Picosecond processes in DNA monitored by transient infra-red absorption spectroscopy

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Introduction

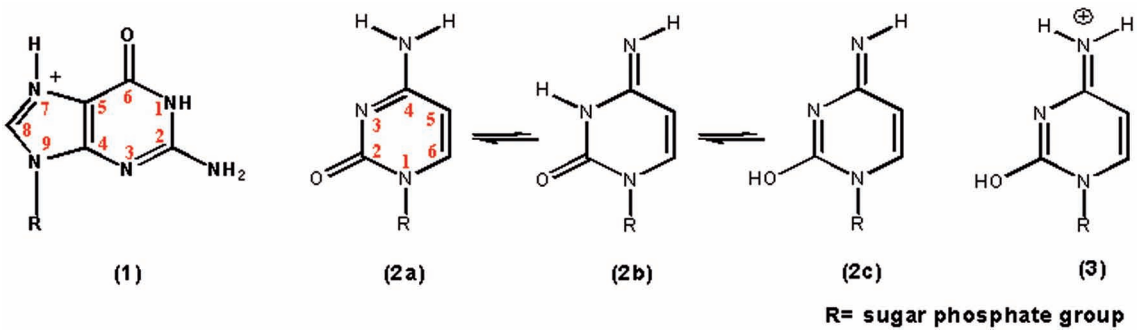
It has long been recognised, especially because of their low fluorescence quantum yields,[1] that the singlet excited state bases in DNA are short-lived. Only in recent years with the advent of femtosecond transient visible absorption[2] and fluorescence upconversion[3] methods has direct observation of these species become possible. These techniques have revealed that the bases and mononucleotides possess sub-picosecond excited state lifetimes. The reasons for these short lifetimes is still a matter of debate, with theoretical studies emphasising the importance of ultrafast non-radiative processes,[4] while the possibility of the formation of tautomers[5] cannot be discounted. The latter area is one where infra-red transient absorption spectroscopy opens up the opportunities to observe any longer-lived species present after radiationless deactivation of the excited state and to characterise them through their vibrational spectra.

With polynucleotides there is evidence that there are longer-lived excited states,[2] although there is still a need to more fully characterise the species, especially in mixed sequence compounds. Again in this area, the ability of PIRATE to identify the excited species is expected to be very powerful.

Previous experiments in Central Laser Facility have shown for the mononucleotides dGMP, dAMP, dCMP and TMP in neutral solution that there is evidence for species substantially longer-lived than their respective excited states.[6] These species were assigned to vibrationally excited ground states, formed after deactivation of the singlet excited state. To learn more about these processes we have investigated (a) the effect of pH on the transient behaviour of GMP and dCMP and (b) the effect of forming stacked structures both in GMP at high concentration and in the polynucleotide polyG.

5'-guanosine monophosphate

Figure 1a shows the spectra of a dilute solution of $GMPNa_2$ (8.4 mM, pH 8.5) at various delays after excitation with 267 nm radiation. (Closely similar behaviour is observed at pH 7.) The spectrum contains regions of transient absorption and ones where depletion ('bleaching') of the ground state bands are dominant. Both decay completely within about 20 ps, indicating the reversibility of the system. It may be noted that the maxima of the transient absorption bands shift to higher wavenumbers within the first 5 ps – a feature that is consistent with our earlier assignment of these bands to a vibrationally excited ground state.[6] This shifting of the position of the transient absorption band affects the shape of the bleaches, which are a convolution of the transient absorption and ground state depletion. This is particularly apparent for the band arising from the ground state vibration at 1577 cm^{-1} (C=C and C=N stretches within the purine ring). This has a major effect on the apparent decay kinetics, which are found to be much shorter at lower wavenumber. (e.g. 1.7 ± 0.2 ps at 1560 cm^{-1}; 4.6 ± 0.4 ps at 1572 cm^{-1}). We assume that the latter value more correctly measures the rate constant for the relaxation of these excited ring vibrations, as the rate of recovery of the lower frequency bleach may be masked by overlap with the neighbouring transient. For the C=O stretch this distortion effect is smaller and the decay constants do not vary so significantly (e.g. 2.1 ± 0.2 ps at 1649 cm^{-1}; 2.7 ± 0.3 ps at 1662 cm^{-1}). It may be observed that the lifetime of the C=O vibrationally excited state is significantly shorter (2.7 ps) than that of the ring vibration (>4.6 ps). This is presumably a consequence of more effective coupling between the C=O and the solvent.



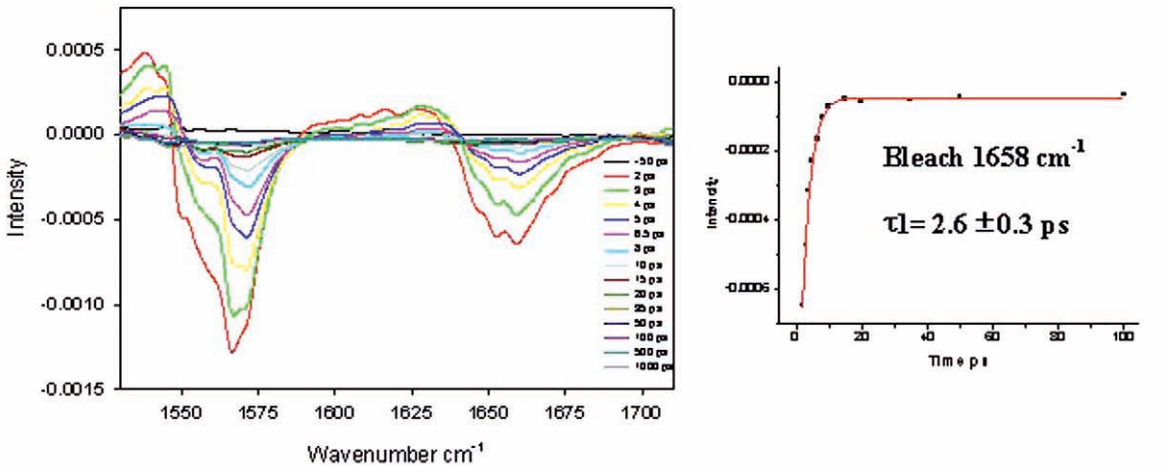


Figure 1. 8.4 mM $GMPNa_2$ in alkaline conditions, 50 mM Na_2HPO_4 in D_2O, pH=8.5. (a) Transient spectra recorded at delays of –50, 2, 3, 4, 5, 6.5, 8, 10, 15, 20, 35, 50, 100, 500, 1000 ps after excitation. (b) Kinetics taken at the carbonyl band (1658 cm^{-1}).

In acid solution it is known that the guanine base is protonated at the 7-position (1), resulting in three bands (at 1577 cm^{-1}, 1607 cm^{-1} and 1690 cm^{-1}) in the ground state FTIR spectrum. The picosecond transient behaviour is also quite different {Figure 2 records this for $GMPH_2$ (10 mM) in H_3PO_4 (0.132 M) in D_2O.}, with both the decay of the transient absorption (bands at 1515 cm^{-1} and 1636 cm^{-1}) and the recovery of the ground state proceeding much more slowly than that found in neutral or alkaline solution. These observations are consistent with the formation of a protonated excited state, which has previously been identified by fluorescence spectroscopy [7a] and by transient visible absorption spectroscopy. [7b] The recovery and decay kinetics of this species recorded in each of the depletion and transient absorption bands give similar first order kinetics (lifetime = 220 ± 20 ps), comparable to that determined by the other techniques. [7] Assignment of the vibrational bands of the transients will require examination by computational methods, but provisionally we assign these to the carbonyl (1640 cm^{-1}) and purine-ring (1515 cm^{-1}) vibrations as in the ground state. It may also be noted that in this acidified solution, the contribution from a very rapid process such as that expected for formation of vibrationally excited ground state, is very small. This is consistent with most of the GMP being protonated in the ground state and suggests that the yield of the protonated excited state is high.

5′-deoxycytidine monophosphate

Figure 3 shows the spectra of dCMP (**2a**) in neutral (buffered pH7) solution. (Identical spectra were also recorded at pH 8.5.) Unlike what is observed for GMP under such conditions, the data clearly shows that there are two kinetic phases. At short times (2-8 ps, shown in red) the behaviour of the transient absorption bands (e.g. shifting on this timescale of the bands to larger wavenumber) is consistent with relaxation of vibrationally excited ground states. The time constant for this is determined to be 2.6 ± 0.4 ps. After this process is complete, however, there is still a strong transient absorption. This longer-lived species decays (and the ground state recovers) with a lifetime of 33 ± 4 ps. Previous reports have only identified the decay of excited states (transient visible absorption) [8] or the vibrationally excited ground state (ps-IR with restricted spectral range). [6] A definite assignment of this longer-lived species will require further study – but we consider it probable that it is a tautomer, such as **2b** or **2c** or possibly a longer-lived non-emissive excited state.

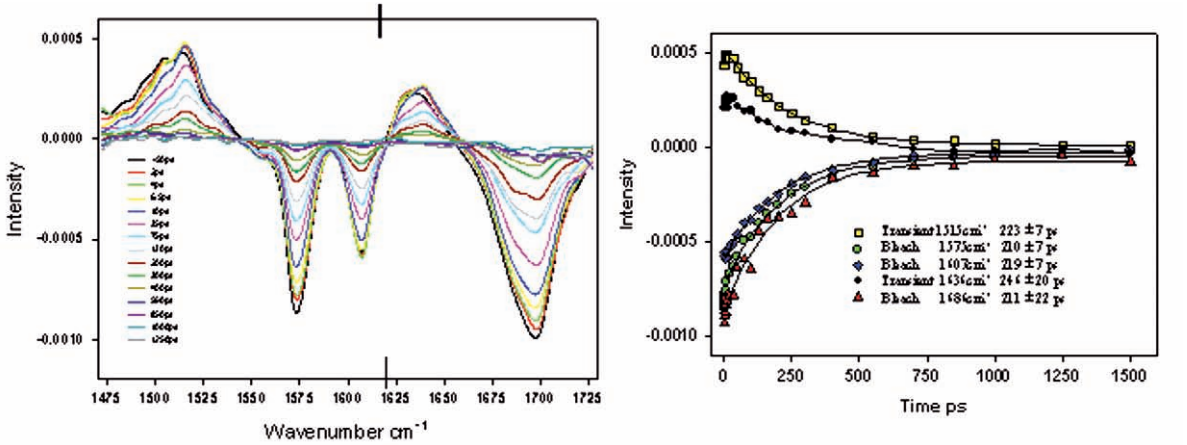


Figure 2. 10 mM $GMPH_2$ under acidic conditions, 0.132M H_3PO_4 in D_2O, pH=2.0. (a) Spectra at delays of 2, 3, 4, 5, 6.5, 8, 10, 20, 35, 50, 75, 100, 130, 160, 200, 250, 300, 400, 550, 700, 800, 1000, 1250, 1500 ps. Taken in windows centred at 1557cm^{-1} and 1633cm^{-1} and joined at 1620 cm^{-1}. (b) Selected kinetic analyses.

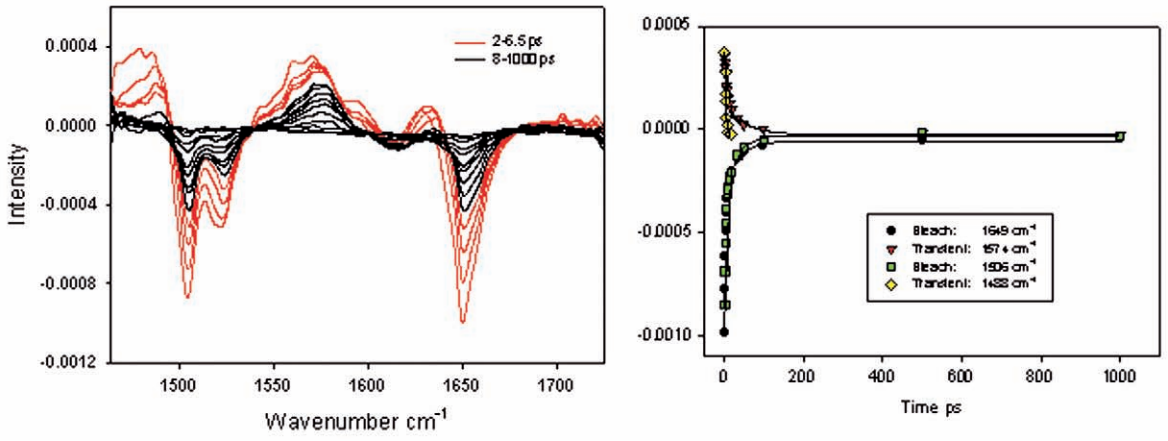


Figure 3. 10 mM CMP in 50mM sodium phosphate buffer in D_2O, pH 6.9. (a) Transient absorption spectra at delays 2, 3, 4, 5, 6.5, 8, 10, 15, 20, 35, 50, 75, 100, 500, 1000 ps. (b) Selected kinetic analyses.

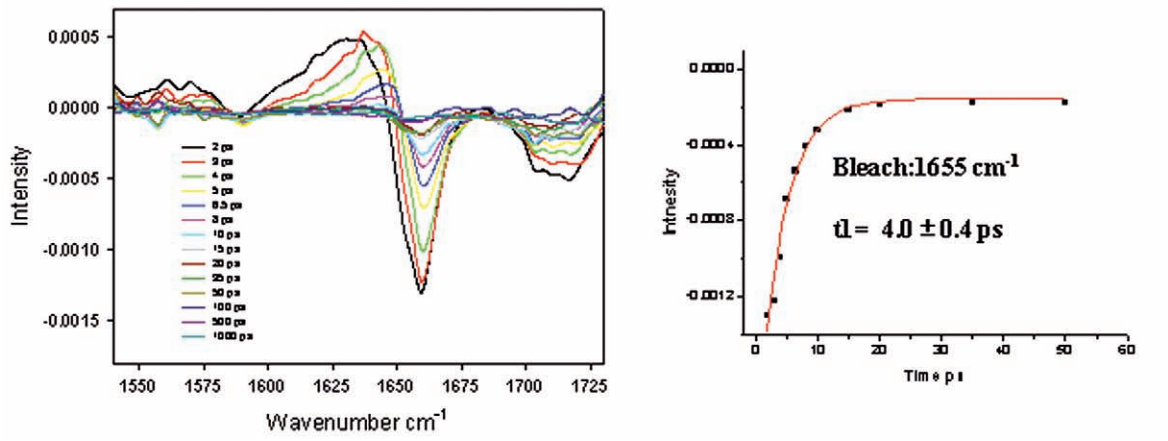


Figure 4. 10 mM dCMP under acidic conditions, 0.132M H_3PO_4 in D_2O, pH=2.0. (a) Spectra at delays of 2, 3, 4, 5, 6.5, 8, 10, 15, 20, 35, 50, 75, 100, 500, 1000 ps. (b) Kinetics recorded at 1655 cm^{-1}.

The effect of protonation of dCMP has also been investigated. When the solution of dCMP is studied in 0.132 mM H_3PO_4, the ground state FTIR spectrum shows bands at 1607 cm^{-1} and 1690 cm^{-1}, as expected for the protonated species (3). The transient behaviour is quite different from that recorded in neutral solution, with no evidence for an appreciable yield of long-lived species. Instead the transient absorption band shifts to higher wavenumber as the signal decays, consistent with relaxation of a vibrationally excited ground state (lifetime = 4.0 ± 0.4 ps).

Effect of increasing concentration of guanine moieties

It is well known that guanine derivatives form complex supramolecular structures at high concentrations.[9] For example at 540 mM $GMPK_2$ forms stacks of guanine tetrads, where the four guanines are ordered through Hoogsteen H-bonding and the K^+ ion sits between tetrads bonding to 8 nucleotides (see Figure 5a inset). This has a major effect on the IR spectrum, with a marked change in the ratio of the base ring vibration to that of the carbonyl stretch being apparent. This effect is reflected in the ps-transient infrared spectra (Figure 5a). Kinetic analysis of the bleaching band at 1672 cm^{-1} shows that over the first 500 ps there is a rapid recovery with a lifetime of 4.0 ± 0.9 ps (60%) and a further portion with a lifetime of 49 ± 9 ps (40%). The first may be ascribed to recovery from the vibrationally excited ground state (the transient absorption of which is observed at lower wavenumber). The second species may either be an excimer-like excited state (as has been observed for other stacked nucleotides) or possibly a tautomer. Differentiation of these possibilities will require ultrafast fluorescence or related measurements.

Finally we report here the behaviour of guanine within the extended polynucleotide (polyG) structure. As can be observed, the spectrum of the K^+-salt of polyG (10 mM nucleotide) (Figure 5b) is also substantially sharper than in GMP at a similar total nucleotide concentration (Figure 1). This may be attributed to the *intramolecular* formation of stacked tetrad structures. The kinetic analysis reveals similar behaviour as for the intermolecular tetrad structures found for 540 mM $GMPK_2$ (5.1 ± 1.0 ps (47%); 37 ± 6 ps (53%) at 1689cm^{-1}, consistent with there being both formation of vibrationally excited ground state and an electronically excited state. It may be noted that certain biologically relevant sequences such as the tandem repeat sequences $(TTAGGG)_n$ found in the telomeres at the end of human chromosomal DNA have the ability to form tetrads. Additionally the excited states of such species

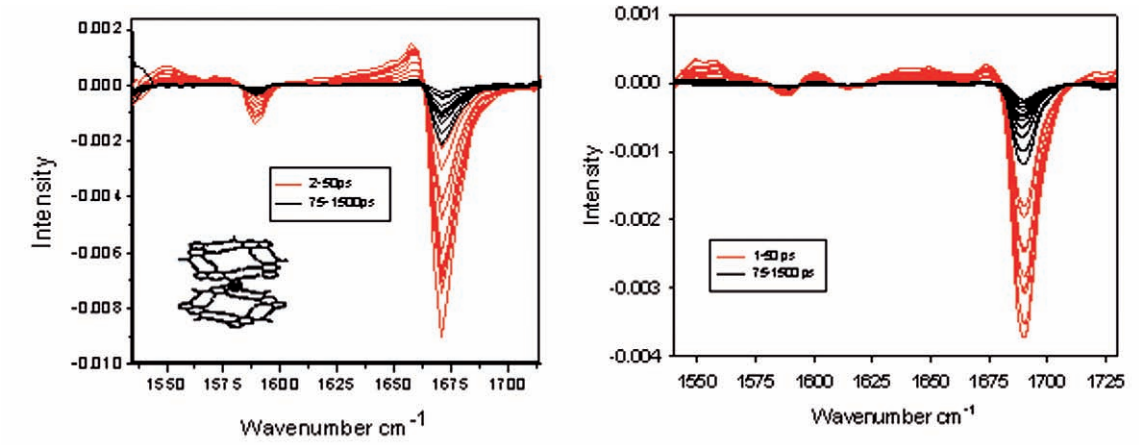


Figure 5. (a) 540mM $GMPK_2$ in 540mM NaCl in D_2O. Delays: 2, 3, 4, 5, 6.5, 8, 10, 15, 20, 35, 50, 75, 100,150, 200, 300, 500, 750, 1000, 1250, 1500 ps (b)10mM polyG in 50 mM potassium phosphate buffer, pH = 6.9. Delays: 2, 3, 4, 5, 6.5, 8, 10, 15, 20, 35, 50, 75, 100,150, 200, 300, 500, 750, 1000, 1250, 1500 ps.

might be important in the putative role of G-rich sequences in acting as traps for oxidative damage to DNA.

Conclusions

The current work further demonstrates the power of ps-TRIR methods to probe the effect of direct UV-excitation of DNA. The technique allows the observation of processes not readily measured by visible transient absorption (e.g. vibrational relaxation of the ground state), excited states (e.g. protonated GMP) and new species (e.g. the probable tautomers of cytosine derivatives). It also opens up the possibility of probing the effect of conformation of the polynucleotide on such processes – e.g. in sequences such as Z-DNA or quadruplex structures, such as those in telomeric DNA.

References

1. M. Daniels and W. Hauswirth, *Science*, 1971, **171**, 675.
2. C.E. Crespo-Hernandez, B. Cohen, P.M. Hare and B.Kohler, *Chem. Rev.,* 2004, **104**, 1977; C.E. Crespo-Hernandez, B. Cohen, and B.Kohler, *Nature*, 2005, **436**, 1141.
3. (a) J. Peon, A. H. Zewail, *Chem. Phys. Lett.,* 2001, **348**, 255; (b) D. Markovitsi, D. Onidas, T. Gustavsson, F. Talbot and E. Lazzarotto, *J. Am. Chem. Soc.,* 2005, **127**, 17130.
4. N. Ismail, L.Blancafort, M.Olivucci, B. Kohler and M.A. Robb, *J. Am. Chem. Soc.,* 2002, **124**, 6818; M. Merchan, L. Serrano-Andres, M.A. Robb and L. Blancafort, *J. Am. Chem. Soc.,* 2005, **127**, 1820.
5. G. Villani, *Chem. Phys.,* 2006, **324**, 438.
6. M. K. Kuimova, J. Dyer, M. W. George, D. C. Grills, J. M. Kelly, P. Matousek, A. W. Parker, X. Z. Sun, M. Towrie and A. M. Whelan, *Chem. Comm.,* 2005, 1182.
7. (a) T. Fujiwara, Y. Kamoshida, R. Morita, M. Yamashita, *J. Photochem. Photobiol. B: Biology,* 1997, **41**, 114; (b) J.-M. L. Pecourt, J. Peon, B. Kohler, *J. Am. Chem. Soc.,* 2001, **123**, 10370.
8. R. J. Malone, A. M. Miller and B. Kohler, *Photochem. Photobiol.,* 2003, **77**, 158.
9. J. T. Davis, *Angew. Chem. Int. Ed.,* 2004, **43**, 668.