The Influence of the Epigenetic Base 5-methly-cytosine on DNA Excited State Dynamics

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Introduction

The photochemistry of DNA has been exhaustively studied due to its importance in skin cancer, the most common form of cancer (40% of cases globally).[1] However, photochemical studies on sequences containing epigenetic bases have yet to be fully explored. This is remarkable as, for example, modified cytosine in the form of methylated-cytosine (5mC) may be called the fifth base of human DNA constituting approximately 1% of the bases in mammalian genomes.[2] Photochemically, in the case of cytidine, C5-methylation is found to destabilize both $n_O\pi^*$ and $n_N\pi^*$ dark states and introduces an energy barrier to the nonradiative decay route that lengthens the excited state lifetime (QY of fluorescence for dCyd of 0.6% increases to 6.4% for 5mdCyd).[3] Understandably the lengthening of the lifetime of 5mC can be expected to influence the susceptibility of electronic excitation of DNA to promote mutagenic events and here we report initial results from time-resolved infrared (TRIR) to evaluate and explore the excited-state dynamics of epigenetic cytosine and its influence within different DNA systems.

Results and Discussion

The FTIR spectra together with the structure of the parent cytosine mononucleotide bases recorded in D_2O are shown in Figure 1. These spectra highlight the significant impact that protonation has on the ground state vibrational spectra for both nucleotides with the appearance of a new vibration at higher wavenumber (ca 1710 cm^{-1}).

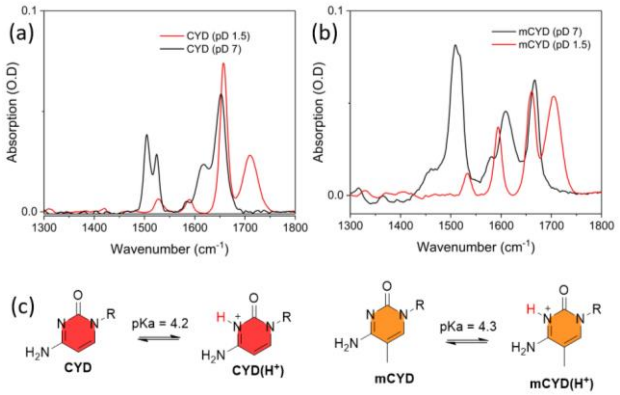

Figure 1: FTIR absorbance spectra of (a) cytidine, b) 5-methyl-cytidine (mCYD) recorded in D_2O buffer, c) structures of epigenetic derivatives and reported p*K*a values.

The TRIR spectra of the cytidine (CYD) with mCYD monomers in different pH environment following 265 nm reveals distinctly different processes, see Figure 2. Firstly, for CYD we observe the longer-lived $n_O\pi^*$ dark excited state (lifetime ~40 ps) produced via decay from a second bright $\pi\pi^*$ state,[4] characterised by a transient signal at 1574 cm^{-1}, Figure 2.

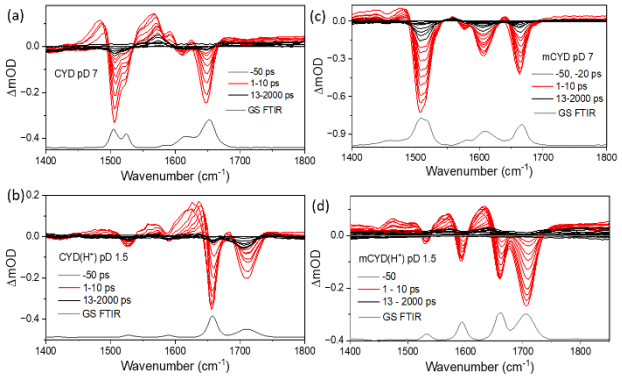

Figure 2: TRIR difference spectra of 10 mM of CYD (a) and (b) and mCYD (c) & (d) in 100 mM K phosphate in D_2O at pD 7 and pD 1.5 (λ_{ex}= 265 nm, 2 kHz,150 fs). Removal of 2000 ps delay subtracted, to remove baseline drift associated with hot D_2O.

This $n_O\pi^*$ spectral feature is due to in-phase stretching of C2−O7, C5−C6, and C4−N3 bonds. Both the ground state bleaching and transient absorption behaviour of CYD under acidic conditions (pD 1.5, Figure. 2b) are quite different from that recorded in neutral solution (Figure 2a). The transient absorption band at 1660 cm^{-1} shifts to higher wavenumber as the spectra decays, consistent with vibrational cooling. Protonation also results in an additional beach absorption band at 1709 cm^{-1}, which overlays with the GS FTIR. Under acidic conditions, formation of the characteristic 1574 cm^{-1} $n_O\pi^*$ transient band is suppressed, suggesting alternative excited state deactivation pathways dominate with no long-lived species being detectable.

Next, the TRIR spectra of mCYD was recorded, (Figure 2c). We observe ground state depletion bands centred at 1508, 1608 and 1665 cm^{-1} which correspond strongly with the underlaid FTIR spectra (also Figure 1b), with only a weak transient absorption band between 1400 to 1475 cm^{-1}. Notably, there is no detectable presence of a transient band in the region of 1574 cm^{-1}. Therefore, we suggest that access to the $n_O\pi^*$ dark state is restricted in this epigenetic variant. This restriction to the $n_O\pi^*$ state is

due to the methyl groups electronic interaction with C5=C6 double bond, increasing the tendency of the pyrimidine ring to remain planar and therefore restricting ring torsional distortion of the pyrimidine ring.[3] The second low-lying dark state, involving the nitrogen lone pair ($n_N\pi^*$), has also been shown computationally to be inaccessible to these excited state dynamics processes. Protonation of the mCYD (Figure 2d), also reveals the formation of transient absorption bands at 1560 and 1660 cm^{-1} which shifts to higher wavenumber as the spectra decay (vibrational cooling). Additional ground-state depletion bands are also seen at 1608, 1665 and 1709 cm^{-1}, these also overlay with the GS FTIR (Figure 1).

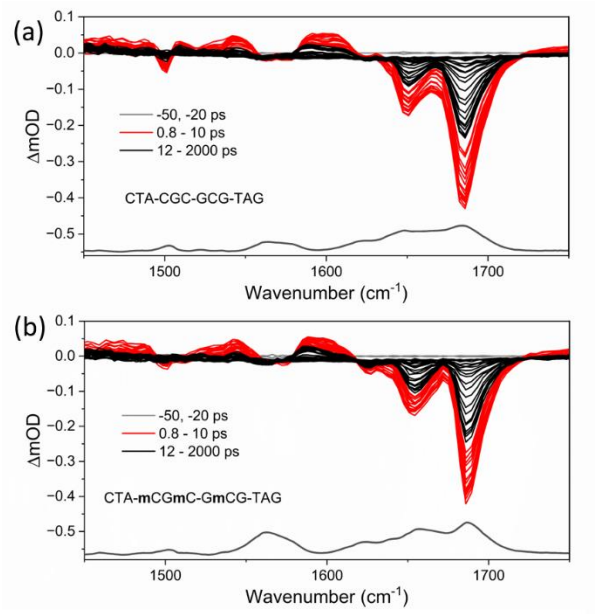

Figure 3: TRIR difference spectra of 10 mM of (a) CTACGCGCGTAG and (b): CTAmCGmCGmCGTAG in 50 mM K phosphate, pD 7, in D_2O (λ_{ex}= 285 nm, 2 kHz,150 fs).

Comparison of TRIR characterisation of the duplex systems

Following on from investigating the individual base systems we turned to identify the influence of 5mC in short oligomer duplexed systems, selecting 285 nm excitation to compare 5-CTA-CGC-GCG-TAG-3 with 5-CTA-mCGmC-GmCG-TAG-3. Figure 3 shows the TRIR spectra are very similar for both sequences. However, closer inspection shows a significant reduction in tracking associated with vibrational cooling, seen with the previously discussed TRIR mono-nucleobase studies (Figure 2), ascribed to the increased stabilisation of individual nucleobase components in the ds-DNA structure by stacking and H-bonding interactions preventing rapid decay to the ground state through conical intersections associated with ring puckering deactivation pathways. The TRIR spectra reveals, ground state depletion bleach bands centred at 1685 cm^{-1} (predominantly associated with the guanine carbonyl, with an additional minor component due to the thymine carbonyls) and 1650 cm^{-1} (predominantly due to the cytosine carbonyl vibration), this band is slightly shifted to 1655 cm^{-1} in the 5 mC containing sequence due to the shifted mC carbonyl vibrational band. There are additional ground state depletion bands associated with the nucleobase ring vibrations, which are significantly suppressed due to the stacking and H-bonding interactions in the ds-DNA structure. These are present at 1505 – 1525 cm^{-1} (cytosine ring-vibrational bands), 1575 cm^{-1} (guanine ring vibrations) and 1627 cm^{-1} (adenine ring vibration). Interestingly, the intensity of the adenine bleach appears to be greater in the methylated sequence, which raises the possibility that charge migration to adenine could be promoted in the methylated sequence, as adenine does not preferentially absorbed at 285 nm.

Comparative single-point kinetic analysis (with 1800 cm^{-1} subtraction) was also performed for the TRIR spectra recorded upon 285 nm excitation by fitting to the 1685 cm^{-1} guanine bleach band. The kinetic analysis revealed biexponential components for the control sequence (8.75 ± 1.82 ps and 54.1 ± 12.9 ps) and methylated sequence (13.24 ± 2.59 ps and 60.4 ± 19.0 ps), with a slightly increased lifetime in methylated version (both sequence decay lifetimes agree within the error margins).

Summary

The TRIR study identifies both similarities and subtle differences between cytosine and its epigenetic form 5-methyl cytosine. The potentially enhanced excited state lifetime of the epigenetically modified sequence highlights the potential for these modifications to result in alternative decay pathways and enhances the probability for these excited states to enter additional photochemical and photodamage pathways. Interestingly, however, the differences between the individual bases in terms of influencing kinetic behaviour when incorporated into DNA-type double strand sequences is not observed. Further work is underway to investigate whether wavelength dependent charge migration can be observed which has previously been shown in sequences containing mC modifications[5] and further studies to look at the excited state dynamics of i-motif structures with 5mC incorporations.

Acknowledgements

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