Monitoring the effect of ultrafast deactivation of the electronic excited states of DNA bases and polynucleotides following 267 nm laser excitation using picosecond time-resolved infrared spectroscopy†

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In this paper we demonstrate the use of picosecond time-resolved infrared spectroscopy (ps-TRIR) to monitor the early structural dynamics of DNA bases and polydeoxynucleotides following UV excitation in solution.

There is interest[1] in the photophysics and photochemistry of nucleic acids since electronic excitation can initiate events that lead to base damage and genetic modification. However, nucleic acid bases are remarkably photostable, this being due to their ultrashort (< 1 ps) excited state lifetimes brought about *via* an ultrafast non-radiative path of the excited singlet state, the precise mechanism of which is not yet fully understood.[2] With some bases the rapid deactivation may be attributable to rapid conversion of the S_1 π–π^* state to a close lying 'dark' n–π^* or $\pi\sigma^*$ state[2–4] that is undetectable by the ultrashort time-resolved spectroscopic technique being employed. Alternatively, internal conversion through rapid crossing from the excited state to the ground state potential energy surfaces through a conical intersection may be most important.[5] The solvent may also play a central role in determining structural and conformational changes of the bases during relaxation, although it has been shown through gas phase studies that the short lifetime is not solely due to solute–solvent interactions but is an intrinsic property of the bases.[5] Calculations indicate that hydrogen bonding with the surrounding water stabilises the keto-tautomer form of guanine[6] and this may be important as UV excitation of both mono- and poly-nucleotides has been shown to break solute–solvent hydrogen bonds in solution[7] and in the gas phase.[8] Fluorescence up-conversion data have been interpreted to show that double-stranded DNA has two characteristic solvation times associated with bulk-type and weakly bound water.[7] In order to provide a different insight into the structural dynamics of processes occurring following direct excitation of DNA and its constituent bases in solution at room temperature, we have employed ps-TRIR.[9] This allows direct monitoring of vibrations that can be correlated with structural and environmental changes occurring within and surrounding the short-lived transient species, such as Watson–Crick H-bonding, solvation and tautomerism.

Fig. 1 shows the ps-TRIR spectra obtained following UV excitation (300 fs, 267 nm) of 5′-dGMP, 5′-dCMP, 5′-dAMP and 5′-dTMP in phosphate buffered D_2O.[10] For each base, the ground state IR absorption bands, which correspond to carbonyl stretching and in-plane ring vibrations, are clearly bleached and transient features are observed on the low wavenumber side of the parent. These transient bands then simultaneously narrow, shift to higher wavenumber and decay. The rate of reformation of the ground state was estimated by fitting the recovery of the parent bands to a single exponential. These rates were found to be 2.9 (±0.2) ps (5′-dGMP), 4.7 (±0.3) ps (5′-dCMP), 4.3 (±0.2) ps (5′-dAMP) and 2.2 (±0.1) ps (5′-dTMP)[11] (see also ESI). The transient absorption bands decay back to the baseline indicating the absence of long-lived products, such as tautomers.[12]

The S_1 excited states of natural DNA bases are now known to have sub-picosecond lifetimes,[2] which allows us to rule out this assignment for the transients observed with ps-TRIR. A more likely explanation is that we are observing vibrationally excited electronic ground states formed after the extremely fast relaxation of the excited nucleobases. This non-radiative decay will cause the immediate environment to sustain a large temperature rise and could lead to large populations of ground state molecules residing in higher vibrational states (S_0, $v > 1$). Indeed Kohler and co-workers have used the spectral broadening and red shift of the UV/visible transient absorption bands to monitor the fast intramolecular vibrational redistribution (IVR) process following

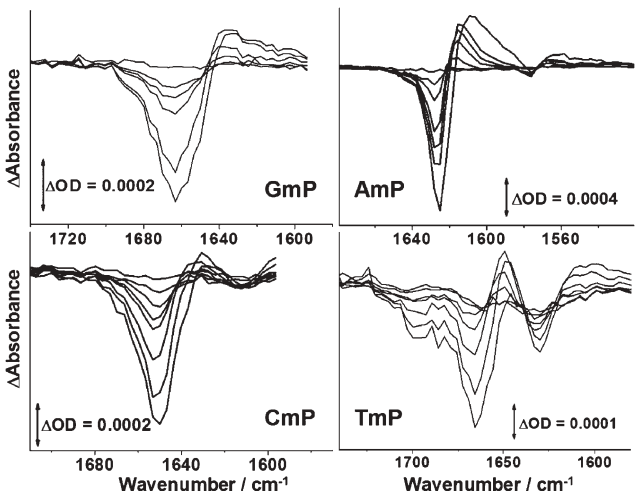

Fig. 1 ps-TRIR spectra (1–50 ps) following 267 nm excitation of 5′-nucleotides (10 mM) in 50 mM phosphate D_2O buffer.

† Electronic supplementary information (ESI) available: TRIR kinetic traces. See http://www.rsc.org/suppdata/cc/b4/b414450c/

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UV excitation. For example, adenosine has a characteristic IVR lifetime of *ca.* 2 ps as monitored at 270 nm in H_2O.[2] The lifetimes obtained in this paper are in a similar range. Differences may reflect our use of D_2O instead of H_2O, which can affect IVR rates[13] and the fact that we are directly monitoring specific vibrations of the nucleobase. Recently, IR pump–IR probe experiments identified[14] the IR features due to the $v = 1 \rightarrow 2$ transition for dCMP and dGMP. These results are similar to those we observe following UV excitation of the bases, although in our experiment we observe lesser shifted transient bands at early times (< 5 ps) and these features have complex decay kinetics. Such observations are typical for a vibrational cascading process[15] and can be assigned to vibrationally hot electronic ground states of the bases following non-radiative relaxation of the upper excited electronic state. A further possibility is that the band shifts are due to activation of low lying modes (< 100 cm^{-1}) which are anharmonically coupled to the observed transient modes thus perturbing their frequency as the cooling process progresses.[16]

The effect of the internal temperature rise of the nucleobase following excited state deactivation (estimated to be 1200–1300 K)[2] on the interaction with solvent and especially on its hydrogen bonding to water should be considered. Breaking of the H-bond is expected to increase the frequency of the affected vibrations, the opposite to what is observed. Our observation of transient bands at lower wavenumber indicates, therefore, that H-bonds must have reformed in a time less than that detectable by our TRIR apparatus (*i.e.* < 1 ps). This is consistent with the ultrafast solvation time of water (50 fs).[17] To determine the role of solvent we have carried out a similar experiment for 5′-dGMP (*ca.* 0.5 mM) in *d4*-methanol. Under these conditions we observe similar spectral changes following excitation but the decay of the transient band and the reformation of the parent occur on a slower timescale, $\tau = 7.6$ (± 0.5) ps. The change in rate on going from D_2O to methanol suggests that the decay is associated with nucleotide–solvent interactions and is consistent with slower vibrational relaxation of the 5′-dGMP in methanol. However, an effect due to changes in the energies of the π–π* and n–π* states cannot be excluded.

We have also investigated the synthetic polynucleotides poly(dG-dC)·poly(dG-dC) and poly(dA-dT)·poly(dA-dT). Fig. 2a shows the transient IR spectra for double-stranded poly(dG-dC)·poly(dG-dC) in 50 mM phosphate buffer, where the polynucleotide is in the B-DNA form. This shows strong bleaching and weak transient features. Analysis of the recovery of the parent bands indicated that this process does not conform to single exponential kinetics. Satisfactory agreement was obtained for double exponential kinetics with lifetimes of 12 (± 2) ps and 50 (± 10) ps (see ESI). A small concentration of long-lived species is also observable at 1000 ps. Interestingly, when the NaCl concentration was increased to 4 M so as to form the left-handed Z conformation of poly(dG-dC)·poly(dG-dC), the recovery of the ground state could be fitted well to single exponential decay lifetimes of 19 (± 3) ps, with no significant improvement found for double exponential fitting. It may be noted that Zewail and co-workers have reported similar lifetimes (10–19 ps)[7] for 'weakly-bound type' hydration in DNA and it could be that we are observing a similar process with the two conformers of poly(dG-dC)·poly(dG-dC). The transient spectrum (Fig. 2b) of

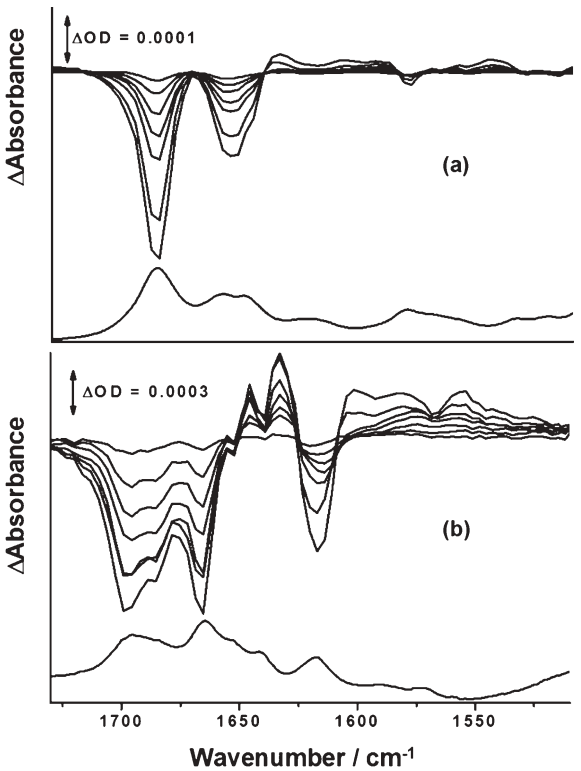

Fig. 2 ps-TRIR spectra (1–1000 ps) obtained following 300 fs, 267 nm excitation of (a) poly(dG-dC)·poly(dG-dC) and (b) poly(dA-dT)·poly(dA-dT) in buffered D_2O.

poly(dA-dT)·poly(dA-dT) also consists of areas with strong ground state bleaches and others where a transient signal is dominant. Close inspection of the dynamics of these modes shows the transient absorption bands at *ca.* 1645, 1630 cm^{-1} rapidly decay with the bleach ground state band at 1620 cm^{-1} recovering on a similar timescale. However, the bleaching of the 1660–1700 cm^{-1} bands has different kinetics and shows a much slower decay. Global kinetic analysis gives lifetimes of 4 (± 1) and 140 (± 10) ps (see ESI). We tentatively ascribe the faster lifetime to a rapid IVR, as observed for the individual bases but the long-lived component is unlikely to be due to this. However, we do not wish to speculate on the nature of the transient(s) responsible for our observations at this stage and merely note here that fluorescence with similar lifetimes has been reported and attributed to excimer or similar excited states.[2] Further experiments are required to fully elucidate these processes and these results are reported to demonstrate the ability of ultrafast TRIR to study poly-oligomer base strands and their differing B and Z forms. In conclusion, this work demonstrates how ultrafast time-resolved infrared absorption can be used to follow the rapid dynamics of DNA and its constituent nucleobases. We have directly observed the rapid formation and decay of vibrationally hot (S_0, $v \geqslant 1$) ground state nucleobases following the relaxation of their electronic excited states. Future work will help define the role of competing mechanisms such as phototautomerism, solvent interactions and especially H-bonding.

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