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Time-resolved photoelectron spectroscopy of adenosine and adenosine monophosphate photodeactivation dynamics in water microjets

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The excited state relaxation dynamics of adenosine and adenosine monophosphate were studied at multiple excitation energies using femtosecond time-resolved photoelectron spectroscopy in a liquid water microjet. At pump energies of 4.69–4.97 eV, the lowest $\pi\pi^*$ excited state, S_1, was accessed and its decay dynamics were probed via ionization at 6.20 eV. By reversing the role of the pump and probe lasers, a higher-lying $\pi\pi^*$ state was excited at 6.20 eV and its time-evolving photoelectron spectrum was monitored at probe energies of 4.69–4.97 eV. The S_1 $\pi\pi^*$ excited state was found to decay with a lifetime ranging from ~210 to 250 fs in adenosine and ~220 to 250 fs in adenosine monophosphate. This lifetime drops with increasing pump photon energy. Signal from the higher-lying $\pi\pi^*$ excited state decayed on a time scale of ~320 fs and was measureable only in adenosine monophosphate. *Published by AIP Publishing.* https://doi.org/10.1063/1.5027258

I. INTRODUCTION

DNA and its constituent components are the fundamental building blocks of life and are capable of a rich variety of photoinduced processes.[1,2] Despite having strong absorption bands in the ultraviolet (UV) regime, DNA is remarkably photostable.[3–7] Consequently, there is considerable interest in developing a fundamental understanding of its photodeactivation. In particular, research efforts are focused on the mechanisms by which energy deposited by electronic excitation is funneled away from nucleic acid (NA) constituents into their surrounding environment and how these dynamics depend on the composition of the NA constituent. Here we use time-resolved photoelectron spectroscopy (TRPES) in water microjets to investigate the ultrafast deactivation dynamics of the nucleoside adenosine (Ado) and the nucleotide adenosine monophosphate (AMP) at photoexcitation energies ranging from 4.69 to 6.20 eV.

The nucleobase adenine (Ade) and its derivatives (Fig. 1) have strong UV absorption bands with maxima at ~260 and ~200 nm (4.77 and 6.20 eV, respectively).[8] These correspond to excitations of the π system of the nitrogenous base. The electronic structure of isolated Ade has been extensively studied[6,9–16] and is considered to be similar to Ado and AMP.[3,5] Both absorption bands are assigned to $\pi\pi^*$ transitions; the lowest $\pi\pi^*$ excitation is accessed near 4.77 eV while a higher-lying excited state of $\pi\pi^*$ character is populated at 6.20 eV.[7,16–18] The lower absorption band can be further sub-divided into two electronic states, ^1L_a and ^1L_b, as indicated schematically in Fig. 2. Most calculations indicate that the ^1L_a state carries the majority of the oscillator strength.[6] The ordering of the excited states in an aqueous environment is altered as compared to the gas phase such that the lowest $n\pi^*$ state is destabilized relative to the ^1L_a and ^1L_b states.[3] However, the exact energies and ordering of ^1L_a and ^1L_b are still disputed.[3,17,19] In Ade

and AMP, both states are present in a similar spectral region but are even less well characterized; for simplicity, we refer to these states collectively as S_1.[3,6,20]

The photoexcitation of Ade, Ado, and AMP near 260 nm has been investigated by experiment and theory. A small fluorescence quantum yield across DNA components suggests that quenching through non-radiative processes dominates the photoinduced dynamics.[3,5] The pathways for these non-radiative processes have been probed in the gas phase using TRPES.[21,22] In aqueous solution, S_1 decays with a lifetime of ~200 fs in Ade and ~250–500 fs in Ado and AMP, as measured by transient absorption (TA),[23–26] fluorescence up-conversion (FU),[27–33] and TRPES[21,34,35] experiments. These lifetimes generally agree with those measured in the gas phase,[22,34,36] hinting at a modest environmental effect on the dynamics of de-excitation.

After considerable investigation, the decay of S_1 has been assigned to internal conversion to the ground state, S_0.[5,6] S_0 was found to fully thermalize within picoseconds in water, and no components of the relaxation mechanism in Ade-based constituents are attributed to long-lived low-lying $n\pi^*$ states or possible triplet states.[23,24] Complementary theoretical studies[6,9–16,20,28,37–39] have corroborated these experimental results and find that S_1 undergoes barrierless internal conversion to vibrationally hot S_0. Relaxation to S_0 is attributed to conical intersections (CIs) between S_1 and S_0 involving an out-of-plane deformation of the purine ring at either C_2 or C_6.[3,6] Notably, in water, the majority of excited states relax through C_2 deformation and the assignment of this CI to a specific excited state is still subject to debate.[17] To our knowledge, no experimental studies of Ado and AMP dynamics subsequent to photoexcitation at 6.20 eV have been reported until now.

The development of the liquid microjet has enabled the study of high vapor pressure liquids in vacuum.[40–42]
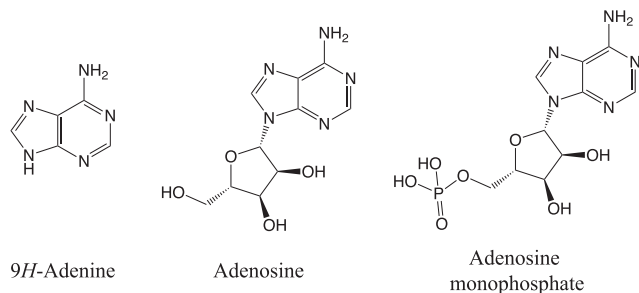
FIG. 1. Structures of the 9H tautomer of adenine alongside adenosine and adenosine monophosphate.

Femtosecond TRPES, which has traditionally been used in gas phase experiments,[43] has been applied to liquid microjets by several research groups and has been shown to be a powerful technique for the interrogation of species in the condensed phase.[44–47] In these experiments, the solute is electronically excited by a femtosecond pump pulse and photoionized by a femtosecond probe pulse at varying pump-probe delays. Provided sufficient probe energy, TRPES can, in principle, track solute dynamics throughout an entire relaxation process and provide the binding energies of all states involved. In this way, excited state lifetimes can be directly measured. Recent work by Buchner and co-workers[35] used TRPES to measure the excited state lifetimes of Ade and Ado in water at variable energies. They observed lifetimes that agreed well with previous TA and FU studies[4,5] and suggested that probe energies above 5.00 eV are sufficiently energetic to observe the full relaxation of S_1.

This paper extends previous work along three directions. First, the TRPE spectra of aqueous AMP are measured for the first time and compared to Ado. Second, excitation of S_1 in both species at pump energies ranging from 4.69 to 4.97 eV (265–250 nm) is followed by ionization at 6.20 eV, a higher probe energy than has been used in previous liquid jet work, in order to assess the effect of probe energy on the observed dynamics. Finally, by reversing the role of the pump and probe pulses, the relaxation dynamics of both species following excitation of the higher energy band at 6.20 eV (200 nm) are investigated, providing evidence for the emergence of a new decay channel involving a higher-lying $\pi\pi^*$ excited state that is particularly pronounced in AMP.
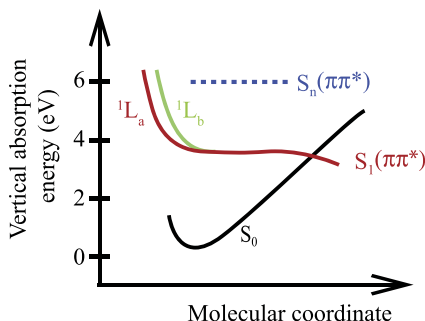
II. METHODS

Our experimental apparatus has been described in detail previously[45,48] and is summarized briefly here. To collect PE spectra, a liquid microjet[40–42] was crossed by two fs laser pulses and the resulting photoelectrons were energy-analyzed with a magnetic bottle time-of-flight (ToF) spectrometer.[49] TRPES experiments were conducted according to the scheme outlined in Eq. (1), where a variable-energy femtosecond UV pump pulse (4.69–4.97 eV), $h\nu_1$, electronically excites Ado or AMP and a 6.20 eV femtosecond probe pulse, $h\nu_2$, photoionizes the excited species. The delay between the pump and probe pulses, Δt, was varied to obtain time-resolved data. This scheme applies to positive pump-probe delays only. Because Ado and AMP are strong absorbers of all energies used, this experiment is pseudo-degenerate. For negative delays, the pump-probe scheme is reversed such that $h\nu_2$ acts as the pump pulse and $h\nu_1$ acts as the probe pulse,

$$A \xrightarrow{h\nu_1} A^* \xrightarrow{h\nu_2, \Delta t} A^\cdot + e^-. \tag{1}$$
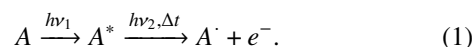
Solutions of Ado (5 mM, Sigma-Aldrich) and AMP (5 mM, Sigma-Aldrich) were introduced into vacuum by applying high backing pressure behind a fused silica capillary with a 20 μm inner diameter. The flow rate for all experiments was held at 0.25 ml/min. All solutions were buffered at pH 8 with Trizma HCl (2 mM, Sigma-Aldrich), and NaCl (100 mM, Sigma-Aldrich) was added as a counterion to mitigate streaming potentials. These provided no TRPE spectra and are shown in the supplementary material.

A commercial femtosecond Ti:sapphire laser (Coherent Astrella) generated 1 kHz, 35 fs pulses centered at 800 nm with 7 mJ/pulse. Part of this output was split and directed to a Light Conversion TOPAS-Prime optical parametric amplifier, which generated the tunable-UV pump (4.69–4.97 eV), and a β-barium borate (BBO)-based sum frequency generation system, which generated the fourth harmonic probe (6.20 eV). The energy of each pulse was maintained between 40 and 60 μJ, as measured at the liquid jet chamber. The cross correlation (measured as σ) of the pump and probe was between 160 and 170 fs, which is taken to be the experimental instrumental response function (IRF). Note that because the pulses are compressed before splitting into the pump and probe arms, the cross correlations are necessarily wavelength dependent. To obtain time-resolved data, the fourth harmonic was routed onto a motorized stage so the pump and probe could be variably delayed between −2 and +2 ps. Static two-photon, one-color background spectra were also taken for the pump and the probe at a delay of 0 ps.

Photoelectrons were directed by the inhomogeneous field of the magnetic bottle ToF spectrometer through a 900 μm skimmer located 1 mm from the jet and into a 66 cm flight tube. The magnetic bottle comprises a 11 000 G rare earth magnet stack and a 10 G solenoid that encompasses the flight tube. Photoelectrons were detected by a microchannel plate (MCP) detector coupled to a phosphor screen; the screen is used for alignment purposes only. Photoelectron arrival time distributions were recorded by measuring the capacitively coupled current off the back MCP as a function of time.

FIG. 2. Schematic representation of the potential energy surface of Ade in aqueous solution, where the surfaces for S_0 and S_1 were adapted from Ref. 6. The upper excited state is indicated by the blue dashed line.

Background scans were subtracted from spectra at each delay, and the resulting ToF data were converted to electron kinetic energy (eKE) using the appropriate Jacobian transformation. Spectra and lifetimes were extracted through a Global Lifetime Analysis (GLA) fitting routine using a minimum number of kinetic components.[50,51] This routine has been described extensively in our previous work.[45] Briefly, GLA can decompose spectrally congested, time-evolving features by simultaneously fitting the entire time-resolved data set according to the following equation:

$$S(eKE, \Delta t) = \sum_{i=1}^{n} DAS_{\tau_i}(eKE) \cdot [e^{-t/\tau_i} \cdot L(t - \Delta t)]. \quad (2)$$

Two assumptions are made. First, the spectral components are assumed to change only in intensity. Each spectral component is then separable into the spectrum of the component which depends only on kinetic energy and the kinetics of the component which depends only on pump-probe delay. Second, it is assumed that the kinetics can be expressed as a sum of monoexponentials. Under this paradigm, data can also be represented as a sum of exponentials, convoluted with the instrumental response function, $L(t - \Delta t)$, scaled by a constant which depends on energy—the decay associated spectrum ($DAS_{\tau i}$)—and indexed by the kinetic time constants ($\tau_i = 1/k_i$), as expressed in Eq. (2). Notably, the DAS is *not* inherently a spectrum; a kinetic scheme must first be assumed before a spectrum can be recovered from the DAS.

III. RESULTS

Background-subtracted TRPE spectra of Ado at 4.78 eV pump and 6.20 eV probe photon energies and of AMP at 4.88 eV pump and 6.20 eV probe photon energies are presented

in Figs. 3(a) and 3(b), respectively. These data are representative of all pump-probe combinations, the rest of which can be found in Figs. S1 and S2 of the supplementary material. In both panels, the spectrum extends just beyond 2.5 eV, but the Ado spectrum peaks ~1.7 eV while the AMP spectrum falls off gradually starting at ~1.0 eV.

The spectra are asymmetric with respect to t_0 and the feature intensity decays with increasing positive delay until it is largely depleted by 500 fs. In AMP, a lower eKE feature, spanning ~0.5–1.5 eV, is seen to evolve over negative pump-probe delays as well.

Spectral lineouts taken at several delays facilitate further inspection of the time-evolving behavior of photoexcited Ado and AMP. Examples at time delays ranging from −300 to 300 fs are shown in Figs. 3(c) and 3(d). Here, the pump is tunable-UV for positive delays and 6.20 eV for negative delays. In Ado, feature A is peaked at eKE ~1.7 eV; in AMP, feature B is peaked at ~1.6 eV and feature C is peaked at ~0.7 eV. Ado does show slight intensity at negative delays, similar to feature C, but this signal is too weak for further analysis.

The vertical detachment energies (VDEs) of features A and B, defined as the probe photon energy minus the peak of the eKE distribution, are ~4.5 and ~4.3 eV, respectively. These values are consistent with detachment from the S_1 $\pi\pi^*$ excited state as observed in previous TRPES studies,[35] and we assign this feature as such. Assignment of feature C is somewhat more elusive and will be addressed in Sec. IV; however, its VDE (~4.2 eV) agrees well with feature B when accounting for the reversed role of pump and probe lasers at negative delays. Each AMP feature appears to change only in intensity, but this assessment is complicated because the negative and positive delay signals overlap near t_0. Because of this
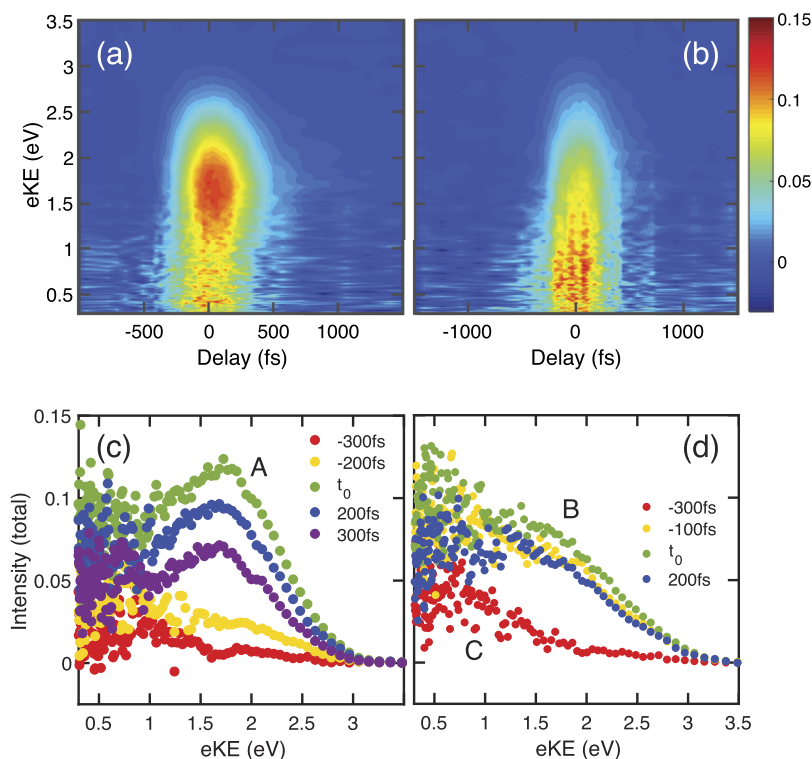

FIG. 3. Filled contour plot of the TRPE spectra of (a) Ado photoexcited at 4.78 eV and photodetached at 6.20 eV and (b) AMP photoexcited at 4.88 eV and photodetached at 6.20 eV for positive pump-probe delays, presented in eKE. One-color, two-photon static background spectra have been subtracted. Selected background-subtracted (c) Ado and (d) AMP PE spectra at pump-probe delays ranging from −300 to 300 fs. Feature B grows in with the IRF and decays over positive delays while feature C grows in with the IRF and decays over negative delays.

complexity, GLA is needed to disentangle the dynamics in these spectra, as discussed in Sec. IV.

IV. ANALYSIS

Ado data were fit using the standard GLA routine described in Sec. II with one kinetic component. Two kinetic components were needed to adequately fit the AMP data by GLA. Figures 4(a) and 4(b) show the total integrated intensity of Ado and AMP, respectively, taken across the entire spectrum at each delay and plotted as a function of delay, with the corresponding GLA kinetic components. The normalized DAS can be found in Figs. 4(c) and 4(d) for Ado and AMP, respectively. Spectral lineouts at ±300 fs are presented alongside the DAS for comparison and are scaled accordingly. These delays were chosen because they lie outside of the Gaussian IRF centered at t_0 and minimize interference
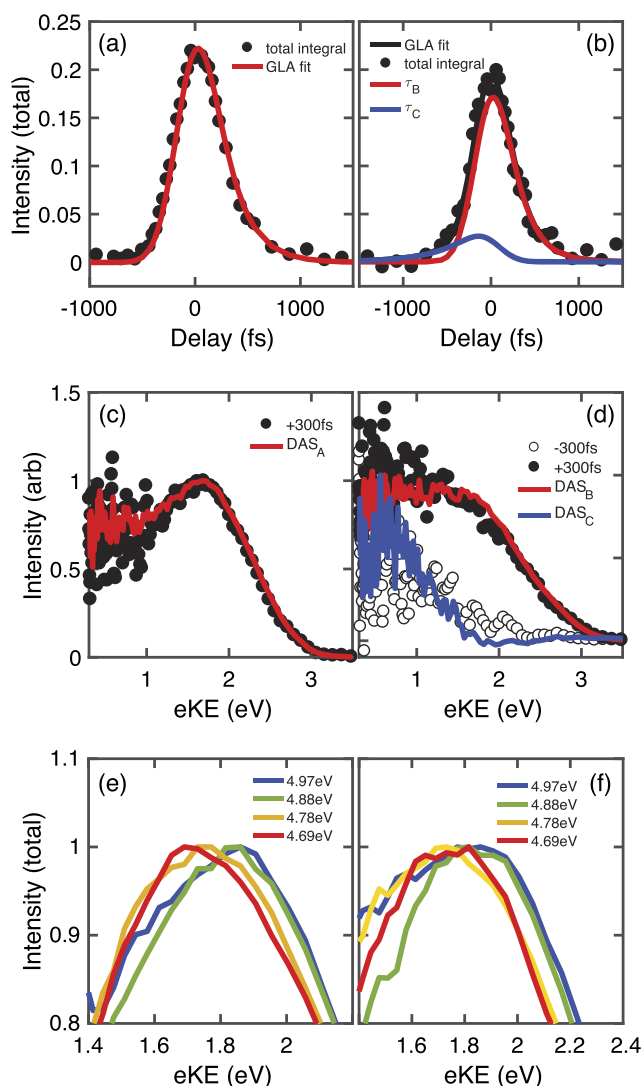

FIG. 4. GLA fit and total integrated intensity of (a) Ado photoexcited at 4.78 eV and (b) AMP photoexcited at 4.88 eV, where both are photodetached at 6.20 eV. The recovered fit for Ado is monoexponential, while the fit for AMP shows exponential decays over both positive and negative delays. Normalized DAS and spectral lineouts for (c) Ado and (d) AMP, where each DAS_x (solid red line) corresponds to τ_x. Comparison of normalized (e) Ado DAS_A and (f) AMP DAS_B at all pump photon energies.

TABLE I. Lifetime and location of the peak intensity for DAS_A in Ado photoexcited and photodetached at UV photons ranging in energy from 4.69 to 6.20 eV. No measureable lifetime was found when the system was photoexcited at 6.20 eV; the IRF is ~170 fs.

Pump (eV)	Probe (eV)	τ (fs)	Peak intensity DAS_A (eV)
6.20	4.69 . . . 4.97	<170.0	n/a
4.69	6.20	210.0 ± 21.2	1.6 ± 0.1
4.78	6.20	218.0 ± 24.3	1.7 ± 0.1
4.88	6.20	227.8 ± 24.1	1.7 ± 0.1
4.97	6.20	236.3 ± 37.6	1.8 ± 0.1

between features at positive and negative delays for AMP. Finally, DAS_A and DAS_B across pump photon energies of 4.69–4.97 eV are shown in Figs. 4(e) and 4(f). The peaks in the DAS shift to higher eKE with increasing pump energy but, notably, do not quite track with the difference in pump energy.

Fitted lifetimes for all pump-probe combinations are presented in Tables I and II. The functional form of an individual kinetic component is a single exponential decay convolved with the Gaussian IRF and a Heaviside function and is described by Eq. S1 of the supplementary material. GLA is considered to accurately report the lifetime of these features because the features do not appear to shift over the time scale of the experiment and the spectra at positive delays are well reproduced by their DAS. GLA recovers a lifetime of <300 fs for the decay of the S_1 $\pi\pi^*$ excited state in Ado (DAS_A) and AMP (DAS_B) for all pump-probe combinations. As the pump photon energy increases, the S_1 lifetime is seen to decrease modestly, although this effect is somewhat less pronounced in AMP.

DAS_C is seen to decay with a lifetime of ~320 fs and is invariant to probe photon energy. This value is longer than that for all other pump photon energies. Feature C peaks near eKE 1.7 eV, and its intensity extends nearly 3 eV above its peak. Although DAS_C agrees reasonably well with feature C, interpretation of DAS_C is not straightforward and will be left to the discussion.

TABLE II. Lifetimes and locations of the peak intensity in eKE for DAS_B and DAS_C in AMP photoexcited and photodetached at UV photons ranging in energy from 4.69 to 6.20 eV.

Pump (eV)	Probe (eV)	τ_B (fs)	Peak intensity DAS_B (eV)
4.69	6.20	220.8 ± 24.6	1.8 ± 0.2
4.78	6.20	231.5 ± 30.4	1.7 ± 0.2
4.88	6.20	256.5 ± 75.0	1.9 ± 0.2
4.97	6.20	245.0 ± 39.6	1.9 ± 0.2

Pump (eV)	Probe (eV)	τ_C (fs)	Peak intensity DAS_C (eV)
6.20	4.69	328.8 ± 75.0	0.7 ± 0.1
6.20	4.78	322.5 ± 190.0	0.6 ± 0.1
6.20	4.88	336.0 ± 249.0	0.7 ± 0.1
6.20	4.97	316.5 ± 273.0	0.8 ± 0.2

V. DISCUSSION

This series of experiments interrogates Ado and AMP in aqueous solution with pump photon energies that access both the 260 and 200 nm (4.77 eV and 6.20 eV) absorption bands in Ado and AMP. It is generally accepted that the S_1 $\pi\pi^*$ transition is responsible for the 260 nm band,[3,6,8,52] while the 200 nm absorption band is attributed to higher-lying excited states also of $\pi\pi^*$ character.[17,18,53] Here, pump energies ranging from 4.69 to 4.97 eV are only able to excite the S_1 state, while the 6.20 eV pump can also promote electrons into higher-lying excited states. Previous studies found a sub-500 fs lifetime for the S_1 $\pi\pi^*$ excited state subsequent to photoexcitation near the band maximum.[23–25,27,29–31,33,35,54] This work investigates the same transition, but extends the probe energy beyond previous work, to 6.20 eV. By contrast, little is known about the photodeactivation dynamics of Ado and AMP excited at 6.20 eV. In this section, the dynamics ensuing from excitation in the two energy regimes are discussed.

The lifetimes of Ado and AMP photoexcited at 4.69–4.97 eV and photodetached at 6.20 eV are found to decay with a sub-ps lifetime. General agreement between TRPES findings and those of both time-resolved transient absorption (TA) and fluorescence up-conversion (FU) measurements in water suggest that all observe the decay of the S_1 $\pi\pi^*$, or fluorescent, excited state.[5] Because excess energy is deposited into the state upon excitation, spectral evolution along the excited state surface is expected to be seen as the wavepacket moves from the Franck-Condon (FC) region to the conical intersection (CI). The TRPE spectra in this work do not exhibit a delay time dependent shift in energy, consistent with rapid relaxation to the CI relative to our time resolution.

Gas phase studies of photoexcited Ado and AMP observe a biexponential decay, comprising a sub-100 fs and sub-ps decay.[21,55–57] The sub-100 fs lifetime is interpreted as motion of the initially prepared wavepacket to the S_1-S_0 CI, which then decays through internal conversion to S_0 on a sub-ps time scale. The measured sub-ps S_1 lifetime in gas phase experiments agrees well with this work, hinting that the solvent plays a minor role in the internal conversion dynamics. However, we see no direct evidence of the feature that gives rise to the sub-100 fs lifetime. Although the presented work was taken with a longer IRF than in the gas phase studies, we note that this feature was also absent in the work of Buchner et al.[35] It thus appears that the signal associated with excited state wavepacket dynamics either decays within the IRF or is not present in water.

However, a closer inspection of these data suggests that some portion of the S_1 state still has excess internal energy when observed, i.e., the vibrational population within this state is not fully relaxed. First, the peaks of DAS_A and DAS_B, shown in Figs. 4(e) and 4(f), show a modest dependence on the pump photon energy. If the population had fully relaxed within S_1, the DAS peak would be expected to be invariant to pump photon energy. Additionally, the photoelectron distribution extends ~2 eV above the peak, which is quite broad for a feature arising from a single electronic state. These observations suggest that features A and B represent largely but not completely relaxed excited state populations at the CI. This incomplete vibrational

relaxation may well be the cause of the decreasing S_1 lifetime with increasing pump photon energy.

The pump-photon energy dependent signals may also reflect dynamics on the ^1L_a and ^1L_b excited states. Strickler-Berg analysis by Cohen et al.[58] strongly suggests that both electronic states contribute to the non-adiabatic de-excitation of Ado photoexcited near 260 nm. Kwok and co-workers also investigated these Ado dynamics in solution by TA and Kerr-gated time-resolved fluorescence spectroscopy and proposed a mechanism involving both $\pi\pi^*$ transitions.[33] The relaxation is posited to follow $^1L_a \rightarrow ^1L_b \rightarrow S_0$. Moreover, ^1L_a is found to undergo vibrational cooling and internal conversion to ^1L_b on a time scale of ~130 fs. The experimental finding that both ^1L_a and ^1L_b play significant roles in the relaxation mechanism of Ado is supported by theoretical studies, which find that these low-lying states couple.[3,17,38,59] However, the energy ordering of ^1L_a and ^1L_b, the extent to which they couple, and the state on which the CI lies are still debated.[19] Recent experimental[28] and theoretical[17,38,59] studies also call into question the relaxation mechanism proposed by Kwok et al.,[33] suggesting instead that the C_2 CI lies on ^1L_a and that the 100 fs lifetime is caused by $^1L_b \rightarrow ^1L_a$ dynamics. The role of the $n\pi^*$ state in this mechanism in the liquid phase is also controversial. Regardless, the dynamics attributed to the interplay between ^1L_a and ^1L_b are too short to be reliably observed with our time resolution.

Taken as a whole, these results support the following mechanistic picture. First, the pump pulse initially populates the $\pi\pi^*$ band with some excess energy. This nascent distribution reaches the S_1 CI with a lifetime that is faster than that of internal conversion to S_0. Theoretical studies suggest that this CI is nearly barrierless.[6] Once at the intersection, the population undergoes internal conversion to hot S_0. The excited state returns to S_0 with a lifetime ranging from ~210 to 250 fs, as measured in this work. This picture agrees with the reported mechanism in previous TA, FU, and TRPES studies.[23–25,27,29–33,35,54] Contributions from multiple excited states, for example, from both ^1L_a and ^1L_b, were not identified in the data. There was also no evidence for contributions from the $n\pi^*$ state, which has been implicated to play a role in the deactivation dynamics of other nucleosides and nucleotides.[3,5] This interpretation corroborates other TA findings,[23,54] which did not see any long-lived spectral contributions corresponding to $n\pi^*$ relaxation in Ado or AMP.

To accurately report the excited state lifetime, the accessibility of the FC window for photoionization should not be limited by the probe photon energy at any point as the state evolves. As pointed out in the work by Martinez and co-workers on uracil,[60] measured lifetimes in a TRPES experiment can be artificially shortened if the energy of the probe photon is insufficient to access the complete manifold of FC-accessible states throughout the entire relaxation process. This consideration motivated our implementation of a 6.20 eV probe photon.

An important point of comparison can be made with a previous TRPES study of Ade and Ado in water microjets by Buchner and co-workers.[35] When Ado was photoexcited at 5.00 eV and photodetached at 4.66 eV, the authors observed a lifetime of ~143 fs. This value is significantly shorter than

the lifetime of ~250 fs reported in this study subsequent to photoexcitation at 4.97 eV and photodetachment at 6.20 eV, suggesting that probe energies below 5.00 eV are insufficient to access the FC window for the entire S_1 decay process. When Buchner *et al.* photoexcited Ado at 4.66 eV and photodetached the electrons at 5.00 eV, they reported a lifetime that agrees, to within error, with the lifetime reported in this work for a 4.69 eV pump and 6.20 eV probe. Thus, a probe photon energy of ~5.00 eV appears to be sufficiently energetic to access the photoionization FC window of S_1, as was proposed by Buchner, and the more energetic 6.20 eV probe used in these experiments does not affect the measured excited state lifetime.

Our S_1 lifetimes for Ado and AMP generally agree with the lifetime of S_1 in the biologically relevant 9H Ade tautomer.[5,25,27,30,35,61] This agreement supports the findings of quantum chemistry calculations,[5] which indicate that the aromatic ring of the nucleobase is principally responsible for the electronic structure and dynamics in NA constituents. Interestingly, the lifetime of S_1 in AMP was found to be slightly longer than in Ado. This result has been seen by others[23,54] as well; however, the relatively small difference in lifetime as compared to differences seen in pyrimidine nucleoside/tide pairs[29,31] suggests that the phosphor moiety has a limited impact on the excited state dynamics of S_1 in the Ade family of NA constituents.

Finally, no evidence of a long-lived signal subsequent to internal conversion and corresponding to vibrationally hot S_0 was found in the data, even at the higher probe photon energy used here. Presumably vibrationally hot S_0 will lie energetically close to S_1 until it relaxes away, and it would not be wholly unreasonable to expect to see a signature of this at low eKE if there were sufficient FC intensity and if the probe photon energy were high enough. Vibrational cooling along S_0 has been observed to proceed with a few picosecond lifetime following internal conversion, as measured by TA and transient electronic and vibrational absorption spectroscopies.[23,61] Although the 6.20 eV probe used in these experiments is quite energetic as compared to previous TRPES studies, a more energetic probe which can access the fully thermalized S_0 population (bound by ~7 eV[56]) is required for a complete description of the photodeactivation of Ado and AMP by TRPES.

Markedly different dynamics are observed for negative delays, suggesting that different states are involved in the relaxation mechanism. This is reasonable, as the 6.20 eV pump pulse is considerably more energetic than the center of the S_1 $\pi\pi^*$ absorption band and can access the 200 nm absorption band. We then look to the transition responsible for the absorption band near 200 nm. Study of this band is sparse,[7,16,18] but in recent work by Mondal and Puranik,[17] Ade in water was photoexcited at 200 nm with resonance Raman and TD-DFT (time-dependent density functional theory) methods, and the excited state in this region was attributed to pure $\pi\pi^*$ character. The transition was found to comprise major orbital contributions from HOMO-1 → LUMO (76%) and HOMO → LUMO + 1 (11%) and is posited to correspond to an in-plane contraction of the heterocycle rings. We tentatively assign the observed negative-time dynamics in this work to the de-excitation of this state.

A close inspection of the data suggests that several states may be involved in the relaxation pathway giving rise to the ~320 fs lifetime. As mentioned above, the VDEs of features B and C agree taking into account that the probe photon energies are different. This suggests that population in this energy region of DAS_C arises from the same electronic excited state as feature B, namely, the S_1 excited state, instead of the initially populated excited state.

Notably, the shape of DAS_C shows little to no intensity in the region above eKE ~2 eV, where, based on energetic grounds alone, one would expect to see a signal from a higher-lying $\pi\pi^*$ excited state immediately after excitation. Although there is intensity extending above 3 eV eKE in the spectrum, its intensity is <10% of the maximal intensity of feature C. Because the oscillator strength to this excited state is primarily attributed to the HOMO-1 → LUMO transition,[17] it is plausible that the initially prepared excited state cannot be ionized to the ground state of the cation by a one-electron transition. Alternatively, the photoionization cross section for the higher-lying $\pi\pi^*$ state may be lower than that of S_1. Either effect would result in little to no photoelectron signal in the region above 2 eV.

Taken as a whole, we postulate the following deactivation pathway. First, the 6.20 eV pump photon excites electrons into a high-lying $\pi\pi^*$ state. This wavepacket then undergoes non-adiabatic relaxation to arrive at the S_1 $\pi\pi^*$ excited state and is depleted by internal conversion to S_0. This pathway is observable in AMP but not in Ado, possibly reflecting slower relaxation of the initially excited state in AMP. Ultimately, more work is needed to fully understand the photodeactivation mechanism of AMP subsequent to excitation of the 200 nm absorption band.

VI. CONCLUSION

TRPES on water microjets has been used to interrogate the de-excitation dynamics of adenosine (Ado) and adenosine monophosphate (AMP) subsequent to photoexcitation at energies ranging from 4.69 to 6.20 eV. This work looks at two absorption bands: 4.69–4.97 eV photons populate the lowest $\pi\pi^*$ excited state, while 6.20 eV photons excite a higher-lying $\pi\pi^*$ transition. In Ado, the S_1 excited state was found to decay with a lifetime of ~210–250 fs, dropping modestly with increasing pump photon energy. Similarly, when AMP was photoexcited by photons in this energy regime, the S_1 excited state population was found to decay with a lifetime of ~220–250 fs. These lifetimes were assigned to the internal conversion of the S_1 $\pi\pi^*$ excited state to vibrationally hot S_0 and agree well with previous experiments. Upon photoexcitation at 6.20 eV, a transient signal at low eKE was evident in both Ado and AMP. Notably, a lifetime for this signal was measureable only in AMP and was found to be ~320 fs. Spectral analysis suggests that a high-lying $\pi\pi^*$ excited state is initially populated, but ultimately relaxes to the S_1 $\pi\pi^*$ excited state before internal conversion to hot S_0. Further study is required for a comprehensive understanding of the dynamics of Ado and AMP subsequent to photoexcitation at 6.20 eV.

SUPPLEMENTARY MATERIAL

See supplementary material for the background-subtracted time-resolved photoelectron spectra at all pump-probe wavelength combinations for adenosine and adenosine monophosphate. Global fits for each dataset are also provided, along with information on the analysis routine.

ACKNOWLEDGMENTS

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