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A Comparative Picosecond Transient Infrared Study of 1-Methylcytosine and 5′-dCMP That Sheds Further Light on the Excited States of Cytosine Derivatives

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Supporting Information

ABSTRACT: The role of N1-substitution in controlling the deactivation processes in photoexcited cytosine derivatives has been explored using picosecond time-resolved IR spectroscopy. The simplest N1-substituted derivative, 1-methylcytosine, exhibits relaxation dynamics similar to the cytosine nucleobase and distinct from the biologically relevant nucleotide and nucleoside analogues, which have longer-lived excited-state intermediates. It is suggested that this is the case because the sugar group either facilitates access to the long-lived $^1n_O\pi^*$ state or retards its crossover to the ground state.

Ultrafast internal conversion is the predominant decay mechanism of singlet $\pi\pi^*$ nucleobase excited states of DNA and is believed to arise from a near-barrierless conical intersection with the ground state.[1,2] The parent nucleobases all have subpicosecond lifetimes, and this property is often invoked as the principal guardian against photodamage. However, two recent observations challenge this assertion: (1) thymine dimers can form on subpicosecond time scales and (2) longer-lived nonemissive states are observed in pyrimidine nucleotides.[3−5] In the case of cytosine, it is possible that such long-lived states may play a role in the production of mutagenic C< >C photodimers and subsequent conversion by deamination to uracil.[6]

Evidence of the nonemissive decay route for electronically excited cytosine derivatives has been obtained from both transient UV−vis (5′-CMP)[4] and time-resolved IR (dCyd and 5′-dCMP) spectroscopies.[5] The relatively long-lived (34−37 ps) species has been assigned as a $^1n\pi^*$ state and is formed in moderate yields.[4] In our time-resolved IR (TRIR) study, a strong transient absorption band centered at 1574 cm^{-1} was observed. However, this feature was found to be absent in the case of the nucleobase cytosine, leading us to conclude that N1 substitution has a major influence on the photophysics of cytosine derivatives (see Figure 1). In relation to this, we now report experiments on the 1-methylcytosine (1-mCyt) analogue, which was chosen to more fully investigate how the N1 substituent can influence the intramolecular dynamics through bond coupling of the pyrimidine ring system.

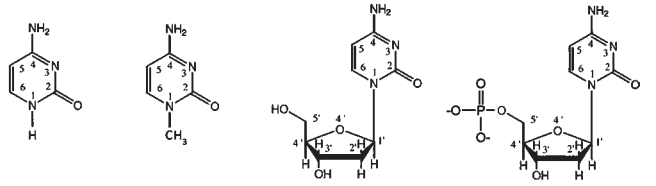

Figure 1. Family of cytosine derivatives with different N1 substituents.

We stress that while 1-mCyt is not a natural base, this analogue is frequently used to represent and model the cytosine base in DNA work (e.g., in computational studies).[7] Thus, there is a need to resolve the behavior of electronically excited states of 1-mCyt in relation to that of the nucleotide (-side) and the parent base cytosine.

Solutions were prepared to a concentration of 10 mM in pH 7 potassium phosphate-buffered D_2O. The solutions are expected to be monomeric in this concentration range, and the Beer−Lambert law is obeyed, in contrast to what is found in chloroform solution.[8] The picosecond TRIR (ps-TRIR) spectrum of 1-mCyt was recorded following 267 nm excitation[9] and is shown along with that of 5′-dCMP in Figure 2. Negative-going bands occur as a result of removal of ground-state population; the mode assignments are carbonyl (1654 cm^{-1}) and ring stretches (1505, 1525, and 1612 cm^{-1}). Analysis of the TRIR spectrum of 1-mCyt shows that it is almost fully recovered within ∼20 ps. By fitting each time delay to a sum of overlapping Lorentzian functions (see Figure 3), we found that the transient bands can all be assigned as hot ground states. The temporal changes of the spectral profile are consistent with a combination of a fast intramolecular vibrational relaxation (IVR) process and also subsequent cooling of the molecule as excess energy is dissipated into the solvent. Both processes cause a shift to higher frequency due to the anharmonicity within the initially excited vibrational modes and the cooling of excited low-frequency modes (unobserved) that couple to the observed higher-frequency modes. Hence, the IR transient absorption maxima are found to shift to higher

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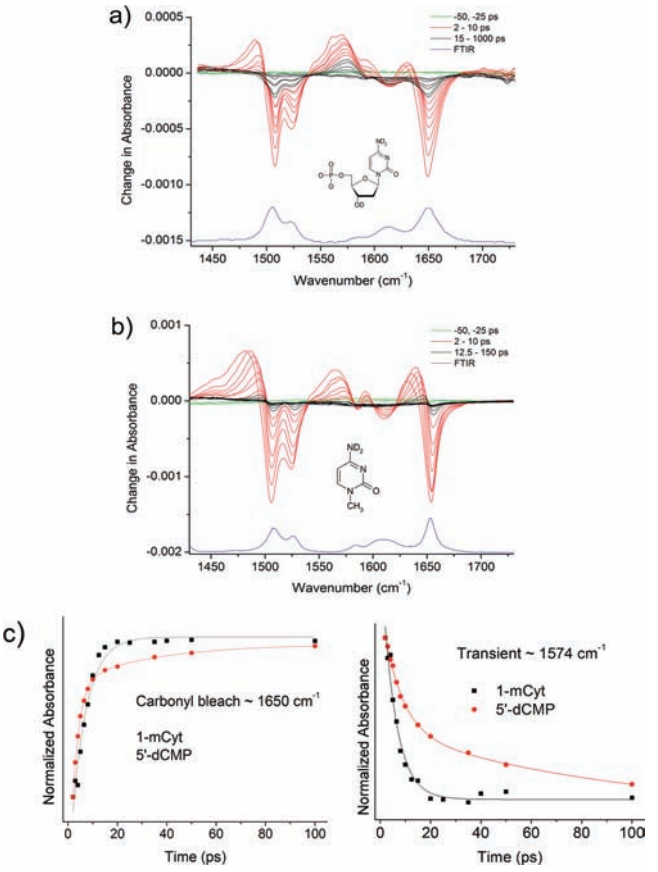

Figure 2. ps-TRIR spectra of (a) 10 mM 5′-dCMP and (b) 10 mM 1-mCyt in 50 mM potassium phosphate D_2O buffer (pH 7); shown below each is the corresponding FTIR spectrum. (c) Comparative kinetics for the transient and bleach recoveries, fit at the band maxima.

wavenumber with time as the molecules thermally equilibrate. (Similar behavior has been observed previously with 5′-GMP and other nucleotides.)[10] The lifetimes of the decay of the fitted bands were calculated, and each demonstrated monoexponential kinetics with a lifetime of 4.0 ± 0.4 ps. This is in good agreement with that measured for the parent nucleobase cytosine, which undergoes rapid single exponential decay with a lifetime of 4.4 ± 0.4 ps.[5] The similarity in the kinetics of cytosine and 1-mCyt leads us to assign the observed TRIR activity to rapid crossing from the electronic excited potential energy surface (PES) to the ground-state PES (via internal conversion from the $^1\pi\pi^*$ state), creating vibrationally hot ground-state molecules formed with up to 34 000 cm^{-1} of excess energy.[10b]

Turning our attention to 5′-dCMP, we see that the TRIR spectra (Figure 2a) are clearly different from those of 1-mCyt. A long-lived IR transient absorption at 1574 cm^{-1} is present in the case of 5′-dCMP (black spectra at longer time delays) that has a double-exponential decay with lifetimes of 5.0 ± 0.4 and 39 ± 5 ps for the deuterated solvent, in close agreement with our previous work.[5] However, this longer-lived transient feature is absent in the 1-mCyt spectrum.

We now discuss these results. As described above, 1-mCyt is frequently used as an analogue of the biologically relevant derivatives of cytosine. The CH_3 group blocks oxo−hydroxy tautomerism between the N1 and O7 sites, and hence, 1-mCyt has access to the same tautomeric forms available to dCyd and 5′-dCMP.[7] However, the current work shows that 1-mCyt does not form the

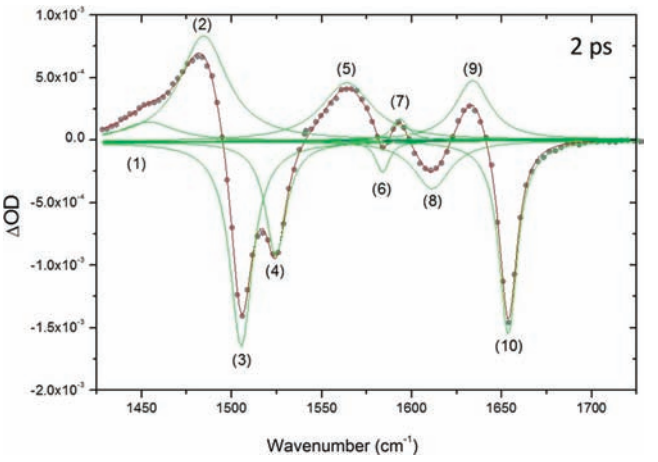

Figure 3. Band fitting of the ps-TRIR data for 10 mM 1-mCyt in 50 mM potassium phosphate D_2O buffer (pH 7) at 2 ps using Lorentzian functions.

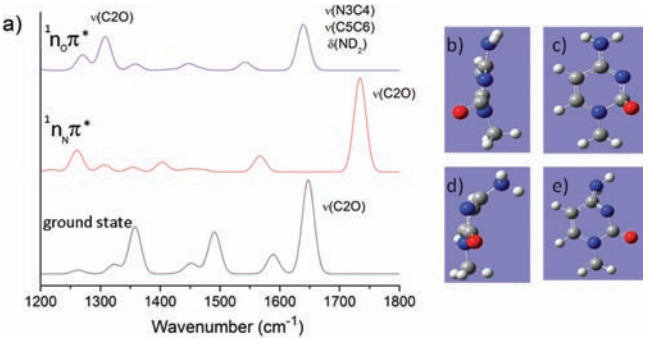

Figure 4. (a) Calculated gas-phase IR spectra of the ground state and $^1n_N\pi^*$ and $^1n_O\pi^*$ excited states of 1-mCyt. Carbonyl stretches and the strong ring vibration of the $^1n_O\pi^*$ state are labeled. (b) Edge-on view of the $^1n_O\pi^*$ state. (c) Face-on view of the $^1n_O\pi^*$ state. (d) Edge-on view of the $^1n_N\pi^*$ state. (e) Face-on view of the $^1n_N\pi^*$ state.

longer-lived state with significant yield, highlighting the fact that simple N1-methyl substitution does not have the same effect as sugar substitution. In line with other reports, we suggest that this longer-lived species is an $n\pi^*$ state. We previously made a tentative assignment to the $n_N\pi^*$ state.[5] This was argued on the basis that the transient band at 1574 cm^{-1} was due to a lowering of the ground-state carbonyl stretch from 1650 cm^{-1}, whereas the $n_O\pi^*$state would be expected to have a vibrational signature at a much lower frequency. Configuration interaction singles (CIS) calculations with the 6-311++G(d,p) basis set have now been performed to estimate the expected band positions for the $n_O\pi^*$ and $n_N\pi^*$states. These calculations (Figure 4) highlight two key points: (i) The vibration of the carbonyl bond for the $n_O\pi^*$state is expected to be displaced to a very low wavenumber (<1400 cm^{-1}). (ii) For the $n_N\pi^*$state, at least in the gas phase, the carbonyl stretch vibration is at a higher wavenumber than for the ground state.[11] Taking account of these findings, we conclude that the transient species we observed is the singlet $n_O\pi^*$state, rather than the $n_N\pi^*$species, though higher-level calculations that take into account solvent influences are required for complete validation.

In contrast to 5′-dCMP or 2′-dCyd, both 1-mCyt and Cyt show rapid routes to the vibrationally excited ground state with no evidence of a long-lived $^1n\pi^*$ state. This could occur because

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the nπ^* state either does not form or alternatively decays on a subpicosecond time scale.

The results presented herein, combined with previously reported results for Cyt and dCyd, categorically show that N1 substitution alone is not sufficient to cause the generation of longer-lived (>10 ps) species. Previously, Kohler and co-workers[4] reported that 1-cyclohexyluracil and 1,3-dimethyluracil show kinetics similar to that of uracil, while much longer lived decay occurs with UMP. Our observations for cytosine mirror those for uracil and now confirm that the ribose group has major significance in the photostability of nucleic acids.

The ultrafast photophysics of pyrimidines has been the subject of a number of detailed computational studies.[12] In the pyrimidine bases, the major nonradiative decay pathway is internal conversion from the $\pi\pi^*$ state to the ground state through a conical intersection arising from pyramidalization of C5 and/or torsion of the C5−C6 bond. The out-of-plane motion of the 5-substituent that accompanies this torsion (ethylenic path) is deemed to be the critical mechanistic step leading toward the conical intersection. Indeed, Gustavsson et al.[13] have correlated the access to the conical intersection from the S_1 minimum to the nature of the 5- and 1-substituents. Additionally, Nieber and Doltsinis found the mechanism of ultrafast decay of uracil and uridine to be dominated by large out-of-plane ring distortions that appear to be closely coupled to changes in the energy gap between the excited and ground states.[14] Interestingly, no qualitative difference between the mechanisms governing nonradiative decay in the gas phase and aqueous solution have been observed. This suggests that the excited-state lifetimes of bases (and more complex forms) are less dictated by hydrogen-bonding influences but rather are linked with their capacity to adopt conformational changes in order to reach the conical intersection.

It is notable that the ultrafast decay of the $\pi\pi^*$ state (<1 ps) has been reported to occur in both cytosine and 5′-dCMP.[1,15] The 39 ps decay observed in dCMP may thus be due to an additional competing pathway.[16,17] Indeed, recent ab initio multiple spawning dynamics calculations by Hudock and Martinez[18] have shown that in contrast to uracil and thymine, cytosine possesses multiple simultaneous competing relaxation pathways, with most trajectories acquiring $n_O\pi^*$ or $\pi\pi^*$ character before quenching through the $n_N\pi^*/S_0$ conical intersection.

One pathway has been predicted to proceed through the $n_O\pi^*$ minimum and therefore should be detectable by picosecond spectroscopy. This is consistent with that observed experimentally for dCyd and 5′-dCMP but different for Cyt and 1-mCyt. It is noteworthy that in this model, the wave function acquires $n_O\pi^*$ character by movement along the N−H out-of-plane vibration. It is intriguing to suggest that the presence of the sugar promotes passage to the $n_O\pi^*$ minimum, whereas that of the H atom or a methyl group is insufficient to direct the molecule to the $n_O\pi^*$ metastable structure. [Alternatively, it may be the case that the $n_O\pi^*$ state is indeed formed for 1-mCyt and Cyt but that its decay to the ground state is very rapid (<1 ps) for 1-mCyt but much slower for 5′-dCMP and dCyd. A distinction between these alternatives is currently not possible given the time resolution of our measurements.]

The role of the ribose group in the decay of the excited state remains unclear. It may be a simple consequence of the mass of the sugar or of some steric property that restricts certain ring motions, resulting in different nuclear configurations. Hare et al.[4a] suggested that hydrogen bonding between the sugar and the solvent may aid vibrational relaxation of the hot nπ^* state in pyrimidines, resulting in trapping on the nπ^* surface. Time-dependent density functional theory calculations on pyrimidine systems have also found that mixing between nonbonding electrons on the base and electrons on the ribose may occur.[19] It is clear that none of these factors are sufficiently influenced by the presence of a methyl substituent at the 1- position.

The participation of different excited states in the photophysics of natural DNA is only beginning to be more rigorously explored with the advancement of spectroscopic techniques, and the role of long-lived dark states in DNA systems was again recently highlighted.[20] The postulate of a localized cytosine excited state in double-stranded $[poly(dG\text{-}dC)]_2$ suggests that it may also be present in genomic DNA.[10a] However, there is still much to be understood about the behavior in the monomeric species.

Importantly, the family of 1-methyl nucleobase derivatives are commonly employed in computational studies.[21] This is primarily to avoid the inherent difficulties associated with performing calculations on very large molecules. Our work highlights the fact that some caution is needed in modeling electronic excited dynamics on the photophysics of such systems. The full potential of transient IR spectroscopy to characterize excited states requires support from computational studies that appropriately account for solvent to allow prediction of structural bands. We recommend revisiting the role of the 1-substitution further using high-level calculations to specifically address the issues highlighted here and thus shed further light on the factors that influence conical intersections.

■ ASSOCIATED CONTENT

Supporting Information. Experimental details and computational methods, kinetics analysis of 1-mCyt, and calculated gas-phase IR spectra of the ground state and $^1n_N\pi^*$ and $^1n_O\pi^*$ excited states of 1-mCyt. This material is available free of charge via the Internet at http://pubs.acs.org.

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