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Time-Resolved Spectroscopy

Picosecond Transient Infrared Study of the Ultrafast Deactivation Processes of Electronically Excited B-DNA and Z-DNA Forms of [poly(dG-dC)]$_2$**

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Unravelling the ultrafast processes within DNA is a challenge that continues to exploit the boundaries of both spectroscopic and computational capabilities. However, such studies are essential for the understanding of the nature and dynamics of the UV-activated processes that precede DNA damage and lead to mutagenesis and ultimately cause a number of diseases.[1] Considerable effort has focused on the photophysics and photochemistry of the individual base components which have very short electronically excited singlet state lifetimes (<1 ps).[2] In contrast, UV excitation of the polynucleotide systems produces additional species that have much longer lifetimes,[3,4] which is currently a main topic of scientific interest and debate. Questions remain as to whether the electronic excited states of these polymeric forms of DNA are localized on a single base or delocalized over a number of bases. Furthermore, the structural features of polymeric DNA raise a number of additional questions, such as their influence on the excited-state properties and relaxation dynamics of base-stacking interactions, hydrogen bonding, hydration, and conformation.

The advantages of picosecond time-resolved infrared spectroscopy (ps-TRIR) are that it can yield structural details about transient species and also that it allows the ground-state depletion to be directly monitored. DNA is only weakly emissive and excited-state decay occurs predominantly through nonradiative channels, thus making ps-TRIR an ideal tool for DNA investigations. ps-TRIR also provides information about such dark processes, which are difficult to observe by the traditional transient absorption and not detectable by transient fluorescence techniques. For these reasons, ps-TRIR has recently been used to study the photodynamics and photoreactions of mononucleotides and polynucleotide DNA.[4–10] By using ps-TRIR, we have identified a number of processes following direct excitation at 267 nm. For example, the cooling of the vibrationally hot ground state (2–4 ps) of mononucleotides was observed.[4] Direct spectroscopic evidence of a longer-lived state (34 ps) in 5′-dCMP was assigned, partially on the basis of the IR band position, to the $^1n_N\pi^*$ dark state.[5] The participation of this latter state in the relaxation dynamics was predicted by computational work[11] and inferred from transient absorption work by Hare et al.[12] We have also studied G-rich polynucleotides that are known to participate in tetrad stacking formation.[6] In addition, the photoionization of the B form of [poly(dG-dC)]$_2$ by direct excitation at 200 nm has been investigated, and resulted in a structural fingerprint to identify DNA damage.[7] The relaxation processes and electron transfer from DNA to an intercalated metal complex excited at 400 nm has also been reported.[8]

Herein, we focus on the ultrafast dynamics of double-stranded poly(dG-dC). This is interesting for a number of reasons. Firstly, the steady-state absorption of the G and C bases in the polymeric form indicates the presence of electronic interactions between the bases, which in turn alter the excited state dynamics from that of the parent bases. It was recently shown that the excited states of the bases in [poly(dG-dC)]$_2$ decay at rates faster than those observed for the individual nucleotides,[13] with a particular role ascribed to charge-transfer states,[14] a process that is predicted to be followed by rapid proton transfer between the G and C units.[14–16] Secondly, if, as predicted, proton transfer acts to modulate the decay of locally excited (LE) states,[14] then the characteristic IR signatures of the deprotonated guanine and protonated cytosine products should be detectable by ps-TRIR. Thirdly, [poly(dG-dC)]$_2$ can adopt the unusual left-handed Z-DNA structure.[17] Thus a comparison of how the base stacking arrangements in the structurally distinct right-handed B- and left-handed Z-DNA forms influences the photophysical processes of the G–C base pairs is possible. Finally, and in particular, the recent characterization of a $^1n_N\pi^*$ dark state for 5′-dCMP, with a strong IR identifiable transient band at 1574 cm^{-1} poses questions as to the possible role of this state in C-containing polynucleotide chemistry.[5]

The transient IR spectra for B-form double-stranded [poly(dG-dC)]$_2$ show strong bleaching and weaker transient features (Figure 1 a). The ground-state IR spectra of the individual bases can be considered to have three regions: the carbonyl stretching region (1640–1700 cm^{-1}), where both C and G bases absorb, the G ring region (1550–1600 cm^{-1}) and

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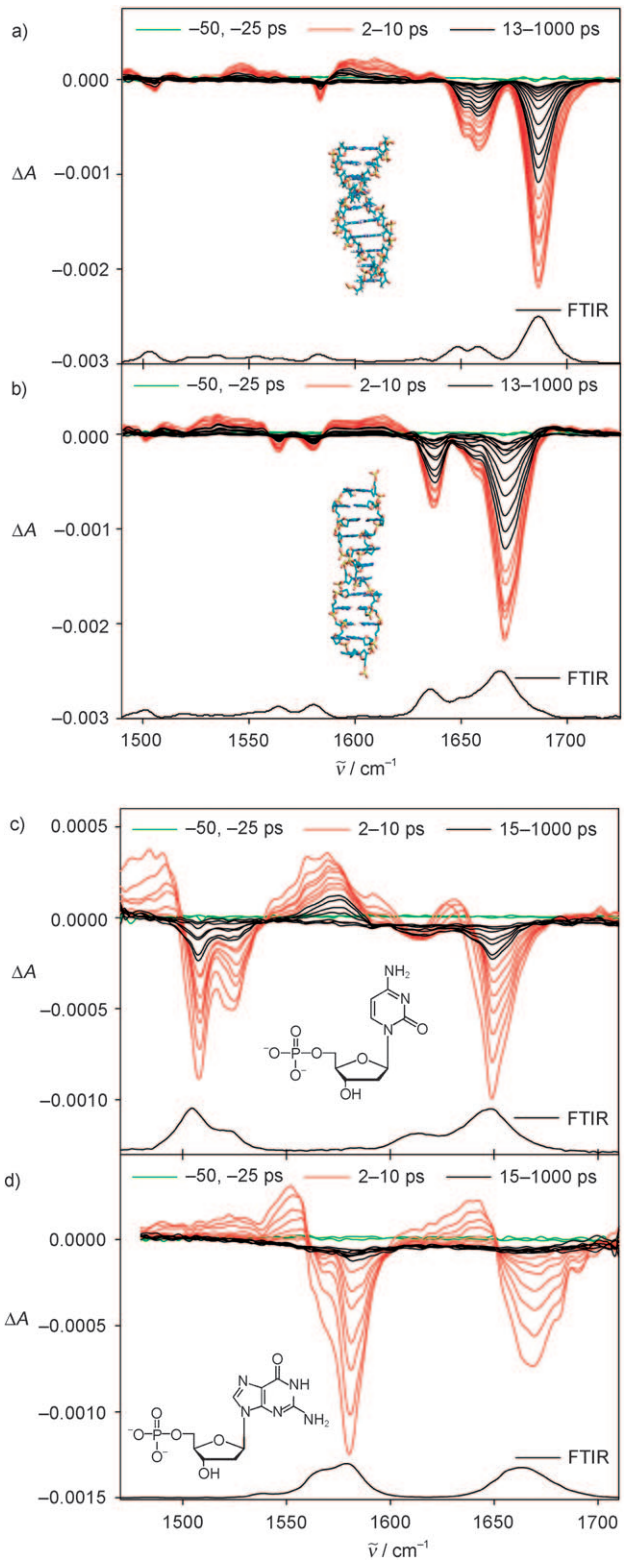


Figure 1. ps-TRIR spectra in 50 mM potassium phosphate D_2O buffer at pH 7 of a) B-form [poly(dG-dC)]$_2$ (10 mM) and b) Z-form [poly(dG-dC)]$_2$ (10 mM) with 4 M NaCl at delays of −50, −25 (green), 2–10 (red), and 13–1000 ps (black), c) 5′-dCMP (10 mM) and d) 5′-dGMP (10 mM) at delays of 50, −25 ps (green), 2–10 ps (red), 15–1000 ps (black) following UV excitation (300 fs, 267 nm). Helices were drawn using HyperChem 8.0.5.

finally the C ring region (1490–1550 cm^{-1}). These are clearly seen in the bleaches shown in Figure 1. The Watson–Crick hydrogen bonding and the stacking interactions in this double-stranded form result in mixing of the vibrational degrees of freedom. This manifests itself in the suppression of the strong ring stretches of the C and G mononucleotides (Figure 1 c, d).[18]

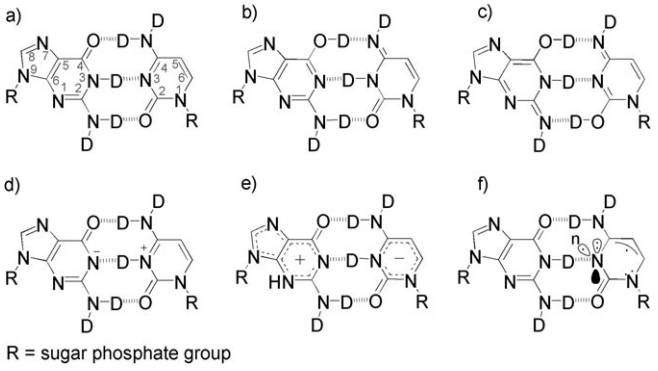
From analysis of the kinetics of the bleaching in both the C (1658 cm^{-1}) and G (1685 cm^{-1}) carbonyl regions of the B form, it is evident that two processes are occurring. These two processes have lifetimes of (7 ± 2) ps and (30 ± 4) ps with approximately the same amplitude (Table 1). As in our previous work,[4] we ascribe the shorter lifetime to the relaxation of a vibrationally excited ground state and the longer lifetime to the decay of an electronic excited state. However, in the spectral regions where transient absorption dominates, the faster process is significantly different from that found in our more recent studies of 5′-dGMP and 5′-dCMP.[5,6] The decay of the transient in mononucleotides is accompanied by a shift in the maximum of the absorption band of the vibrationally hot ground state to higher wavenumbers (Figure 1 c, d). Strikingly, this "tracking" behavior is not found between 2–10 ps for the polynucleotides. One possible explanation for this is that in the double-stranded polymer the vibrational energy is very rapidly (< 1 ps) distributed between the nucleobases, so that on average no base has more than one quantum of vibrational energy, at least in the higher (> 1500 cm^{-1}) modes. This would be expected if very effective phonon coupling exists between the stacked bases and/or it could arise from rapid delocalization of the initially formed Franck–Condon excited state over a number of bases (as predicted by exciton theory[19]). A second observation is the behavior of two discernible transient absorption bands in the region 1590–1625 cm^{-1}. Unlike the bleaches, both of these transient bands are found to obey monoexponential kinetics ((21 ± 4) ps at 1597 cm^{-1} and (6 ± 1) ps at 1612 cm^{-1}; the kinetics are shown in the Supporting Information).

These observations indicate that there are two channels, of roughly equal magnitude, along which the initially formed excited state can decay nonradiatively on the ultrafast timescale. While longer-lived species (observed by fluorescence on the nanosecond timescale) have previously been ascribed to an electronic excited state species such as an

Table 1: Summary of observed IR bands and kinetics.

DNA	IR band position [cm^{-1}]	τ [ps]
5′-dCMP	1574	(2.6±0.3) 80%, (33 ± 4) 20%
5′-dGMP	1669	(3.1±0.3)
[poly(dGdC)]$_2$	1685	(7±1) 54%, (30 ± 4) 46%
B form	1658	(7±1) 49%, (31 ± 3) 51%
	1612	(6±1)
	1597	(21±4)
[poly(dGdC)]$_2$	1670	(18±2)
Z form	1636	(20±2)
	1617	(10±2)
	1593	(16±2)

exciplex,[20] our results show that greater than 96% of the ground state has recovered by 1 ns. Any contribution from such states is small although at this stage the biological relevance for the formation of nucleic acid products needs to be determined. The species with kinetics of 21 ps has a relatively well-defined infrared absorption feature at approximately 1597 cm^{-1}. The origin of this spectral component needs to be considered regarding tautomers, electron or/and proton transfer products (e.g., the guanine radical cation, guanine radical, protonated cytosine, see Scheme 1 a–f) or



Scheme 1. a) Watson–Crick G–C base pairing, b) and c) tautomeric products, d) proton transfer, e) charge transfer, and f) $^1n_N\pi^*$ form for the excited state of $[poly(dG\text{-}dC)]_2$.

other dark excited states. Of the two most likely C-based tautomers (imino–oxo and imino–enol forms), the first is less likely as calculations predict a band at higher frequency than that of the canonical form.[5] Further computation will be required to unequivocally dismiss the imino-enol possibility. Additionally, the IR spectra of protonated cytosine or guanine anions suggest that it is unlikely that either species is responsible for the characteristic absorption at 1597 cm^{-1}. The location of this band is also close to that of the dark $^1n_N\pi^*$ state of 5′-dCMP (1574 cm^{-1}) that we have previously assigned.[5] Therefore we suggest that the 1597 cm^{-1} band observed in the B form of $[poly(dG\text{-}dC)]_2$ is the $^1n_N\pi^*$ dark state of the 2′-deoxycytidine moiety. This shows the importance of this state in the deactivation processes of C-containing polynucleotides.

The faster (7 ps) process observed in the B form of $[poly(dG\text{-}dC)]_2$ is consistent with the formation of a vibrationally excited ground state in a polynucleotide structure. This can be accessed by rapid internal conversion from the initially formed excited state (either localized on one of the nucleobases or delocalized over several). Alternatively the "hot" species could, in theory, result after a subpicosecond back reaction from a rapidly formed photoproduct. One such option is the initial formation of a charge-transfer state (CT, with a single electron moving from the guanine base to the cytosine base), which is then followed by the transfer, within tens of femtoseconds, of the imino proton from guanine to cytosine with formation of a neutral radical.[14] Recent fluorescence upconversion measurements of modified G–C pairs that are hydrogen bonded in chloroform solution concluded that the enhancement in the decay rate was due to proton transfer in the base-pair—a mechanism not available to the individual base.[15] This overall process is estimated to occur within a 200 fs time frame[14] (much faster than the time resolution of these experiments), although structural and hydration effects in polynucleotide systems might prolong this process. The timescale of our measurements do not allow us to expand further on the processes.

These results should also be considered in light of the recent findings of Crespo-Hernandez et al. that UV excitation of $d(GC)_9{\cdot}d(GC)_9$, gave a monoexponential (6 ps) recovery of the ground state as observed at 250 nm.[21] This was attributed to deactivation of the electronic excited state by (dark state) intrastrand exciplexes rather than interstrand proton transfer. Intriguingly, the long-lived component of (31 ± 26) ps (observed for the mononucleotide mixture by using the same technique) was not observed in the $(GC)_9$ duplex. In our experiment, we are able to directly observe spectral signatures that distinguish both transient species. It should be pointed out that the $^1n_N\pi^*$ state is not readily detectable by visible absorption transient spectroscopy. The lack of a comparable bleaching signal could be explained by the overlap of ground-state and transient absorption at 250 nm. Such overlap is generally less common in infrared measurements because of unique molecular fingerprints for each species. Another factor accounting for this difference may be the different medium. Our experiments necessitate the use of D_2O whilst the UV/Vis transient absorption experiments were performed in H_2O. This may lead to different dynamics that stem from the reordering of the electronic states changing deactivation pathways.

The B and Z forms of $[poly(dG\text{-}dC)]_2$ were next compared. DNA is a polymorphic structure and in the presence of NaCl (4 M) the left-handed Z conformation of $[poly(dG\text{-}dC)]_2$ is obtained.[22] The ps-TRIR spectrum of this form is given in Figure 1 b. The different base-pair stacking and solvation environment results in the bleach bands that are located at 1685 cm^{-1} and 1658 cm^{-1} in the B form being shifted to 1670 cm^{-1} and 1636 cm^{-1} in the Z form. Moreover, in contrast to the B form, the recovery kinetics of the ground state monitored at the two dominant bleaches, 1670 cm^{-1} ((18 ± 2) ps) and 1636 cm^{-1} ($(21(\pm 2)$ ps) followed a single exponential decay with no significant improvement found for double-exponential fitting. The transient bands analyzed in the region of 1520–1560 cm^{-1} gave a single-exponential lifetime of (16 ± 2) ps and a similar value was obtained in the region 1587–1605 cm^{-1} (Table 1, kinetics are shown in the Supporting Information). However, some evidence of shorter values was obtained in the region 1610–1620 cm^{-1}, for example, at 1617 cm^{-1} (central peak) a decay of (10 ± 2) ps was observed. Furthermore, a small signal remains after 1000 ps, which shows depletions at 1636 and 1670 cm^{-1} and an absorption band at 1695 cm^{-1} (see below).

It is reasonable to expect that the structural differences between these forms will influence the excited-state dynamics. In the B form, the intrastrand stacking of G–C bases are uniform with a base separation of 3.4 Å, while the Z form is characterized by a repeating dinucleotide unit with the spacing being 3.5 Å within and 4.1 Å between such units,

with improved overlap of the stacked bases in the dinucleotide unit. In the Z form, the bases are located at the periphery of the helix, which positions the phosphate groups closer together. The resulting electrostatic repulsion is reduced by the presence of the sodium ions (4 M NaCl). Furthermore, the nature of hydration is also very different for the two conformers.

The results for the Z form show that the IR transient absorption predominantly decays with a lifetime of 16 ps although some evidence of a faster decay (ca. 10 ps) in the 1617 cm^{-1} region is also noted. The transient spectra of B- and Z-DNA at 2 ps and 16 ps, are compared in Figure 2. The

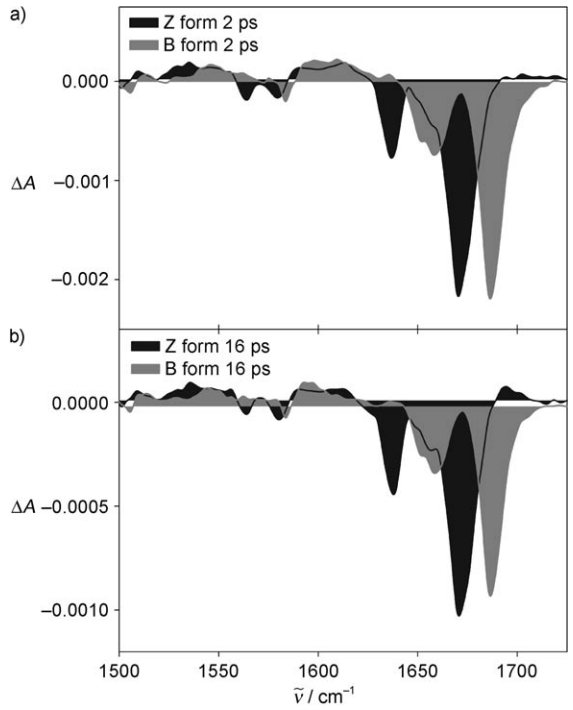


Figure 2. Comparison of ps-TRIR spectra of both forms of [poly(dG-dC)]$_2$ at a) 2 ps and b) 16 ps time delays following UV excitation (300 fs, 267 nm). Both forms (10 mM) were in potassium phosphate buffer (50 mM, pH 7, D_2O), with NaCl (4 M) for the Z case. The thin black line shown within the B-form spectra represents the peak profile of the Z form.

intensity of the absorbing species is comparable at both times. As for the B form, the Z form shows absorptions in the region of the spectra associated with the $^1n_N\pi^*$ state but the structured band at 1597 cm^{-1} is replaced by a broader less structured feature in Z-DNA. If this is a single species (e.g., the vibrationally hot ground state) then its decay is significantly slower than in the B-conformer. Alternatively it is possible that two species give rise to this band, the $^1n_N\pi^*$ species being one likely candidate.

There is considerable speculation regarding the contribution of base stacking to the stabilization of the long-lived excited states in polynucleotides. Theoretical studies on $(dGdC)_5$ in the B form have predicted that 50% of the excited states produced are delocalized over at least two bases.[23] The broad nature of the transient spectrum in Z-DNA is consistent with an exciplex species. In Z-DNA, the interdinucleotide step staggers the bases far enough apart to reduce electronic communication.[20,22] Thus, location of the excitation may be restricted to two bases. However, in the B form the more widely delocalized excited state has a shorter lifetime. It is worth noting that the common structural feature present in both the B- and Z-DNA forms is Watson–Crick hydrogen bonding. Interstrand hydrogen bonding is predicted to lead to strong coupling of the G and C transition dipoles in the excited states.[24] This has been experimentally observed in previous 2D IR studies.[25] The formation of delocalized excited states by electronic coupling has also been studied in detail in the work of Markovitsi and co-workers, [13,23,24,26] which further supports the interpretation that the broad IR band shapes indicate exciplex formation.

In both systems, a small amount of bleaching (ca. 4%) was found to persist after 1 ns. However, for the Z-DNA system the appearance of a transient absorption band at 1695 cm^{-1} was also observed. We have previously reported the appearance of a band in a similar position from the direct photoionization by 200 nm UV excitation[7] and the sensitized photooxidation[8] of [poly(dG-dC)]$_2$ in the B conformation. Thus, this species may be related to direct photoionization of G that yields an oxidized G photoproduct. Alternatively, given the early timescale signals, it may be evidence of an excited-state charge–transfer transition from C to G that was identified in recent theoretical reports,[14] and/or subsequent H transfer. Our ability to clearly resolve this band may stem from the shifting of the guanine based carbonyl band in the Z form relative to its position in the B form, where it would most likely be masked. The specific issue of the feature at 1695 cm^{-1} will be addressed in a future publication.

In conclusion, we have shown that UV excitation of the Watson–Crick G–C base pairs in double-stranded DNA produces transient IR absorption signals long after the $^1\pi\pi^*$ excited state is expected to have decayed. Evidence for two dark-state processes that contribute approximately equally to the decay of the initially formed excited states is found for B form [poly(dG-dC)]$_2$. The first may be rationalized in terms of the decay of a vibrationally excited ground state. Dispersion of excess energy over a number of base pairs could account for the observed absence of tracking that is characteristic of vibrational cooling. However, it is also possible that the 7 ps species in B-DNA could arise from a delocalized exciplex formed over more bases. The second transient in the B form, which has a well-defined band at 1597 cm^{-1}, results quite clearly from a localized excited state. We attribute this band to the $^1n_N\pi^*$ dark state of the 2′-deoxycytidine moiety. This demonstrates that a very significant proportion of the excited states decay by a very different route than that proposed in most recent theoretical treatments.[11,14]

There is sensitivity of the decay processes in the G–C base pair to changes of the structure of DNA. In Z-DNA, both the transient bands and the bleaches conform very well to a single exponential decay of 16–20 ps with some evidence for a faster process. While we cannot rule out the role again here of the $^1n_N\pi^*$, we favor the presence of a dinucleotide-localized exciplex-type excited state that manifests itself as the spectrally broad transient. The influence of the deuterated

solvent needs also to be fully considered with regard to the recently proposed proton-transfer dynamics between the G–C base pair.[14] The above ps-TRIR measurements show that all transients formed in both B- and Z-DNA $[poly(dG\text{-}dC)]_2$ are deactivated much more readily than those found in A–T-rich DNA systems.[27] It is striking that the significant structural changes in base stacking and solvation between the two forms studied does not appear to dramatically influence the timescale of the relaxation dynamics. To what extent the hydration and the proximity of the sodium ions used to generate the Z form have in influencing the photophysical properties and in controlling the nonradiative decay properties of the excited states including the $^1n_N\pi^*$ state remains to be fully elucidated.

Experimental Section

Measurements were made using the picosecond infrared absorption and transient excitation (PIRATE) system.[28] The data were collected in a number of 150 cm^{-1} spectral windows by using the delay line for optical delays between 2 ps and 1 ns. The difference signal was calibrated using water lines present in the probe spectrum, and the spectral windows were interleaved using overlapping transients recorded at the same delay time. The sample was raster scanned in the *x*- and *y*- directions at an approximate rate of 100 mm ms^{-1}. The 1-pixel accuracy was ± 4 cm^{-1}. Ground state UV and FTIR measurements were recorded using a Perkin–Elmer Lambda2 and a Nicolet Avatar 360 respectively (see the Supporting Information for further experimental details).

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