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Competing $\pi\sigma^*$ States in the Photodissociation of Adenine

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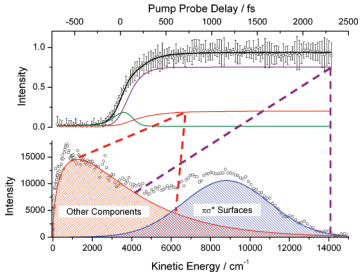
ABSTRACT Despite the extensive investigations thus far, there is still debate regarding the role of the repulsive $\pi\sigma^*$ state in the photochemistry of adenine. We present evidence that suggests that H-atom elimination via both the azole group (N^9) and the often overlooked amino group (N^6) occurs following excitation at 200 nm. In contrast, excitation at 266 nm supports the view that the role of the $\pi\sigma^*$ states is minimal.

SECTION Dynamics, Clusters, Excited States



The ultraviolet (UV) photostability of the DNA bases and other biomolecules such as amino acids is of fundamental importance. The stability of these species following UV excitation is governed by the complex interplay between different relaxation mechanisms that dissipate potentially harmful electronic energy following irradiation.[1,2]

The photostability of the DNA base adenine (Ade) has been extensively studied by both spectroscopy and theory. Despite these extensive investigations, there remain various open questions regarding the participation of and energetic thresholds for certain relaxation pathways following UV excitation. In the following letter, we present strong evidence that suggests that excitation at 200 nm results in H-atom elimination on an ultrafast time scale through both the azole group and the often overlooked amino group. In contrast, excitation at 266 nm shows very little, if any, H-atom elimination.

Domcke and Sobolewski's computed potential energy surfaces of heteroaromatic systems were the first to suggest the involvement of repulsive $\pi\sigma^*$ states (located along the N−H coordinates,) in their nonradiative relaxation.[3−5] Experiments targeting similar $\pi\sigma^*$ states in Ade have largely focused on the azole, N^9−H coordinate (Figure 1a). These experiments have included time-resolved mass spectroscopy (TR-MS),[6−8] Doppler spectroscopy,[9] TR-photoelectron spectroscopy,[10] total kinetic energy release (TKER) spectroscopy[11] in addition to power dependence studies by our group.[12] With the exception of the TKER studies of Nix et al., which covered the excitation wavelength range $280 > \lambda > 214$ nm and found an onset for the N_9−H fission at 233 nm, these studies have largely targeted the role of the azole $\pi\sigma^*$ state following excitation at 267 nm with somewhat conflicting conclusions. Here we introduce the first attempt to measure TR H-atom elimination from Ade, 6-dimethylaminopurine (6-DMAP) and 9-methyladenine (9-MA) (Figure 1a,b,c) following excitation at 200 nm using TR-MS and velocity-map ion imaging (VMI) and 266 nm using TR-MS.

The kinetic energy (KE) of the H atom photoproducts, following photodissociation at 200 nm and probing with 243.1 nm at a pump/probe delay of 2.5 ps, was measured for Ade, 9-MA, and 6-DMAP using VMI and is shown together

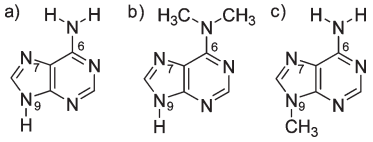


Figure 1. Structures of (a) Ade, (b) 6-DMAP, and (c) 9-MA.

with the corresponding H^+ image in Figure 2. The KE spectra were obtained after deconvolution of the raw H^+ images using a polar onion peeling algorithm.[13] Each image shown takes approximately 60 min to acquire and is a sum of 1500 individual images. Observation of a high KE component ($\sim$8000 cm^{-1}) in all three species clearly implicates the participation of $\pi\sigma^*$ states along both the N^9 (azole) and N^6 (amino) coordinates following excitation at 200 nm.[5,14] The TKER spectra have been fit using a statistical decay model function for the low KE channel and a Gaussian for the high KE channel (dark and light green plots, respectively, the details of which are outlined in the Supporting Information (SI)). The ratios of the fits to the high and low KE components are 1:1.3 for Ade, 1:1.8 for 9-MA, and 1:1.0 for 6-DMAP, the measured KE spectra matching qualitatively with those recorded previously using TKER spectroscopy.[11]

In Figure 3, we present H^+ transients using TR-MS, following excitation at 200 nm and probing at 243.1 nm. The error bars reported correspond to a 95% confidence limit, i.e., two standard deviations of the mean. There is a clear step at time zero, indicative of H-atom loss following excitation at 200 nm. Fits to these data consist of a decay component and either one or two step functions. The decay component is most likely due to direct production of H^+ through multiple-photon absorption via a short-lived intermediate state (lifetime <100 fs). The step function(s) correspond to neutral H-atom eliminations, the details of which will be discussed below. Interestingly, when we excite with 266 nm, there is no step,

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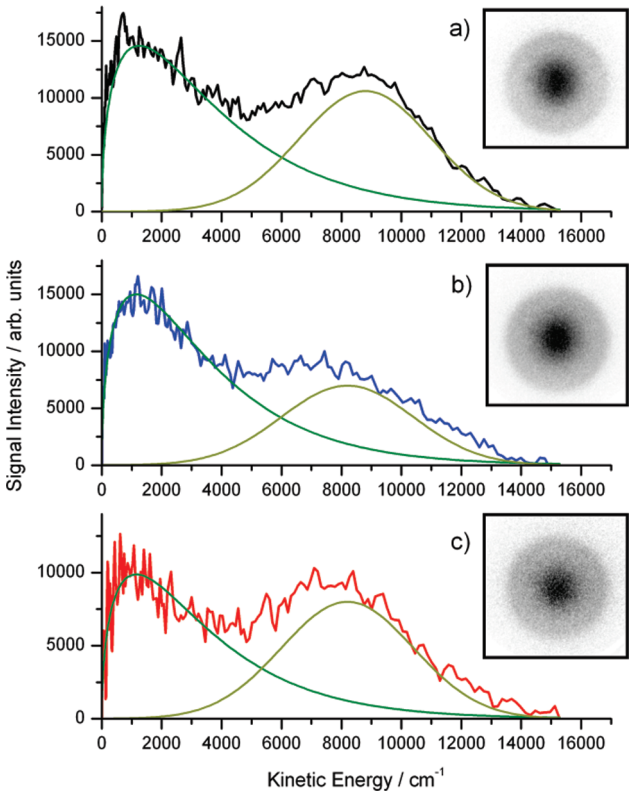

Figure 2. H-atom KE spectra and raw H^+ images inset for (a) Ade, (b) 9-MA, and (c) 6-DMAP. All images were taken at an extended time delay, where the pump (200 nm) precedes the probe (243.1 nm) by 2.5 ps. Each image is a sum of >1500 individual images taken over the course of 1 h. The low and high TKER spectra have been fit using a statistical and Gaussian function (dark and light green plots, respectively).

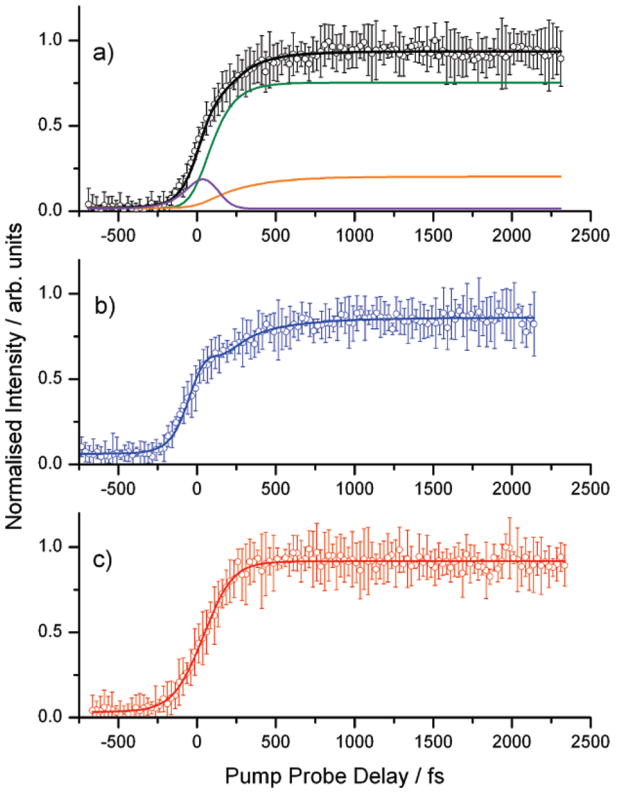

Figure 3. TR-MS H^+ signal following excitation at 200 nm and probing at 243.1 nm with corresponding fits. (a) Ade, with component fits of an exponential decay (violet) and two step functions, τ_1 = 110 fs ± 63 fs (green) and τ_2 = 265 fs ± 135 fs (orange); (b) 9-MA fitted with an exponential decay and two step functions, τ_1 = 95 fs ± 48 fs and τ_2 = 326 ± 205 fs; (c) 6-DMAP fitted with an exponential decay and a single step function τ_1 = 95 ± 21 fs. Error bars correspond to a 95% confidence limit.

illustrating the inactivity of the $\pi\sigma^*$ state at this excitation energy (Figure 4).

In Ade and 9-MA, two step functions are required to fit the H^+ transients, yielding time-constants of τ_1 = 110 fs ± 63 fs and τ_2 = 265 fs ± 135 fs in Ade and τ_1 = 95 fs ± 48 fs and τ_2 = 326 fs ± 205 fs in 9-MA with the fast and slow time-components having differing relative contributions. In Ade we require an 80% contribution from the fast time-component and 20% from the slow component, while in 9-MA this ratio changes to 65% and 35%, respectively. 6-DMAP requires only a single step function component (τ_1 = 95 fs ± 21 fs) to fit the observed time signature.

It is immediately tempting to assign one time scale to one KE component, assuming that the high-KE H atoms are produced on the shorter time scale, but this simple model is insufficient. There is significant discrepancy between the ratios obtained for the two observed KE channels and the ratio of signals with different time constants used to fit the time-resolved data in the three molecules studied.

Considering 6-DMAP, it is clear that the low KE signal observed appears with a time constant similar to that of the high-KE channel, as only a single time constant is required to fit the temporal profile. These two channels, which show similar time signatures, occur in a ~1:1 ratio as evidenced by the TKER spectrum. Given that the amino group is blocked to H atom loss by methyl groups, and since it has been previously shown[15,16] that deuterating ring C−H's resulted in the persistence of a significant low KE signal, it is highly likely that both these channels originate from azole (N^9−H) bond fission.

In 9-MA, the azole channel is blocked, allowing insight into the loss of H from the amino site. In this case, the low-KE channel dominates over the direct (high-KE) channel by a factor of ~1.8. However, in contrast to 6-DMAP, a slower (326 fs) time constant is also required to fit the time trace. This time component is required to be half the amplitude of the fast component. Assuming that the high-KE channel is entirely represented by a fast (95 fs) time constant, it is clear that assigning the entire low-TKER signal to τ_2 (326 fs) produces the wrong ratio of time constants. However, from 6-DMAP and previous work,[15] we know that there is potential for a low-KE channel with a fast time constant. By assigning ~50% of the low-KE channel to τ_1 (95 fs) and ~50% to τ_2 (326 fs), we can account for the observed ratio of time constant contributions in 9-MA. It seems that there are three dissociation channels involving the amino group at λ = 200 nm. One is direct (via the $^1\pi\sigma^*$ state), resulting in prompt, high-KE H atoms. The remaining two channels both produce low-KE "statistical" H

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atoms, most probably via multiphoton excitation and subsequent internal conversion. One of these channels occurs on a 95 fs time scale and the other with a longer (326 fs) time constant.

To deconvolute the contributions in Ade directly from the KE and temporal data would be very difficult. However, on the basis of the preceding discussion for 6-DMAP and 9-MA and the assumption that the dynamics in Ade will essentially behave as a linear combination of the two substituted systems, it is possible to understand the dynamics and make some observations about the relative contributions of the azole and amino sites to H atom loss at $\lambda = 200$ nm.

The ratio of high to low KE signal in Ade is 1:1.30. By taking a weighted sum of the KE ratios for 6-DMAP and 9-MA, with coefficients of 1.0 and 0.91, respectively, the ratio for Ade is recovered, indicating almost equal (overall) contributions from the azole and amino sites. Applying the same coefficients to the temporal decompositions for 6-DMAP and 9-MA gives an expected ratio of 83% fast (110 fs) to 17% slow (265 fs), which agrees very well with the fit of the Ade temporal data. This cross check suggests that Ade can be modeled well using the behavior of the two substituted systems as a basis.

We can also draw some general conclusions regarding the subcomponents of the H^+ transient in Ade. In 6-DMAP, a single time constant is required to fit the H^+ transient with an ~1:1 ratio in the high and low-KE signal. In addition, given the contributions of the 6-DMAP and 9-MA to the Ade KE and H^+ transient signals, we can anticipate almost 50% of the H^+ transient arising through $\pi\sigma^*$ state dissociation, a considerable component of which is from the amino site. This process occurs in <100 fs. The remainder of the H^+ transient in Ade is from low KE H-atoms from both the azole and amino sites. A fast subcomponent ($\tau < 100$ fs) originates from both the azole and amino sites, while a slower component ($\tau \sim 300$ fs) arises only from the amino site.

In Figure 4 we present the H^+ transient measured in Ade following excitation at 266 nm and probing with 243.1 nm. Once again, error bars indicate a 95% confidence limit, with the significantly larger errors reflecting the decreasing two-color pump/probe signal at these wavelengths. If H-atoms are released by photodissociation and then probed, one would expect to see a rise in the H^+ signal at positive delays (as in Figure 3). Instead we observe a clear decay at negative delays, i.e., when the probe precedes the pump. This is most likely due to multiphoton dissociative ionization yielding H^+ directly, where the decay results from a short-lived resonance state accessed following absorption of one or two photons of 243.1 nm. Because of the sensitivity of these experiments to the production of H-atoms, this result strongly suggests that there is no participation of the $\pi\sigma^*$ state in the relaxation dynamics of Ade (within the time frame of our measurements, 3.5 ps) following excitation at 266 nm and supports the TKER measurements of Nix et al.,[6] who reported the observation of a high KE H-atom channel at excitation wavelengths below 233 nm. While the lack of signal with a fast time-constant is not too surprising, the total lack of any two-color signal when the pump precedes the probe is surprising, as we would expect multiphoton low KE H-atoms to appear with a fast time constant, as suggested following excitation at 200 nm. It is also worth noting that these experiments are not sensitive to pathways which do not result in the production of H-atoms. Therefore, this result does not exclude the participation of other ultrafast processes, which have been reported elsewhere[6−12] and hence we are unable to quantify the quantum yield of the H-atom elimination channels in Ade.

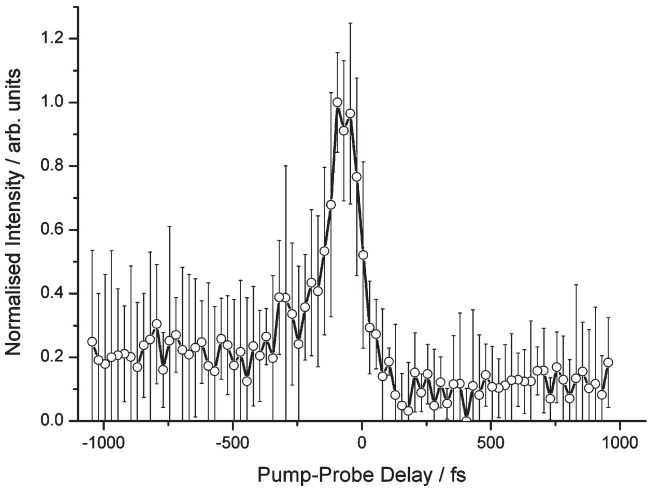

Figure 4. TR-MS H^+ signal (266 nm pump and 243.1 nm probe) in Ade. Error bars correspond to a 95% confidence limit.

The results presented here support the view that the role of the $\pi\sigma^*$ states following excitation at 266 nm is minimal. Data obtained via excitation at 200 nm strongly imply participation of the azole *and* amino $\pi\sigma^*$ states. The substantial contribution of the amino group to the total H-atom elimination yield may have further implications. Although it occurs at high energy, this H-atom channel could become important in the relaxation dynamics in solution phase Ade, as these states are known to fall in energy in solution. This has already been suggested to explain photoionization spectroscopy measurements of Ade−water complexes[17] and fluorescence upconversion spectroscopy measurements of Ade in aqueous solution.[18]

While the work described above shows that Ade can be well-modeled using the dynamics observed for both 9-MA and 6-DMAP, this is only an approximation and highlights the need for more theoretical studies to be carried out. This work also highlights the need to extend our measurements to record complete TR-VMI spectra. Given that a complete TR-VMI scan would take in excess of 120 h, our group is currently developing an oven source capable of sustaining a sufficient sample density for experiments to be carried out over these time scales.

EXPERIMENTAL SECTION

The experimental configuration is described in detail in the Supporting Information. Briefly, a commercially available Even−Lavie valve was used to introduce Ade, 9-MA, and 6-DMAP seeded in helium, into a femtosecond time-resolved mass-spectrometer. The molecules of interest were optically

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excited at either 200 or 266 nm (pump), and the resultant neutral H-atom photoproducts were resonantly ionized at 243.1 nm with a temporally delayed probe pulse. The H^+ yield was measured as a function of time delay between the pump and probe pulses by a position sensitive detector at the terminus of a 500 mm flight tube. The laser used was a commercially available Spectra-Physics regenerative amplifier system which was operated at 500 Hz, centered at 800 nm, and delivered 35 fs pulses. The output was split into three beams of equal intensity, two of which were used in these experiments. The pump and probe were generated through frequency up-conversion using a home-built unit and a commercial optical parametric amplifier (TOPAS), respectively.

SUPPORTING INFORMATION AVAILABLE Experimental procedures and additional experimental results. This material is available free of charge via the Internet at http://pubs.acs.org.

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