A study of the pH dependence of electronically excited guanosine compounds by picosecond time-resolved infrared spectroscopy†

David A. McGovern,[a] Gerard W. Doorley,[a] Aine M. Whelan,[a] Anthony W. Parker,[b] Michael Towrie,[b] John M. Kelly*[a] and Susan J. Quinn*[a]

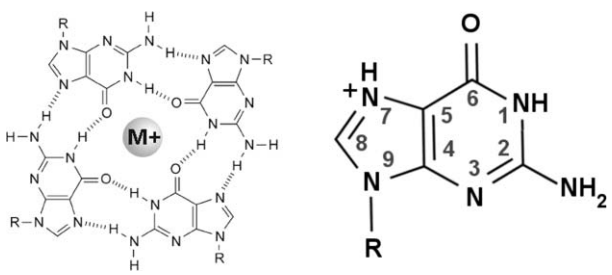
Received 10th October 2008, Accepted 30th January 2009
First published as an Advance Article on the web 6th March 2009
DOI: 10.1039/b817756b

The photophysical properties of 5′-guanosine monophosphate (5′-GMP) and polyguanylic acid {poly(G)} in D_2O solutions of varying pH have been studied using picosecond transient infrared absorption spectroscopy. Whereas in neutral or weakly alkaline solution only the vibrationally excited electronic ground state of 5′-GMP is observed, in acidic solution the relatively long-lived (229 ± 20 ps) electronic excited state of protonated 5′-GMP, which possesses strong absorptions at 1517 and 1634 cm^{-1}, could be detected. The picosecond transient behaviour of polyguanylic acid in acidic solution is also very different from that of the polynucleotide in neutral solution due not only to the protonation of guanine moieties yielding the protonated excited state but because of the disruption of the guanine stacks which are present in the species in neutral solution.

Introduction

Picosecond time-resolved infrared (ps-TRIR)[1–8] is emerging as a powerful technique for the study of the photophysical processes in nucleic acids. TRIR has followed on from the study of DNA systems by both UV-vis transient absorption and fluorescence techniques.[10–13] Our work seeks to understand how photochemically driven reactivity is altered by environmental perturbations and/or interaction with other nucleic acid bases, as found naturally in polymeric and stacked forms of the bases. To this end guanine nucleotides and polynucleotides are particularly interesting. Guanine has the lowest ionization potential of the four DNA bases and consequently is most susceptible to photo-oxidation, a process that leads to the formation of radical cations which may lead to DNA damage, mutation and the onset of cancer.[4,14] Guanine is also interesting for its ability, through Hoogsteen binding, to form tetrad structures (**1**).

The mononucleotides 5′-GMP and 5′-dGMP are only very weakly luminescent and this has been attributed to the ultra-short lifetime (860 fs for 5′-dGMP from fluorescence upconversion)[15] of the emitting singlet excited state, caused by very rapid internal conversion to form a vibrationally excited electronic ground state.[16] The relaxation of this latter species through coupling to the solvent occurs on the picosecond time scale and has previously been characterised by ps-TRIR measurements.[1,3] However, it is known that very simple modifications of nucleic acids, such as chemical substitution or the presence of a ribose group or protonation, may significantly change the excited state lifetimes.[17] In this light the protonation of 5′-GMP to form 5′-GMPH$^+$ (**2**) ($pK_a = 2.3$) is known to have a dramatic effect on the lifetime of the electronic excited state. The protonated form is significantly more fluorescent than the neutral base. In the first transient spectroscopy study of this system, Fujiwara *et al.*[18] used a synchroscan streak camera to monitor the effect of pH on the lifetimes of the excited states of guanine, guanosine and 5′-GMP. They observed that the lifetime of the excited state of 5′-GMP was significantly longer at acidic pH (*ca.* 200 ps) than for the neutral molecule. Guanosine (Guo) at pH 3 has also been investigated by Peon and Zewail using femtosecond up-conversion transient fluorescence methods.[15] Here, three lifetime components were recorded [τ_1= 660 fs (67%), τ_2= 3.4 ps (18%) and τ_3= 209 ps (15%)]. The long-lived component was in agreement with fluorescence observations made using a streak camera and attributed to the GuoH$^+$ species. The subpicosecond component was the same as that recorded for the unprotonated Guo in neutral solution and as such attributed to the presence of this species. The 3.4 ps component was attributed to relaxation processes within the S_1 state of protonated guanine. Pecourt *et al.* in their femtosecond pump–probe transient absorption study of 5′-GMPH$^+$ again observed the presence of the long-lived component, $\tau = 194$ ps.[16] These results are therefore all consistent with the lifetime of the fluorescing singlet excited state of 5′-GMPH$^+$ being *ca.* 200 ± 20 ps.



Under certain conditions, guanine-based Hoogsteen tetrads may form extended structures through stacking interactions.[19,20] Such stacking interactions have been considered in light of the presence of repeat runs of guanine in the human telomeric

[a]*School of Chemistry and Centre for Chemical Synthesis and Chemical Biology, Trinity College, Dublin 2, Ireland. E-mail: jmkelly@tcd.ie, quinnsu@tcd.ie; Fax: +35 316712826; Tel: +35 318961947*
[b]*Central Laser Facility, Science & Technology Facilities Council, Rutherford Appleton Laboratory, Harwell Science and Innovation Campus, Didcot, Oxfordshire, UK OX11 0QX*

† Electronic supplementary information (ESI) available. See DOI: 10.1039/b817756b

sequence found at the end of chromosomal DNA.[21] FTIR is a powerful technique for probing the DNA secondary structures present in solution, as bands in the region 1500–1700 cm^{-1} are very sensitive to stacking interactions. For example, the characteristic IR bands of 5′-GMP undergo marked changes in frequency and intensity when guanine is present in ordered structures.[20] In our initial ps-TRIR study of guanine-rich systems we observed that systems containing stacked guanine tetrads, such as 5′-GMP at high concentration or polyguanylic acid [poly(G)] showed a long-lived transient in addition to the vibrationally excited ground state. This transient species was assigned to an excimer within the stacked structure.[3]

In the present publication, we use ps-TRIR to follow the excited state processes in 5′-GMP in neutral, acidic and alkaline D_2O solutions. We then consider the effect protonation of the guanine base has on the photodynamics of poly(G), a system that is known to adopt interesting secondary structures based on stacked guanine tetrads.[3] Note that under our experimental conditions all exchangeable protons are replaced by deuterons.

Experimental

Materials and sample handling

The 5′-H_2GMP was supplied by MP Biomedicals Inc. as the 5′-H_2GMP free acid, purity 98–100%. The 5′-Na_2GMP, polyguanylic, phosphate buffer components, D_2O and other reagents were all supplied by Sigma-Aldrich. Samples were prepared by weighing out an amount of the nucleotide base or polynucleotide into an eppendorf followed by the addition of a known amount of D_2O or D_2O buffer solution and the concentration was determined by UV measurements.

Picosecond time-resolved infrared (ps-TRIR) experiments

The ps-TRIR experiments were carried out at the Central Laser Facility of the Rutherford Appleton Laboratory. This apparatus has been described previously[9] and a brief description is given in the ESI.† The sample was excited with a 150 fs 267 nm pulse of energy approximately 2 μJ with a spot size of between 150–200 μm. The data were collected in a number of 150 cm^{-1} wide spectral windows centred at approximately 1625 and 1565 cm^{-1} using the delay line for optical delays between 2 ps and 1.5 ns, normally at 2, 3, 4, 5, 6.5, 8, 10, 12.5, 15, 20, 35, 50, 100, 150, 200, 500, 1000 and 1500 ps. The sample in D_2O was placed between two 25 mm CaF_2 plates in a Harrick cell. For low concentration experiments a 12 μm Teflon spacer was used and no spacer was used in the case of high concentration experiments. Ground state UV-vis and FTIR spectra were recorded before and after all experiments using a Perkin Elmer Lambda 2 and a Nicolet Avatar 360 respectively to ensure that no photodegradation occurred during the ps-TRIR experiments.

Results

5′-GMP and 5′-GMPD$^+$

5′-GMP excited by a 150 fs pulse of 267 nm radiation shows results very similar to those of 5′-dGMP which has previously been characterised at pH 7 by ps-TRIR[1,3] (see also ESI). In these experiments the bleached bands of the ring (1581 cm^{-1}) and carbonyl (1669 cm^{-1}) stretches were found to decay rapidly (<6 ps). This rapid decay was attributed to cooling of the vibrationally hot ground state after relaxation from the electronically excited state. Interestingly, the rate of recovery to the thermally equilibrated ground state is different for the ring-based vibration (lifetime 3.1 ± 0.3 ps) than for the carbonyl-based vibration (4.7 ± 0.4 ps), suggesting that there is some degree of mode-specific interaction with the solvent.[3] The IR transient absorptions also show a characteristic tracking behaviour with the absorption maximum moving to higher wavenumber as the transient decays.[3]

To explore the influence of pH on this system, 5′-GMP was first monitored in acidic D_2O solution (pD 2) at various delays after 267 nm excitation (Fig. 1). The behaviour observed at this pH was found to be very different from that in neutral solution. The protonation of 5′-GMP, at the N7 position of the imidazole ring to form 5′-GMPD$^+$, results in major changes in the ground state FTIR spectrum. The ring band at 1578 cm^{-1} is accompanied by an additional band at 1608 cm^{-1} and the carbonyl band is found to shift from 1669 to 1689 cm^{-1}. The three bands manifest themselves as bleaches in the ps-TRIR and two intense transient absorption bands are present at 1517 and 1634 cm^{-1}. Both transient bands for this excited state occur at lower frequency than for the ground state. While assignment of the transient bands will require examination by computational methods, we provisionally assign these to the purine-ring based (1517 cm^{-1}) and carbonyl based (1634 cm^{-1}) vibrations as in the ground state. In the case of the carbonyl this suggests an excited state with less double bond character than the ground state.

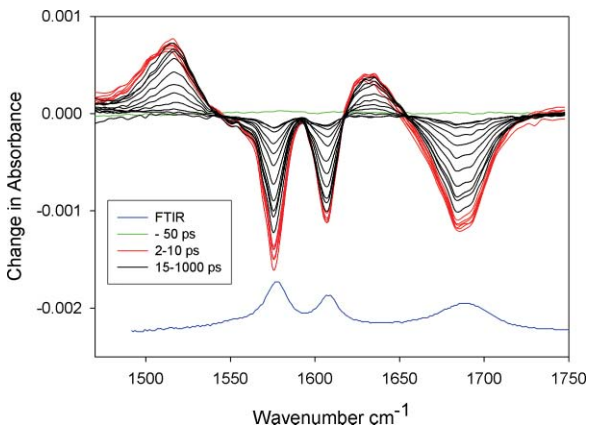


Fig. 1 ps-TRIR of 10 mM 5′- Na_2GMP under acidic conditions, 0.132 M H_3PO_4 in D_2O, with FTIR below (blue). Spectra at delays of −25 ps (green), 2–10 ps (red) and 15–1000 ps (black).

Analysis of the kinetics of the bleach and transient decays reveals a monoexponential recovery to the ground state, with a lifetime of 229 ± 20 ps (Fig. 2). This is considerably longer than observed for 5′-GMP under neutral conditions and is consistent with the presence of a protonated excited state, which has previously been identified by fluorescence[15,18] and transient visible absorption spectroscopy.[17]

Next 5′-H_2GMP was studied in unbuffered D_2O solution. Under these conditions a mixture of the protonated and neutral species is present, as can be seen from the FTIR (bottom of Fig. 3a). The ps-TRIR indicates the presence of two different decaying

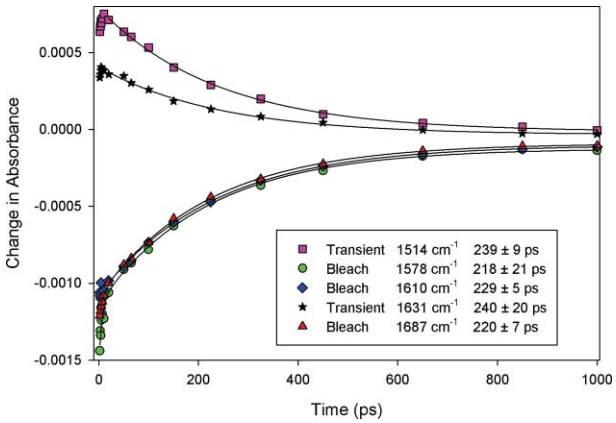


Fig. 2 Kinetic analysis of ps-TRIR data for 10 mM 5′-Na_2GMP under acidic conditions, 0.132 M H_3PO_4 in D_2O. Kinetics recorded at the carbonyl and ring bleach and associated transient positions 1687 cm^{-1}(▲), 1631 cm^{-1}(★), 1610 cm^{-1}(♦), 1578 cm^{-1}(●) and 1514 cm^{-1} (■). 1578 and 1687 cm^{-1} traces calculated over the range of 2–1000 ps with 1514, 1610, and 1631 cm^{-1} calculated over the range 20–1000 ps.

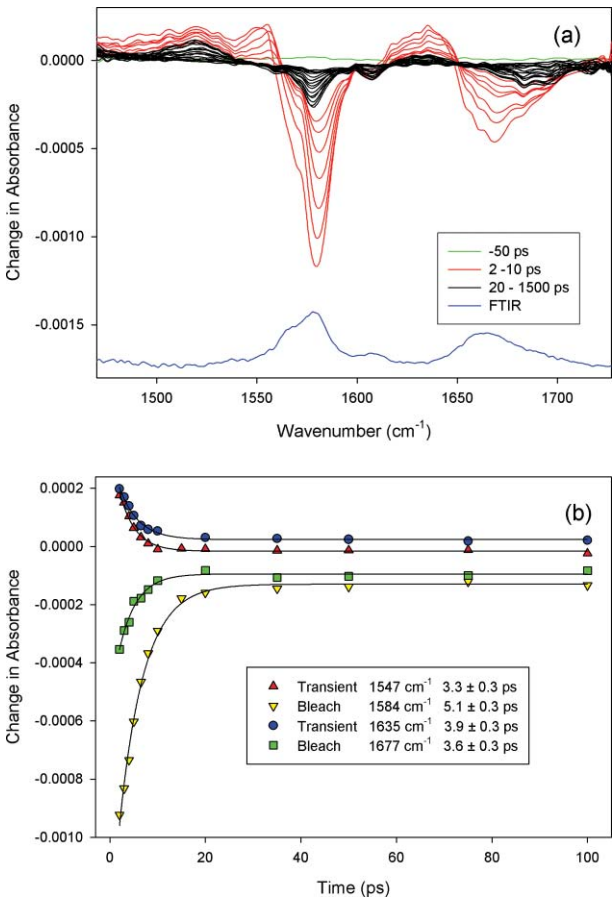


Fig. 3 ps-TRIR and FTIR of 10 mM 5′-H_2GMP in unbuffered D_2O. (a) Spectra at delays of –25 ps (green), 2–10 ps (red) and 20–1500 ps (black). (b) Kinetics recorded at the carbonyl and ring bleach and associated transient positions: 1677 cm^{-1} (■), 1635 cm^{-1} (●), 1584 cm^{-1} (▼) and 1547 cm^{-1} (▲).

species, one fast (1580 and 1669 cm^{-1}, appears in red) and one slow (1578, 1608 and *ca.* 1689 cm^{-1}, appears in black). This difference in behaviour is most striking in the carbonyl region (1650–1700 cm^{-1}) of the ps-TRIR spectra (Fig. 3a), where the vibrationally excited ground state (red component of the transient) is easily discernible from the longer-lived protonated excited state (black component of the transient). Analysis of the bands characteristic of the neutral species yields short decay kinetics. For example for the carbonyl (1677 cm^{-1}) and ring (1584 cm^{-1}) bleached bands, lifetimes of 3.6 ± 0.3 ps and 5.1 ± 0.5 ps were found. The decay times of the corresponding transient absorptions also display similar kinetics with decay times of 3.5 ± 0.3 ps and 3.3 ± 0.3 ps observed at 1635 cm^{-1} and 1547 cm^{-1} respectively. In turn, examination of the features associated with protonated species give long-lived lifetimes in agreement with those reported above. For example, the carbonyl bleach at 1690 cm^{-1} recovers with a lifetime of 224 ± 23 ps (measured between 20–1500 ps) while the associated transient at 1635 cm^{-1} decays with a lifetime of 205 ± 41 ps. The transient feature at 1517 cm^{-1} displays features of the neutral and protonated form with a short decay times of 3.1 ± 0.5 (56%) and a long 243 ± 24 (44%) respectively. The results of the kinetic analysis at each of the transient absorption and bleaching maxima are presented in Table 1.

5′-GMP was also studied under mildly alkaline conditions (pH 8.5). The results obtained at this pH were found to be very

Table 1 Decay times for 5′-GMP in neutral solution and poly(G) in neutral and acidic solution

Guanine system	FTIR/ cm^{-1}	Lifetime (ps) and % contribution
5′-GMP neutral[a]	1577	**1553 (T)** 3.5 ± 0.3, **1587 (B)** 4.7 ± 0.4
	1662	**1642 (T)** 3.3 ± 0.3, **1676 (B)** 3.1 ± 0.3
5′-Na_2GMP in 0.132 M H_3PO_4[b]	1577	**1514 (T)** 239 ± 9
	1607	**1610 (B)** 229 ± 5
	1688	**1578 (B)** 6.5 ± 2.2 (27%), 218 ± 21 (73%)
		1631 (T) 240 ± 20
		1687 (B) 7.0 ± 1.5 (15%), 220 ± 7 (85%)
5′-H_2GMP in D_2O[c]	1577	**1517 (T)** 3.1 ± 0.5 (56%), 243 ± 24 (44%)
	1607	**1547 (T)** 3.3 ± 0.3
	1662	**1579 (B)** 3.3 ± 0.1 (89%), 220 ± 27 (11%)
	1688	**1584 (B)** 5.1 ± 0.3, 194 ± 24
		1635 (T) 3.5 ± 0.3 (83%), 205 ± 41 (17%)
		1677 (B) 3.6 ± 0.3
		1690 (B) 224 ± 23
Poly(G)[d]	1581	**1553 (T)** 3.6 ± 0.7 (68%), 35 ± 9 (28%)
	1610	**1599 (T)** 3.7 ± 0.5 (59%), 36 ± 4 (41%)
	1687	**1608 (B)** 2.5 ± 1.4 (60%), 52 ± 18 (40%)
		1648 (T) 4.5 ± 0.6 (72%), 36 ± 8 (28%)
		1687 (B) 5.1 ± 1.4 (47%), 37 ± 6 (53%)
Poly(G)H^+ [e]	1577	**1559 (T)** 9 ± 2 (58%), 133 ± 30 (42%)
	1603	**1584 (B)** 24 ± 9 (56%), 190 ± 75 (44%)
	1673	**1606 (B)** 18 ± 4 (70%), 201 ± 85 (30%)
	1698	**1640 (T)** 17 ± 6 (46%), 92 ± 25 (54%)
		1672 (B) 18 ± 4 (80%), 190 ± 131 (20%)
		1689 (B) 16 ± 4 (69%), 118 ± 48 (31%)
		1702 (B) 13 ± 4 (57%), 104 ± 31(43%)

(B) = bleach; (T) = transient.[a] Range of 2–100 ps. [b] Range of 20–1000 ps for long single exponential times and for biexponential result range of 2–1000 ps. [c] Short single exponential range of 2–100 ps, long single exponential range of 20–1500 ps and any biexponential range of 2–1500 ps. [d] Range of 2–500 ps. [e] The range was 3–500 for 1640 cm^{-1} and 3–1500 ps for all other bands.

similar to those observed at neutral pH (ESI, Fig. S1a†). Regions of transient absorption and depletion ('bleaching') of the ground state ring and carbonyl bands are present. The bleached bands were found to recover with lifetimes of $\tau = 2.7 \pm 0.3$ ps (1675 cm^{-1}) and 5.2 ± 0.5 ps (1587 cm^{-1}) respectively (ESI, Fig. S1b†). These data are comparable to the results found for 10 mM 5′-GMP in neutral conditions {*i.e.* 3.1 ± 0.3 ps (1676 cm^{-1}) and 4.7 ± 0.5 ps (1587 cm^{-1})}.[1,3] The results complement those already in the literature and clearly show that chemical changes can be readily identified and monitored using TRIR. However, as biological systems are based on polynucleotide sequences we now consider the effect of protonation on a guanine-based polymer as seen by ps-TRIR.

Poly(G) and poly(GH^+)

Guanine bases are known to self-assemble in solution to form stacked tetrads through Hoogsteen hydrogen bonding.[19] This phenomenon is also found for poly(G) which forms a four-stranded helical structure. The formation of this structure is readily observable using circular dichroism.[21] The spectrum is dominated by an exciton couplet characterized by a positive band at *ca.* 260 nm with a shoulder at *ca.* 290 nm, and a negative maximum at *ca.* 240 nm (Fig. 4). The presence of these bands under the experimental conditions indicates the existence of the assembled form in solution.

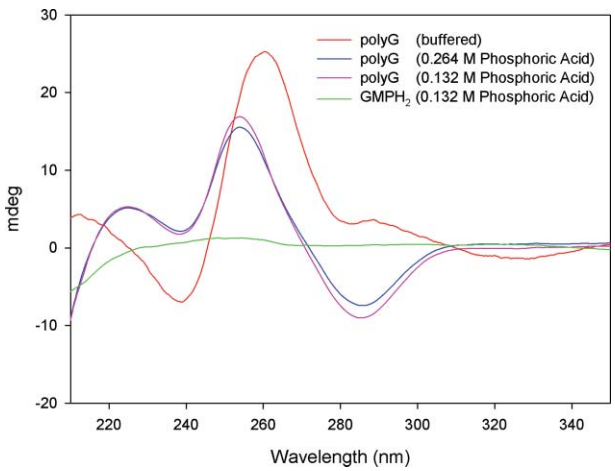


Fig. 4 Circular dichroism of 10 mM 5′-H_2GMP in 0.132 M H_3PO_4, 11.3 mM poly(G) in 0.132 M H_3PO_4, 11.3 mM poly(G) in 0.264 M H_3PO_4, 11.3 mM poly(G) in 50 mM potassium phosphate buffer, all samples in D_2O.

The presence of guanine stacked tetrads is also readily identified by FTIR spectra. The guanine carbonyl and ring stretches both undergo changes upon the formation of the stacked Hoogsteen hydrogen bonded guanines.[20] A narrowing of the carbonyl band is observed and the band shifts to higher wavenumbers (1663 cm^{-1} to 1688 cm^{-1}), while the ring bands are found to undergo significant hypochroism (see ESI, Fig. S2†). In Fig. 5a and 5c the ps-TRIR, 2 ps after excitation, of poly(G) and 5′-GMP, at neutral pH are compared. As expected from the FTIR, the bleaching of the ring-based vibrations of poly(G) are noticeably suppressed and there is a sharpening of the carbonyl vibration, which shifts from 1669 cm^{-1} to 1690 cm^{-1}, indicating the formation and stacking of

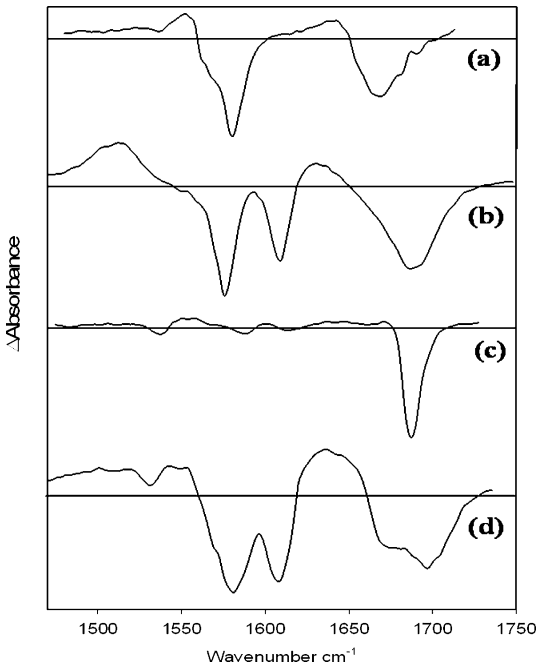


Fig. 5 ps-TRIR spectra all recorded at 2 ps of (a) 10 mM 5′-GMP in 50 mM potassium phosphate D_2O buffer at pH 7, (b) 10 mM free acid 5′-GMP in 0.132 M H_3PO_4 in D_2O, (c) 11.4 mM poly(G) in 50 mM potassium phosphate D_2O buffer at pH 7 and (d) 11.3 mM poly(dG) in 0.132 M H_3PO_4 in D_2O.

guanine tetrads.[20] The kinetic behaviour of the poly(G) also differs markedly from that of 5′-GMP in dilute solution. (However, it is similar to that of the stacked system formed in the presence of high 5′-GMP concentration.[3]) Analysis of the recovery of the carbonyl bleach at 1687 cm^{-1} reveals a biexponential process with a short component of 5 ± 1 ps (47%) and a longer component of 37 ± 6 ps (53%). Interestingly, whilst the short lifetime is comparable to that observed for the vibrationally hot ground state of 5′-GMP there is an absence of any tracking of the bands. This phenomenon has been recently observed in other polymer systems and may be explained by a delocalization of the excess vibrational energy over neighbouring bases in the polymer form.[8] The longer-lived species is assigned to an excimeric excited state.[3]

Next the effect of protonation of the poly(G) has been studied in D_2O. The CD of poly(G) (Fig. 4) shows that in the presence of acid there is a change in sign of the band at 290 nm and a shift in the intensity and position of the positive band at 260 nm to 255 nm. Furthermore, the band at 240 nm disappears. These results are in good agreement with those observed previously under similar conditions.[21] The spectra are closely similar at both 0.132 M and 0.264 M H_3PO_4, showing that the extent of protonation is independent of the acid concentration in this range. As expected, the decrease in pH results in changes in the ground state FTIR spectrum. The ring vibrations are no longer suppressed with two bands observed at 1577 and 1603 cm^{-1} and the region associated with the carbonyl bond is dominated by a broad structured band at 1700 cm^{-1} with a shoulder of lower intensity between 1663–75 cm^{-1} (Fig. 6). These changes are attributed to protonation of guanine within the polymer and a disruption of the tetrad stacking. The ps-TRIR, 2 ps after excitation, of poly(G) under acidic conditions is shown in Fig. 5d. All four ground state IR absorption bands

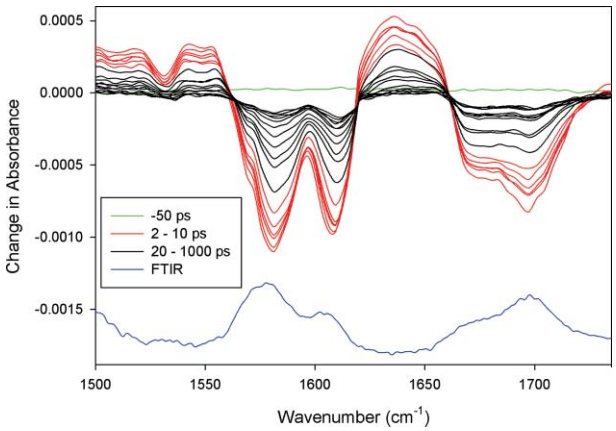


Fig. 6 (a) ps-TRIR of 11.3 mM poly(G) under acidic conditions, 0.132 M H_3PO_4 in D_2O with FTIR below. Spectra at delays of −50 ps (green), 2–10 ps (red) and 20–1000 ps (black). Spectra recorded in windows centred at 1557 cm^{-1} and 1633 cm^{-1} and joined at 1561 cm^{-1}.

appear as bleaches in the ps-TRIR spectra and, in addition, there are two strongly absorbing transient signals observed, one between 1500–1550 cm^{-1} and a second broad band centred at 1640 cm^{-1}. These bands are assigned to a ring-based vibration and a carbonyl vibration in the excited state respectively. Significantly, both the bleaching and transient absorption regions of the spectrum are found to be similar to that observed for 5′-GMPD$^+$ at the same time of 2 ps (Fig. 5b).

The ps-TRIR profiles of protonated poly(G) recorded at delays 2–1500 ps are shown in Fig. 6. Examination of the data reveals complex kinetics, with biexponential decay and recovery, observed in the bleach and transient features (Table 1). The faster decaying component is markedly longer-lived and the slower-decaying species is significantly shorter-lived, than their counterparts in neutral poly(G). The short times are possibly extended due to the superposition of a number of short-lived states which result from incomplete protonation.

Discussion

The mechanism by which nucleic acids dissipate excess energy is a subject of increased interest.[12,23] In the case of 5′-GMP it is now recognised that the initially formed $^1\pi\pi^*$ state undergoes rapid internal conversion at a conical intersection forming the electronic ground state with considerable excess vibrational energy.[16] It is this 'hot' ground-state transient behaviour that can be monitored at early, <5 ps, times in our ps-TRIR experiments.[1,3] The speed of energy loss from this species has been shown to be dependent on the vibrational mode involved and is slightly faster for the vibration associated with the carbonyl group than for that of the ring (C=C, C=N).[3] The current study further supports the contention that for 5′-GMP the species observed under these neutral/weakly alkaline conditions is the vibrationally excited ground electronic ground state.

By contrast in strongly acidic D_2O solution, quite different behaviour is observed. Under these conditions the ground state is protonated at the 7-position, giving 5′-GMPD$^+$.[24] This results in removal of electronic charge from the pyrimidine ring. The subsequent decoupling of the C=O mode with the solvent through less hydrogen bonding leads to a shifting of the C=O mode by 21 cm^{-1}, representative of more double bond character. The transient observed following 267 nm excitation shows that the excited state deactivates with a lifetime of 229 ± 25 ps. This is much longer than that observed from 5′-GMP in neutral solution and is closely similar to that observed by fluorescence[15] and transient visible absorption.[16,17] This protonated excited state is characterised by two strong absorption bands in the IR at 1518 and 1634 cm^{-1}. At this stage, in the absence of computational studies we are unable to unambiguously assign these features that probably relate to the excited state being a $^1\pi\pi^*$ state of 5′-GMPD$^+$. Interestingly, there is little evidence of the band tracking that is characteristic of vibrational cooling.[2,3,16,25] This would suggest that either when the protonated excited state is formed, it does so with only a small amount of excess vibrational energy or that the solvent coupling is sufficiently increased in this species that vibrational relaxation is extremely efficient, and as a result we are unable to observe the tracking behaviour within the time-resolution of our experiments.

In unbuffered solutions of 5′-H_2GMP both the 5′-GMP and 5′-GMPD$^+$ are present. Probing the transient absorption of this mixture yields spectra and kinetics that are entirely consistent with the behaviour expected for both species. The characteristic short-lived vibrationally excited electronic ground state of the 5′-GMP is produced and the longer-lived (*ca.* 200 ps) electronic excited state of 5′-GMPD$^+$ is also clearly resolved. The kinetic behaviour (Table 1) is that expected for the separate 5′-GMP and 5′-GMPD$^+$.

Incorporation of 5′-GMP into a polymeric structure places the guanine base in a new environment in terms of hydrogen bonding, stacking interactions and solvation, which are all dependent on the biopolymer's secondary structure. Circular dichroism is particularly sensitive to secondary structure and has the advantage of an extensive library of diagnostic band positions. The buffered poly(G) sample was found to have a negative band at 240 nm and a positive band at 260 nm. These CD signals are characteristic of parallel G4-DNA sequences of the G_n type.[21] This secondary structure, in particular the formation of stacked guanine tetrads, results in major changes in the ground state IR and the ps-TRIR behaviour, with the dominating feature being a narrow carbonyl band at 1690 cm^{-1}.[3,20] A biexponential decay is observed with a short-lived transient (3.7 ± 0.5 ps) and an additional longer-lived transient (36 ± 4 ps). This can be understood in terms of the new environment of the guanine base in the polymer, which is different to that of the monomer in a number of ways. Importantly, both the solvation and hydrogen bonding arrangements are altered. Furthermore, base-stacking may lead to electronic coupling due to orbital overlap. In the case of poly(G) this manifests itself in a redshift in the UV absorption spectrum (see ESI†).[26] Longer-lived transient signals are commonly found for stacked nucleotides and have been ascribed to excimer-type excited states.

Moving from neutral to acidic solution is expected to result in a disruption of the Hoogsteen hydrogen bonding that is present in the G4-DNA. The CD spectra (Fig. 4) are consistent with a change in structure which has previously been attributed to the formation of a protonated double-stranded form.[22] Therefore, not only is the chemical nature of the guanine bases expected to differ from that of poly(G), the structural environment will also be changed. The FTIR of poly(G) in acid solution shows distinctly different features from those of the polynucleotide in neutral solution. The dominant bands resemble those of protonated 5′-GMP, with some

evidence of bands similar to unprotonated 5′-GMP (*e.g.* bleach bands at *ca.* 1675 cm^{-1} and at *ca.* 1580 cm^{-1} and transients at *ca.* 1550 cm^{-1} and *ca.* 1640 cm^{-1}). It is possible therefore that under these acid conditions some guanine residues are not protonated. Analysis of the recovery kinetics for the corresponding bleaches in the ps-TRIR reveal a fast and a slow phase of the ground state recovery. The longer recovery has the same rate constant (within error) as that observed for the transient band at 1587 cm^{-1} with decay times of 220 ± 20 ps for 5′-GMP in acid and 233 ± 18 ps for poly(G) in acid.

Finally one may note a major difference in the transient absorptions between the monomeric 5′-$GMPH^+$ and the protonated polymer. Thus while 5′-$GMPH^+$ shows two distinct transient absorption bands at 1520 and at 1640 cm^{-1}, only the latter band is clearly defined in the protonated polymer. The reason for this is unclear and will require further study.

Conclusions

This study demonstrates the ability of ps-TRIR to unravel the complexities of medium effects on the excited states of nucleic acids, and in particular how protonation of guanine affects the photophysical properties of the compounds on both mononucleotide and polymeric systems where base-stacking and Watson–Crick base-pairing can be significant. The different dynamics observed between the non-protonated and protonated form of guanine nucleotide have long been recognised, because of the latter's strongly luminescent behaviour. This work reveals that the protonated excited state has characteristic vibrational absorption bands, which should assist computational chemists to determine the structure and electronic properties of this excited state. The results reported here for 5′-H_2GMP in unbuffered solution demonstrate a particular strength of ps-TRIR by allowing for the resolution of both the ground state depletion and the transient behaviour of a mixture (namely the unprotonated and protonated forms).

It is well known that the photochemical behaviour of the polymeric forms of nucleotides is particularly complicated. Our study with poly(G) reveals how the aggregation of the individual guanine moieties markedly changes the photophysical properties. The structure of poly(G) is known to be complex with the formation of guanine tetrads being a major (but not the only) feature. We have analysed the transient kinetics with a biexponential function, although it is likely that this is an approximation. The short-lived species is ascribed to the vibrationally hot ground state, indicating that sub-picosecond processes (most probably internal conversion) play a major role. The longer-lived transient is assigned to an excimer-like state, the confirmation of which would require luminescence studies which were not possible in the current programme.

Finally, we have examined the case of protonated poly(G) where both protonated and unprotonated guanines are expected to be present and the extent of stacking compared to that of poly(G) in neutral solution is much reduced. This additional complexity makes it difficult at this stage, without computational support, to explain all the spectral features we observe. However, it is possible to identify the modes that are most influenced by protonation and determine their dynamics, which must be directly related to structural modifications and changes in solvent coupling.

Acknowledgements

We are grateful to the EU access programme of the Rutherford Appleton Laboratory (JMK) which allowed these measurements to be made. We thank Dr Kate Ronayne for her help in making these measurements. We acknowledge financial support from Science Foundation Ireland (07/RFP/CHEF437).

Notes and references

1 M. K. Kuimova, J. Dyer, M. W. George, D. C. Grills, J. M. Kelly, P. Matousek, A. W. Parker, X. Z. Sun, M. Towrie and A. M. Whelan, Monitoring the effect of ultrafast deactivation of the electronic excited states of DNA bases and polynucleotides following 267 nm laser excitation using picosecond time-resolved infrared spectroscopy, *Chem. Commun.*, 2005, 1182–1184.
2 S. Quinn, G. W. Doorley, G. W. Watson, A. J. Cowan, M. W. George, A. W. Parker, K. L. Ronayne, M. Towrie and J. M. Kelly, Ultrafast IR spectroscopy of the short-lived transients formed by UV excitation of cytosine derivatives, *Chem. Commun.*, 2007, 2130–2132.
3 D. A. McGovern, S. Quinn, G. W. Doorley, A. M. Whelan, K. L. Ronayne, M. Towrie, A. W. Parker and J. M. Kelly, Picosecond infrared probing of the vibrational spectra of transients formed upon UV excitation of stacked G-tetrad structures, *Chem. Commun.*, 2007, 5158–5160.
4 M. K. Kuimova, A. J. Cowan, P. Matousek, A. W. Parker, X. Z. Sun, M. Towrie and M. W. George, Monitoring the direct and indirect damage of DNA bases and polynucleotides by using time-resolved infrared spectroscopy, *Proc. Nat. Acad. Sci. U. S. A.*, 2006, **103**, 2150–2153.
5 B. Elias, C. Creely, G. W. Doorley, M. M. Feeney, C. Moucheron, A. Kirsch-DeMesmaeker, J. Dyer, D. C. Grills, M. W. George, P. Matousek, A. W. Parker, M. Towrie and J. M. Kelly, Photooxidation of guanine by a ruthenium dipyridophenazine complex intercalated in a double-stranded polynucleotide monitored directly by picosecond visible and infrared transient absorption spectroscopy, *Chem.–Eur. J.*, 2008, **14**, 369.
6 W. J. Schreier, T. E. Schrader, F. O. Koller, P. Gilch, C. E. Crespo-Hernández, V. N. Swaminathan, T. Carell, W. Zinth and B. Kohler, Thymine dimerization in DNA is an ultrafast photoreaction, *Science*, 2007, **315**, 625–629.
7 M. Hare, C. T. Middleton, K. I. Mertel, J. M. Herbert and B. Kohler, Time-resolved infrared spectroscopy of the lowest triplet state of thymine and thymidine, *Chem. Phys.*, 2008, **347**, 383–392.
8 G. W. Doorley, D. A. McGovern, M. W. George, M. Towrie, A. W. Parker, J. M. Kelly and S. J. Quinn, Picosecond transient infrared study of the ultrafast deactivation processes of electronically excited B-DNA and Z-DNA forms of $[poly(dG–dC)]_2$, *Angew. Chem., Int. Ed.*, 2009, **48**, 123–128.
9 M. Towrie, D. C. Grills, J. Dyer, J. A. Weinstein, P. Matousek, R. Barton, P. D. Bailey, N. Subramaniam, W. M. Kwok, C. Ma, D. Phillips, A. W. Parker and M. W. George, Development of a broadband picosecond infrared spectrometer and its incorporation into an existing ultrafast time-resolved resonance Raman, UV/visible, and fluorescence spectroscopic apparatus, *Appl. Spectrosc.*, 2003, **57**, 367–380.
10 D. Onidas, D. Markovitsi, S. Marguet, A. Sharonov and T. Gustavsson, Fluorescence Properties of DNA Nucleosides and Nucleotides: A Refined Steady-State and Femtosecond Investigation, *J. Phys. Chem. B*, 2002, **106**, 11367–11374.
11 W.-M. Kwok, C. Ma and D. L. Phillips, Femtosecond time- and wavelength- resolved fluorescence and absorption spectroscopic study of the excited states of adenosine and an adenine oligomer, *J. Am. Chem. Soc.*, 2006, **128**, 11894–11905.
12 C. E. Crespo-Hernández, B. Cohen, P. M. Hare and B. Kohler, Ultrafast excited-state dynamics in nucleic acids, *Chem. Rev.*, 2004, **104**, 1977–2020.
13 C. E. Crespo-Hernandez, B. Cohen and B. Kohler, Base stacking controls excited state dynamics in A–T DNA, *Nature*, 2005, **436**, 1141–44.
14 P. O'Neill, A. W. Parker, M. A. Plumb and L. D. A. Siebbeles, Guanine modifications following ionization of DNA occurs predominantly *via* intra- and not interstrand charge migration: an experimental and theoretical study, *J. Phys. Chem. B*, 2001, **105**, 5283–5290.

15 J. Peon and A. H. Zewail, DNA/RNA nucleotides and nucleosides: direct measurement of excited-state lifetimes by femtosecond fluorescence up-conversion, *Chem. Phys. Lett.*, 2001, **348**, 255–262.
16 J. M. L. Pecourt, J. Peon and B. Kohler, DNA Excited-state dynamics: ultrafast internal conversion and vibrational cooling in a series of nucleosides, *J. Am. Chem. Soc.*, 2001, **123**, 10370–10378.
17 B. Cohen, P. M. Hare and B. Kohler, Ultrafast excited state dynamics of adenine and monomethylated adenines in solution: implications for the nonradiative decay mechanism, *J. Am. Chem. Soc.*, 2003, **125**, 13594–13601.
18 T. Fujiwara, Y. Kamoshida, R. Morita and M. Yamashita, Temporally-separated ultrafast-emission dynamics of neutral and cationic molecules in guanine and its derivative aqueous solutions, *J. Photochem. Photobiol., B*, 1997, **41**, 114–121.
19 J. T. Davis, G-Quartets 40 years later: from 5′-GMP to molecular biology and supramolecular chemistry, *Angew. Chem., Int. Ed.*, 2004, **43**, 668–698.
20 (*a*) M. R. Guzman, J. Liquier, S. K. Brahmachari and E. Taillandier, Characterization of parallel and antiparallel G-tetraplex structures by vibrational spectroscopy, *Spectrochim. Acta, Part A*, 2006, **64A**, 495–503; (*b*) R. Ghana, C. Walss and J. A. Walmsley, Sodium and potassium ion-promoted formation of supramolecular aggregates of 2′-deoxyguanylyl-(3′-5′)-2′-deoxyguanosine, *J. Biomol. Struct. Dyn.*, 1996, **14**, 101–110.
21 (*a*) G. Gottarelli, S. Lena, S. Masiero, S. Pieraccini and G. P. Spada, The Use of Circular Dichroism Spectroscopy for Studying the Chiral Molecular Self-Assembly: An Overview, *Chirality*, 2008, **20**, 471–485; (*b*) R. Giraldo, M. Suzuki, L. Chapman and D. Rhodes, Promotion of parallel DNA quadruplexes by a yeast telomere binding protein: A circular dichroism study, *Proc. Natl. Acad. Sci. U. S. A.*, 1994, **91**, 7658–7662; (*c*) D. Gray, A circular dichroism study of poly dG, poly dC, and poly dG:dC, *Biopolymers*, 1974, **13**, 2087–2102.
22 E. A. Lesnik, I. M. Kochkina, A. S. Tikhonenko and Y. M. Varshavskii, Structure of polyriboguanylic acid in solution, *Mol. Biol. (Moscow)*, 1980, **14**, 820–829.
23 (*a*) D. Markovitsi, F. Talbot, T. Gustavsson, D. Onidas, E. Lazzarotto and S. Marguet, Molecular spectroscopy:Complexity of excited-state dynamics in DNA, *Nature*, 2006, **441**, E7; (*b*) D Markovitsi, T. Gustavsson and F. Talbot, Perspective: Excited states and energy transfer among DNA bases in double helices, *Photochem. Photobiol. Sci.*, 2007, **6**, 717.
24 G. Barbarella, M. L. Capobianco, A. Carcuro, F. P. Colonna, A. Garbesi and V. Tignoli, ^{15}N–^{13}C Nuclear Magnetic Resonance of Deoxydinucleotide Monophosphates. I. Protonation, of Deoxycytidylyl–(3′,5′)-guanosine in dimethylsuphoxide, *Can. J. Chem.*, 1988, **66**, 2492–2497.
25 C. T. Middleton, B. Cohen and B. Kohler, Solvent and solvent isotope effects on the vibrational cooling dynamics of a DNA base derivative, *J. Phys. Chem. A*, 2007, **111**, 10460–10467.
26 D. Markovitsi, T. Gustavsson and A. Sharonov, Cooperative effects in the photophysical properties of self-associated triguanosine diphosphates, *Photochem. Photobiol.*, 2004, **79**, 526–530.

