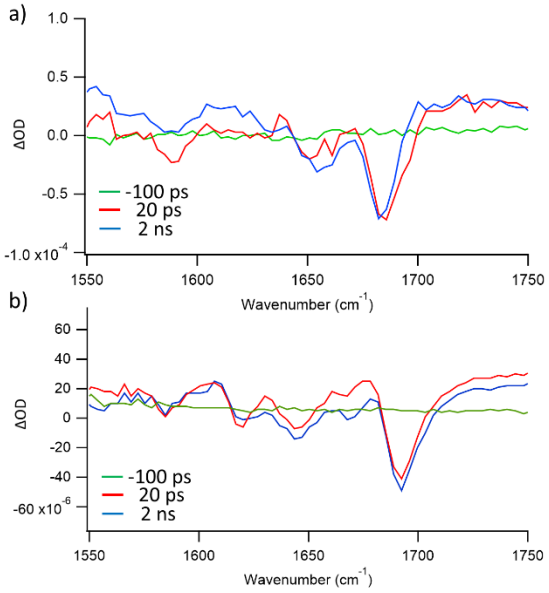

Figure 4. Ps-TRIR spectra of Λ-**1** in the presence of (a) **I6** (b) **A6** at selected delays after 400 nm excitation.

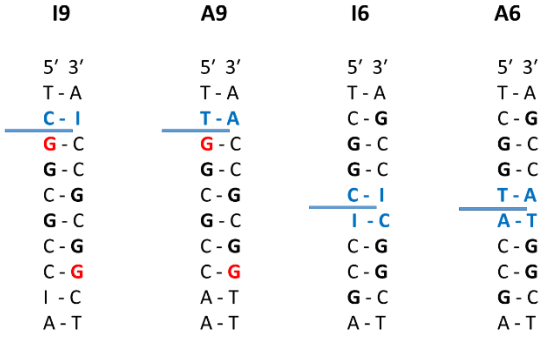

Figure 5. Proposed primary binding sites for Λ-**1** in IC/AT substituted sequences.

Conclusions

This study has shown how small changes in a DNA sequence can have a large effect on the photodynamic action of an intercalated metal complex. This is significant for the design of diagnostic/therapeutic agents that target modified DNA, such as may occur at mismatches or point mutations. This work also highlights that use of inosine as an effective control for guanine oxidation may require knowledge of whether the sensitiser binds from the minor or major groove.

Acknowledgements

Programme access to CLF (Apps 15230018 & 16130042), BBSRC (grants BB/K019279/1 & BB/M004635/1), Royal Irish Academy /Royal Society International Exchange Scheme award, Science Foundation Ireland PI Awards 10/IN.1/B2999 and 13/IA/1865, Irish Research Council, and UCD College of Science.

References

1. P. M. Keane, J. P. Hall, F. E. Poynton, B. C. Poulsen, S. P Gurung, I. P. Clark, I. V. Sazanovich, M. Towrie, T. Gunnlaugsson, S. J. Quinn, C. J. Cardin and J. M. Kelly, *Chem. – Eur. J.*, 2017, **23**, 10344.

2. P. M. Keane, F. E. Poynton, J. P. Hall, I. P. Clark, I. V. Sazanovich, M. Towrie, T. Gunnlaugsson, S. J. Quinn, C. J. Cardin and J. M. Kelly, *Faraday Discuss.*, 2015, **185**, 455.

3. (a) P. M. Keane and John M. Kelly, *Coord. Chem. Rev.*, 2018, **364**, 167 (b) C. J. Cardin, J. M. Kelly and S. J. Quinn, *Chem. Sci.*, 2017, **8**, 4705.

4. J. P. Hall, F. E. Poynton, P. M. Keane, S. P. Gurung, J. A. Brazier, D. J. Cardin, G. Winter, T. Gunnlaugsson, I. V. Sazanovich, M. Towrie, C. J. Cardin, J. M. Kelly and S. J. Quinn, *Nat. Chem.*, 2015, **7**, 961.

5. P. M. Keane, F. E. Poynton, J. P. Hall, I. V. Sazanovich, M. Towrie, T. Gunnlaugsson, S. J. Quinn, C. J. Cardin and J. M. Kelly, *Angew. Chem. Int Ed.*, 2015, **54**, 8364.

6. H. Niyazi, J. P. Hall, K. O'Sullivan, G. Winter, T. Sorensen, J. M. Kelly and C. J. Cardin, *Nat. Chem.*, 2012, **4**, 621.