Fluorescence quenching of 2-aminopurine identifying transient species formed in DNA

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Introduction

2-Aminopurine has been used to study the structural dynamics of DNA and RNA because of its fluorescence properties and ability to induce excited-state charge transfer through DNA, that can be activated using 310 nm radiation, to the red of the first UV absorption of the natural nucleobases[1]. We are developing a method based on picosecond time-resolved infrared spectroscopy, ps-TRIR, and *ab initio* computed vibrational spectra to unravel the interactions of 2aminopurine with flanking bases in DNA. Here we present examples of the ps-TRIR spectra obtained after short DNA oligomers (di- and trinucleotides) containing 2-aminopurine where irradiated at 310 nm.

Experimental

All experiments were performed on the PIRATE system at CLF. Samples (about 1 mM concentration) were dissolved in D_2O-phosphate buffer. The samples were excited using 310 nm light and ps-TRIR spectra were recorded over the region 1525-1730 cm^{-1} between 1 and 2000 ps after excitation. Calculated vibrational spectra were obtained using both the MP2 and B3LYP methods (ground state species) and CIS method (excited state species) using the 6-31G(d,p) basis set. The solvent was modelled implicitly using the pcm method[2]. All N-^{1}H bonds were replaced with N-^{2}H bonds in the calculations to reflect the fact that the experimental spectra were recorded in D_2O.

Results

2-aminopurine deoxyriboside, $2AP_{dr}$: figure 1 shows the difference between infrared spectra recorded after excitation of $2AP_{dr}$ at 310 nm and spectra recorded before. The bleaches shown in figure occur because excitation depopulates the ground state of 2APdr. In addition to the expected bleaches a small transient peak is present about 1550 cm^{-1}. Based on the computed spectrum, also shown in figure 1, this transient peak is tentatively assigned to the electronically excited state S_1, of $2AP_{dr}$. Note that this species is no longer present 2 ns after the excitation pulse, even though the ground state has not yet fully recovered, indicating that the initially formed excited state undergoes some photophysical/photochemical reaction before ultimately returning to the ground state.

2-aminopurine-thymine dinucleotide, 2AP-T: Figure 2 shows the change in infrared absorption of the dinucleotide 2AP-T following excitation at 310 nm, a region where thymine alone does not absorb. In addition to bleaches about 1580 and 1620 cm^{-1}, corresponding to vibrational modes of 2-aminopurine, a clear bleach is seen at ~1660 cm^{-1}. However, this bleach at ~1660 cm^{-1} has fully recovered by 2 ns, and is therefore unlikely to correspond to vibrational modes of 2aminopurine, c.f. figure 1 data.

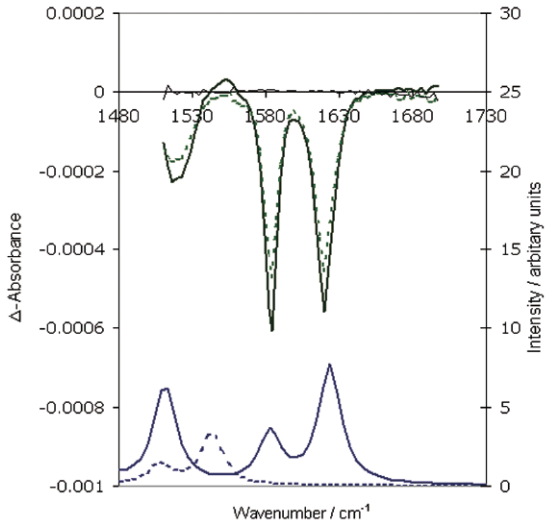

Figure 1. Upper trace: Difference ps-TRIR spectra of 2APdr. Solid black line -50 ps, solid green line 50 ps and dashed green line 2000 ps after excitation at 310 nm. Lower trace: Computed infrared spectra of ground state 2AP, solid blue line, and electronically excited 2AP, dashed blue line. (All computed frequencies have been scaled by 0.92).

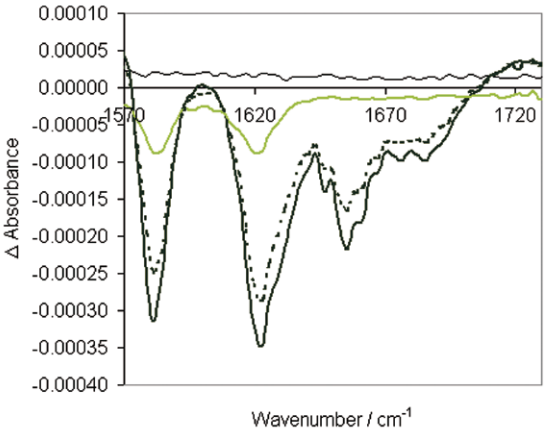

Figure 2. Difference ps-TRIR spectra of 2AP-T solid black line -50 ps, solid dark green line 5 ps, dashed green line 50 ps and solid light green line 2000 ps after excitation at 310 nm.

Guanine-2-aminopurine-guanine trinucleotide, G-2AP-G: Figure 3 shows the difference ps-TRIR spectra obtained in two different regions (~1525-1680 cm^{-1} and 1575-1730 cm^{-1}) for the G-2AP-G trinucleotide. In addition to bleaches corresponding to vibrational modes of ground state 2aminopurine, bleaches corresponding to vibrations of the ground state guanine residue are also seen. The transient peak present at about 1550 cm^{-1} is presumably due to the S_1 excited state of 2-aminopurine. The identity of the other transient features shown in figure 3 has not yet been confirmed. The transient about 1700 cm^{-1} may, however, be the same species seen when samples of poly dGdC are irradiated at 200 nm [3].

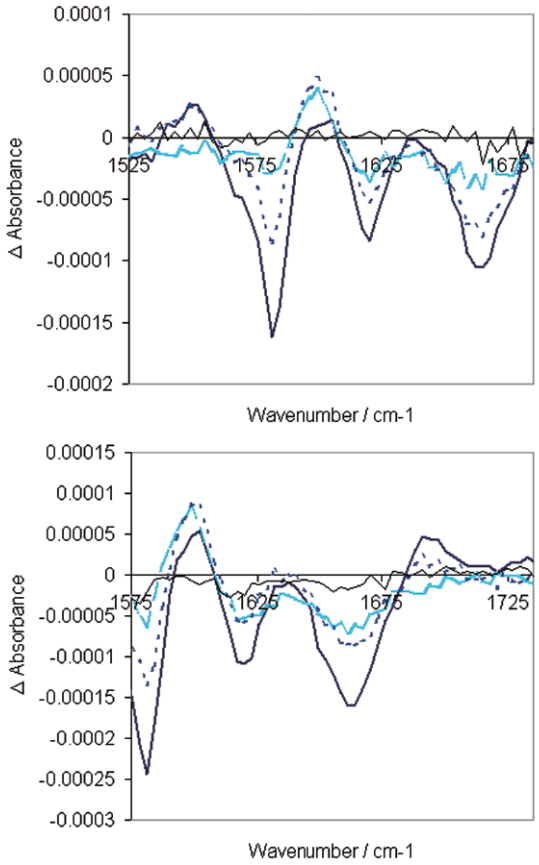

Figure 3. Difference ps-TRIR spectra of G-2AP-T in two different infrared windows. Solid black line -25 ps, solid dark blue line 5 ps, dashed blue line 50 ps and solid light blue line 2000 ps after excitation at 310 nm.

Conclusions

Excitation of $2AP_{dr}$ gives rise to a transient species which absorbs in the IR window 1525-1680 cm^{-1}. This transient species is thought to be the electronic excited state of $2AP_{dr}$.

The ps-TRIR spectra recorded for the DNA oligomers 2AP-T and G-2AP-G indicate that excitation at 310 nm causes the ground states of both the 2-aminopurine residue and the natural nucleobase residues to be depopulated within 5 ps. This finding suggests that the quenching of the fluorescence of 2-aminopurine when base stacked with natural nucleobases is caused, at least in part, by the excited state being delocalised across the 2-aminopurine residue and the flanking bases. In the case of stacking with guanine additional transient features are seen in the infrared spectra, indicating that species are formed when 2-aminopurine is base stacked with guanine that are not formed when 2-aminopurine is stacked with thymine. The most likely explanation is that an oxidised guanine moiety is formed via excited-state electron transfer and we are observing the guanine radical cation or the neutral guanine moiety following rapid deprotonation. We do not observe this in the case of thymine as this base is not so readily oxidized. The exact chemical identities of the species giving rise to the transient peaks seen in the infrared is currently under investigation.

References

1. Rist and Marino, *Curr. Org. Chem*, **6**, 775 (2002).
2. For more details see a standard computational chemistry text such as *Introduction to Computational Chemistry*, Jensen, Wiley, 1999.
3. Kuimova, Cowan, Matousek, Parker, Sun, Towrie and George, *Proc. Natl. Acad. Sci, USA*, **103**, 2150 (2006).