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Tracking DNA Excited States by ps-Time-Resolved Infra-Red Spectroscopy: Signature Band for a Charge Transfer Excited State in Stacked Adenine- Thymine Systems

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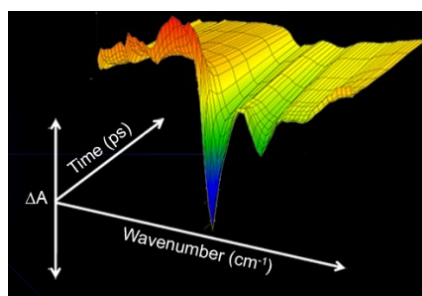
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ABSTRACT

UV photoexcitation of an adenine-thymine heterodimer (ApT) in D₂O yields a complex transient infrared signature in the 1500-1600 cm⁻¹ spectral region. The spectral dynamics fit well to a biexponential decay assignable to two transient species. The first, a short-lived species with a lifetime of ca. 5 ps, originates from the vibrationally hot electronic ground state of the unstacked form of the dinucleotide. The second species is longer-lived (ca. 75 ps) and its yield correlates to the amount of stacked dinucleotide present in solution. We assign the longer lived component to a charge transfer (A^{•+}pT^{•-}) state by comparison with calculated spectra for the adenine radical cation and thymine radical anion. Significantly, the CT feature is also identified in UV excited [poly(dA-dT)]₂. This experimental observation gives a powerful insight into how base-base interactions lead to extended-lifetime electronic excited states of the nucleic acid bases and how a dimeric structure controls the relaxation pathway.

TOC GRAPHICS



KEYWORDS (Word Style “BG_Keywords”). Picosecond, Transient spectroscopy, excited state, polynucleotide. DNA, Charge Transfer

There continues to be significant debate on the nature of the electronic excited states in DNA.¹⁻¹⁰ To unravel the complexity of the excited dynamics of DNA it is necessary to build on experiments of model systems using a variety of time-resolved spectroscopic methods. However, sometimes results from these various techniques have yielded apparently conflicting conclusions regarding how DNA responds to electronic excitation – for example the role played by exciplexes/excimers, charge transfer (CT) states, and excitons and whether the energy remains localized at the point of photon absorption or is delocalized across two or more bases. Despite the controversy regarding the nature of the states, common to all studies is the affirmation that interactions between bases in polymer systems result in increased excited state lifetimes following UV excitation and this has important ramifications for UV induced mutation of DNA. This latter point makes identification of spectroscopic signatures for such states an important task. Transient vibrational spectroscopy provides a powerful solution-phase structural tool to characterize the various intermediates via an infrared fingerprint of excited state intermediates of mononucleotides, dinucleotides and polymeric systems.¹⁰⁻¹³ In this paper we investigate the origins of the long-lived excited state present in UV excited alternating A-T systems by studying the dApT dinucleotide using ps-TRIR, FTIR and computational methods [see Methods in SI]. Using this model system we are able to extrapolate our findings to determine the transient species formed in double-stranded [poly(dA-dT)]₂.

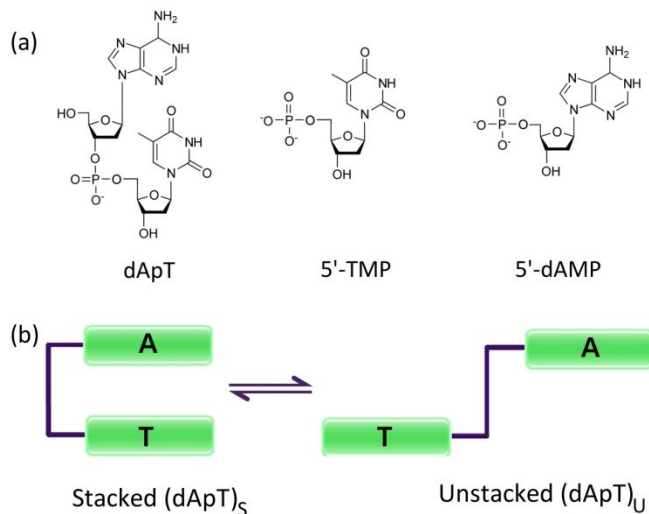


Figure 1. (a) Adenine and thymine systems (b) representations of stacked and unstacked dApT dinucleotide.

In solution the dinucleotide dApT exists in equilibrium between two structures, a stacked and unstacked form, viz; $(dApT)_S \rightleftharpoons (dApT)_U$, see Figure 1. ^1H NMR in buffered D_2O indicates that dApT is 38% stacked and 62% unstacked [see Table S1 in supporting information SI]. The power of IR over UV-vis spectroscopy is revealed in studying this dimer as, while base stacking in dApT results in only a minor change in the electronic absorption spectrum ($\sim 4.8\%$ hypochromism),^{14,15} far greater changes are observed in the infrared spectrum, which is very sensitive to base stacking in the $1500\text{-}1700\text{ cm}^{-1}$ region. Thus in the case of adenine, stacking interactions result in a significant suppression of the adenine ring vibration at 1626 cm^{-1} , which can be seen by comparing the FTIR spectra of a 1:1 mixture of A and T mononucleotides with the dApT dinucleotide, see Figure 2a. (D_2O is used as solvent as H_2O absorbs strongly in the IR region of interest). The FTIR spectrum of the dinucleotide comprises a mixture of 62% $(dApT)_U$ and 38% $(dApT)_S$ thus the $(dApT)_S$ spectrum was obtained by subtracting a weighted spectrum of the mononucleotides, assuming that $(dApT)_U$ has the same spectrum as 5'-dAMP and 5'-TMP.

This derived species (Figure 2a) shows a very marked suppression of the adenine absorption bands when compared to those of (dApT)_U.

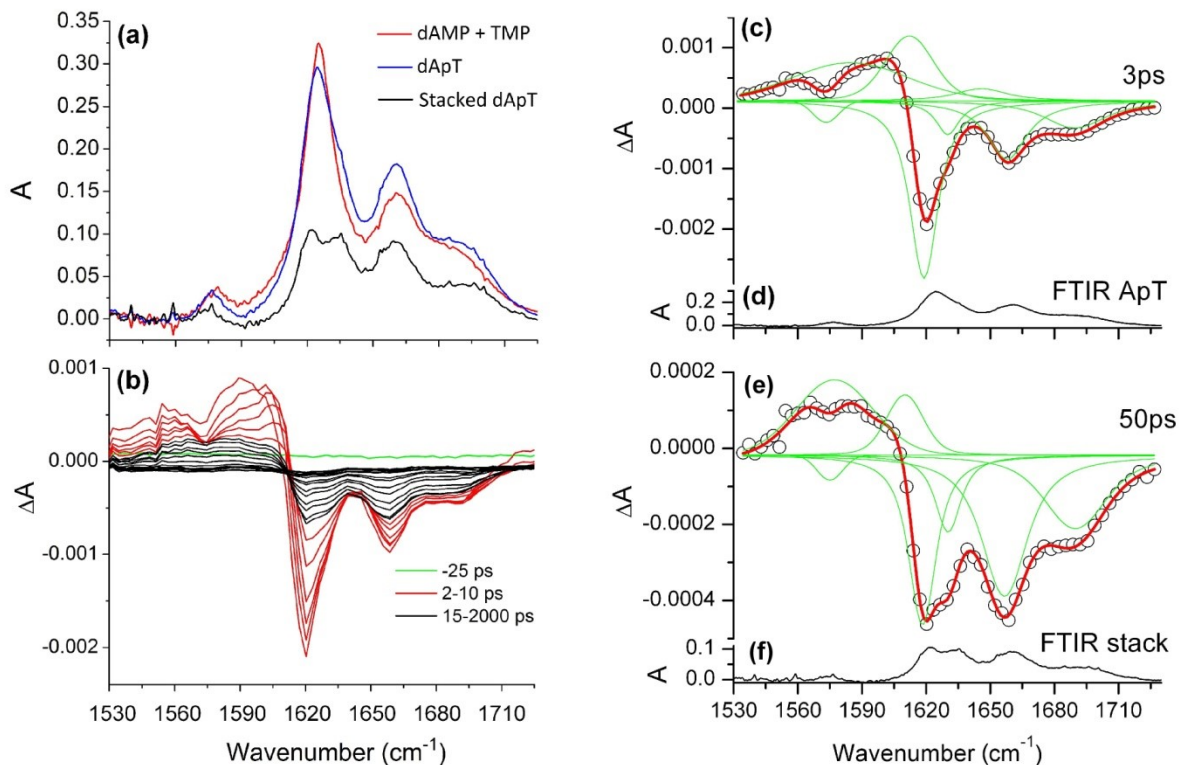


Figure 2. (a) Ground state FTIR spectrum of dApT systems compared to that of an equi-molar mixture of 5'-dAMP and 5'-TMP. The spectrum of the stacked species (dApT)_S is derived as explained in text. (b) ps-TRIR spectra of 10 mM dApT following UV excitation (150 fs, 267 nm). All in D₂O 50 mM phosphate buffer pH 7, at 20° C. (c) Voigt fitted data of ps-TRIR spectrum at 3 ps (d) ground state FTIR spectrum for dApT (e) Voigt fitted data of ps-TRIR spectrum at 50 ps and (f) ground state FTIR spectrum of stacked component of dApT as derived, see (a) above.

The ps-TRIR difference spectra obtained after UV excitation of dApT in D₂O 50 mM phosphate buffer are shown in Figure 2b. At early delay times (≤ 10 ps) shifting of the transient bands to higher wavenumbers is observed (red lines) which is characteristic of cooling of a vibrationally hot ground state species.^{16,17,18} Previous ps-TRIR measurements of the 5'-dAMP and 5'-TMP revealed mono-exponential kinetics for the reformation of the ground state with a

rapid recovery time of 4.1 ± 0.2 and 2.2 ± 0.2 ps respectively.¹⁸ Electronic relaxation of both mononucleotides is known to occur on the femtosecond timescale¹⁹ and thus the recovery observed for the mononucleotides is attributed to vibrational cooling of the hot electronic ground state that is formed after ultrafast internal conversion from the $1\pi\pi^*$ state.¹⁸ In the case of 5'-TMP recovery is not complete as there is a small amount of very long-lived component, (> 1000 ps), which is attributed to triplet state and photoproduct formation, [Figure S1 in supporting information (SI)].¹⁸ This same behavior is observed for the 1:1 mixture of mononucleotides under identical conditions (Figure 3).

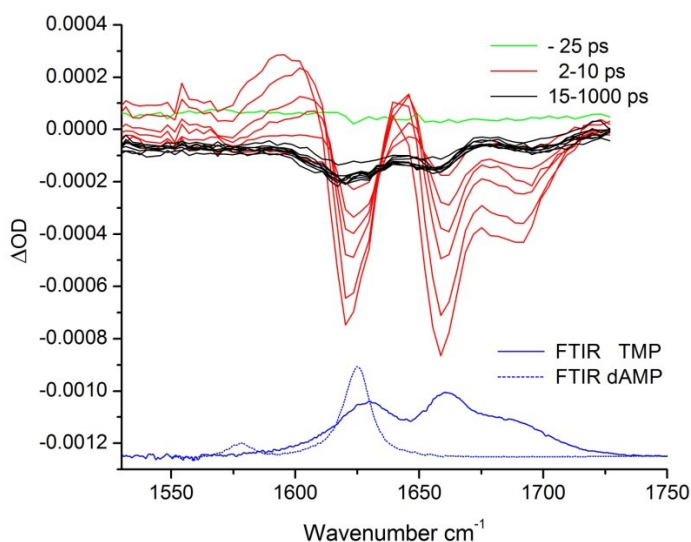


Figure 3. ps-TRIR spectra following UV excitation (150 fs, 267 nm) of a 1:1 mixture of 5'-dAMP and 5'-TMP and ground state spectra all in 50 mM potassium phosphate D₂O buffer at pH 7, at 20° C.

For dApT, however, the persistence of bleached (representing loss of ground state material) and transient bands after 15 ps (black lines in Figure 2b) indicate the presence of a longer-lived species, which is not present in the mononucleotides. Furthermore, the system is highly reversible with $>99\%$ recovery being detectable 1000 ps after excitation at all band positions

indicating that thymine side products are minimized in this system. It should also be noted that the ps-TRIR difference spectra at and after 15 ps have a very different profile than that recorded shortly after the laser pulse excitation.

To resolve and interpret these structural features of the dinucleotide species a Voigt peak fitting function was applied to the ground state [Figure S2 in supporting information SI] and transient IR spectra. At short times (3 ps), fitted bleached bands are found to closely resemble the fitted absorption bands in the ground state FTIR of the 1:1 mixture (Figure 2c, 2d) while the transient bands also correspond well with those observed for the mononucleotides of the absorption present (between 0-10 ps a shift to higher wavenumber with time is observed). At later delay time (50 ps) the bleached structure is found to resemble that of the ground state FTIR determined for the stacked species (Figure 2e, 2f), with the fitted difference spectrum indicating the presence of contributions from both the adenine (bleaches at 1577 and 1626 cm^{-1}) and thymine (at 1629, 1661 and 1695 cm^{-1}). These bleached bands are accompanied by a similarly long-lived transient band found between 1530 and 1612 cm^{-1} centered at approximately 1580 cm^{-1} which when fitted can be resolved into two transient bands, one at 1575 cm^{-1} and another weaker one at 1615 cm^{-1} , the latter overlapping the strong adenine bleach at 1626 cm^{-1} .

Global analysis of the single-pixel kinetics for dApT yielded a lifetime for the shorter-lived component, of 3.9 ± 0.2 ps, [Figure S3 in supporting information (SI)]. This value, which compares well with the lifetimes obtained by the same analysis of 5'-dAMP and 5'-TMP, reflects an average of the lifetimes observed for unstacked thymine and adenine bases. In a similar fashion global analysis of the single-pixel kinetics was performed for the longer-lived species and gave a value of 75 ± 7 ps [Figure S3 in supporting information (SI)].

We now consider the nature of this longer-lived (75 ps) species. We hypothesize that this feature is due to the formation of a charge separated (CT) state A^+pT^- state, resulting from an excited state electron transfer from adenine to thymine. Such a charge-transfer state has been proposed for dinucleotides by Takaya et. al.,²⁰ and predicted by calculations of Lange and Herbert.²¹ Experimentally, if a CT state exists we would expect the TRIR spectra to have characteristics of the adenine radical cation and thymine radical anion. To investigate this possibility we first calculated the mid-IR spectra of ribosyl adenine and its radical cation and ribosyl thymine and its radical anion. The necessary optimisations and vibrational frequency calculations were performed using DFT(BPW91) with 6-311G+(2d,p) basis in the Gaussian 09 package.²²⁻²⁴ Solvent effects were considered using a polarizable continuum model (PCM) within the integral equation formalism (IEF-PCM). All calculations and optimizations were conducted on structures where labile protons had been replaced with deuterium atoms. The DFT method was chosen based on evidence in the literature which indicated that DFT non hybrid methods yield vibrational spectra in close agreement with experimental solution phase results for amide and carbonyl containing systems.²⁵ DFT methods have recently been used by others to probe the properties of A and T excited states and radical ions.²⁶⁻³⁰ The spectra in the region of our experimental observations between 1500-1750 cm^{-1} calculated by DFT(BPW91) methods are shown in Figure 4. [For full spectral calculation outputs and relevant assignments see Tables 2-3 in supporting information (SI)]

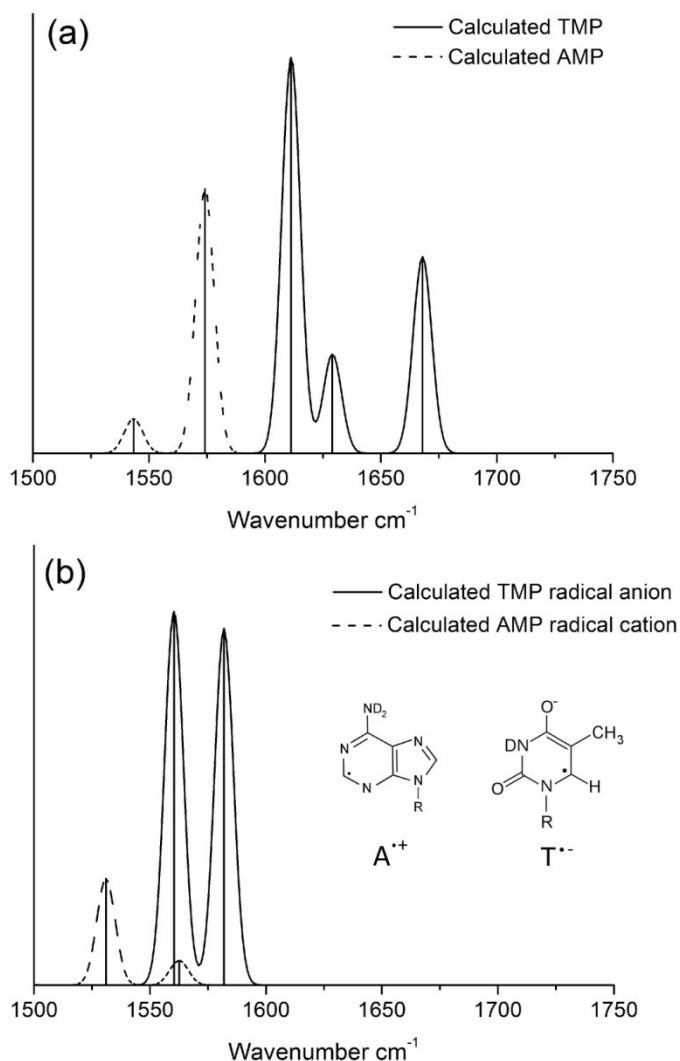


Figure 4. Calculated mid-IR spectra of (a) ribosyl (R) thymine and adenine and (b) ribosyl (R) adenine radical cation and thymine radical anion. The necessary optimizations and vibrational frequency calculations were performed using the DFT functional, BPW91.

The calculated spectrum for the adenine ground state shows two bands at 1574 cm^{-1} and 1543 cm^{-1} ($\text{C}=\text{C}/\text{C}=\text{N}$ ring vib). This spectrum is shifted by approximately 50 cm^{-1} from the FTIR recorded in D_2O solution [see Table S4 in supporting information SI]. The calculated groundstate spectrum of 5'-TMP shows three bands due to carbonyl and ring stretch contributions. These bands are shifted from the FTIR in D_2O to a lesser extent though the relative intensities are quite different. This is attributed to the different effect of solvent on the different modes. Such

differences between raw (non-scaled) calculated spectra and solution phase experiment are common.²⁶⁻³⁰ In light of these results a scaling factor was not applied to the computational data.³¹

The spectrum for the adenine radical cation shows two bands at 1562 cm^{-1} and 1530 cm^{-1} which are slightly shifted (12-13 cm^{-1}) to lower wavenumbers from the groundstate and exhibit a very marked change in relative intensity (Figure 4a). In the case of thymine the calculated spectrum for the radical anion shows two strong bands at 1560 and 1582 cm^{-1} of similar intensity, which are largely carbonyl in character [Table S3 in supporting information (SI)]. There is a marked lowering of the frequency (69 and 87 cm^{-1}) of the bands for the thymine radical anion carbonyl modes, as is expected as they lose some of their double bond character. This is in agreement with previous DFT calculations for thymine which showed that upon reduction to the radical anion a 70 cm^{-1} red shift was observed in the position of the carbonyl bands³⁰ Note: the ring stretch present in the groundstate is shifted to lower wavenumbers outside the region of interest. The calculations demonstrate that we may expect significant differences between the IR band positions for the ground state and their radical ions.

In the TRIR spectrum of the stacked form of dApT (Figure 2e) we note that the transient bands at 1575 and 1615 cm^{-1} are displaced from the ground state thymine-localized vibrations by 86 and 80 cm^{-1} respectively. In addition, the transient spectra are devoid of transient species above 1610 cm^{-1} . Consideration of the data in Figure 4 reveals similar observations where there is a loss in intensity of the intense groundstate adenine band upon radical cation formation and a similar shift in the case of the calculated thymine radical anion spectra, with no absorbance in the high wavenumber region for either species. Of course the influence on the adenine and thymine vibrational modes of electrostatic interactions between stacked bases cannot be discounted but are expected to be small.

We now turn to the assignment of the long-lived species in (dApT)_s. The formation of a triplet state of thymine is discounted as its reported lifetime is on the ns timescale,^{2, 29} while the mixed sequence also rules out the assignment of an excimer. However, the calculations do provide sufficient evidence to assign the TRIR difference spectrum of (dApT)_s to the formation of an excited state species possessing adenine cation and the thymine anion character. This charge-transfer character probably evolves rapidly (< 2ps) from an initial exciplex state. Identification of the charged species provides strong evidence for the role of CT states in the photochemistry of DNA, and supports other research.^{20, 21, 32}

We now examine the relevance of the structural and kinetic observations made for the dinucleotides to A/T polynucleotide systems. The chemistry of A/T has come under scrutiny in the past few years^{27, 33-38} stemming from an intense interest in the different excited state behavior exhibited by the alternating [poly(dA-dT)]₂ and homopolymer [(polydA).(polydT)] systems.³⁴ Our data, Figure 2e, establishes an IR transient marker band from 1530-1612 cm⁻¹ for the A-T dinucleotide localized CT state and we can now consider whether a similar marker band is present in other AT systems. Indeed a previous ps-TRIR study of [poly(dA-dT)]₂ revealed a transient absorption band in this region.¹⁸ A comparison of the transients recorded for dApT to that observed for the [poly(dA-dT)]₂ shows them to be similar, see Figure 5 (a-b). In the case of the polymer a broad transient is clearly present in the range 1530-1605 cm⁻¹ with other transient spectral features observed between 1625 and 1650 cm⁻¹. The fact that the polynucleotide TRIR spectrum is similar to the dinucleotide of dApT supports the hypothesis that after excitation of the polymer the initially formed electronic excited state evolves to a charge-transfer state that is localized between two bases.

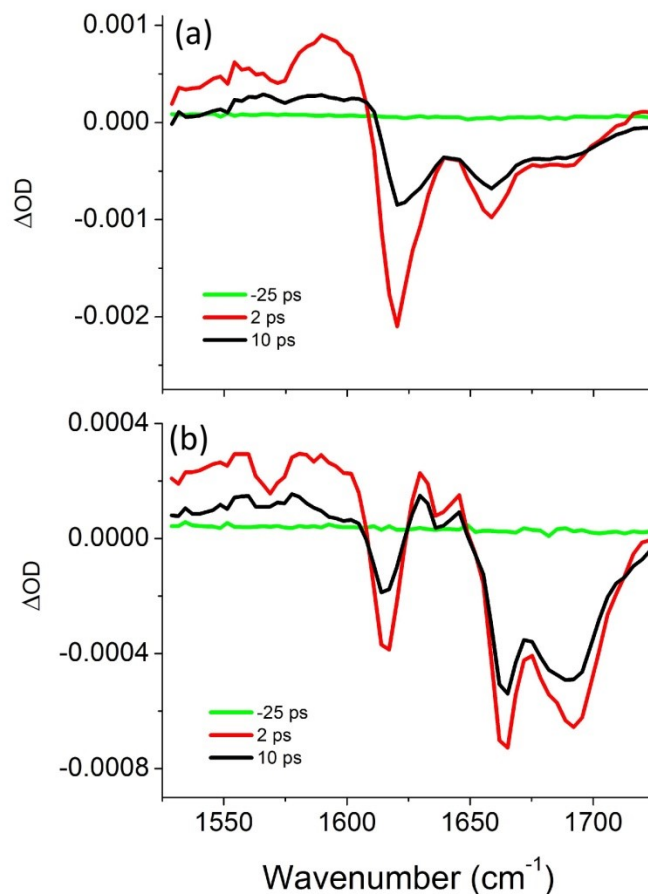


Figure 5. The ps-TRIR spectra obtained following UV excitation (300 fs, 267 nm) of (a) 10 mM dApT and (b) 10 mM poly[(dA-dT)₂] in 50 mM potassium phosphate D₂O buffer at pH 7.

This proposal that the excited state in polynucleotide systems is characteristic of a localized dinucleotide stack is further supported by the kinetic data. The lifetime of 75 ± 7 ps found for dApT correlates with previous observations for d(AT)_n.d(AT)_n polymer samples ($n = 9$ or 10) where similar conformations of the dinucleotide components are present. (lifetimes ranging from 51 to 72 ps).^{33,35} Importantly, the result is also in close agreement with our earlier reported ps-TRIR for [poly(dA-dT)]₂ which comprises ~ 2000 base pairs and was found to possess a longer-lived component of 50 ± 10 ps.¹⁸ Recent work by Kwok et al. on d(AT)₁₀.d(AT)₁₀ suggested an interplay between ultrafast base-localized (SLE) bright states (< 50 fs) and longer

excimer/ exciplex (SE) dark states (3 and 72 ps).³⁶ Our observations show that the increased lifetime in poly(dA-dT)]₂ results from a single interaction with a neighboring base.

In summary, we have shown how structurally sensitive transient IR spectroscopy can be used to assign transient species. Comparison of the transient spectra dinucleotide dApT and [poly(dA-dT)]₂ provides convincing evidence that a dinucleotide localized charge transfer excited state is the origin of the longer-lived species found in the larger polynucleotide. This has important implications for the comparative role of base pairing and hydrogen bonding interactions in stabilizing excited states. The emerging picture is one where stacking interactions between two bases stabilize the excited state³⁹ providing sufficient strength to negate charge sequestration while hydrogen bonding interactions between two bases are more likely to act as a mechanism for fast decay through solvent/environment interactions and/or to provide a secondary means to reduce columbic anion/cationic charges (in the case of G-C).⁴⁰ Our results indicate that for double-stranded AT systems, Watson-Crick associations do not result in alternative deactivation pathways but serve to re-enforce the electronic interactions created by stacking which govern the formation of a dinucleotide localized excited state. This state determines the longer-lived dynamics exhibited in the larger polymeric structure. Yet further subtleties must exist. For example; photolysis of dApT fails to yield a cyclobutane-photoadduct but photolysis of TpdA results in adduct formation although with quite a low quantum yield of 7×10^{-4} mole/E.⁴¹ Future work should consider the influence of conformation within isomer sequence on the excited state behavior of localized dinucleotide excited states. Moving from homopolymers to DNA, recent results of transient fluorescence experiments⁵ suggest the existence of CT excited states in calf-thymus DNA, while transient infrared has recently been used to study photodamage in DNA

systems.⁴² The identification of infrared marker bands for specific CT excited states offers the potential to identify the role of species in particular sequences and sequence of DNA.

ASSOCIATED INFORMATION

Supporting Information Available:

Supporting information includes NMR data to determine the degree of stacking, additional ps-TRIR data and analysis and tabulated computational data. This material is available free of charge via the Internet at <http://pubs.acs.org>

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