Vacuum-Ultraviolet Photoionization Studies of the Microhydration of DNA Bases (Guanine, Cytosine, Adenine, and Thymine)†

Leonid Belau,‡ Kevin R. Wilson,‡ Stephen R. Leone,‡,§ and Musahid Ahmed*,‡

Chemical Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, California 94720, and Departments of Chemistry and Physics, University of California, Berkeley, California 94720

Received: January 23, 2007; In Final Form: March 1, 2007

In this work, we report on a photoionization study of the microhydration of the four DNA bases. Gas-phase clusters of water with DNA bases [guanine (G), cytosine (C), adenine (A), and thymine (T)] are generated via thermal vaporization of the bases and expansion of the resultant vapor in a continuous supersonic jet expansion of water seeded in Ar. The resulting clusters are investigated by single-photon ionization with tunable vacuum-ultraviolet synchrotron radiation and mass analyzed using reflectron mass spectrometry. Photoionization efficiency (PIE) curves are recorded for the DNA bases and the following water (W) clusters: G, GW_n ($n = 1-3$); C, CW_n ($n = 1-3$); A, AW_n ($n = 1,2$); and T, TW_n ($n = 1-3$). Appearance energies (AE) are derived from the onset of these PIE curves (all energies in eV): G (8.1 ± 0.1), GW (8.0 ± 0.1), GW_2 (8.0 ± 0.1), and GW_3 (8.0); C (8.65 ± 0.05), CW (8.45 ± 0.05), CW_2 (8.4 ± 0.1), and CW_3 (8.3 ± 0.1); A (8.30 ± 0.05), AW (8.20 ± 0.05), and AW_2 (8.1 ± 0.1); T (8.90 ± 0.05); and TW (8.75 ± 0.05), TW_2 (8.6 ± 0.1), and TW_3 (8.6 ± 0.1). The AEs of the DNA bases decrease slightly with the addition of water molecules (up to three) but do not converge to values found for photoinduced electron removal from DNA bases in solution.

Introduction

The molecular structures of many biological molecules—DNA, RNA, peptides, and amino acids—are very well-known, in light of 50 years of research that have elapsed since Watson and Crick's original postulation for the structure of DNA. However, fundamental aspects of the "nuts and bolts" molecules that are the building blocks of life are not yet well-understood.[1] Both intramolecular properties (how a molecule interacts with itself) and intermolecular properties (how a molecule interacts with its surroundings) are unknown for a vast majority of biomolecules. Studying these molecules under well-defined conditions can distinguish between intrinsic and externally imposed properties (i.e., solvation). Investigation of complexes of increasing size can mimic the transition from the isolated molecule to solution or bulk, pointing the way from in vacuo to in situ.

The ionization of DNA bases is a key initial step that leads to DNA damage and mutation.[2] The electron hole that is introduced by the ionization process in DNA migrates along the DNA's helix through various hopping mechanisms leading to tautomerization through proton transfer and dissociation in the strand of the helix itself.[3] Apart from the evolutionary and carcinogenic effects that this damage might induce in living systems, there is also much interest in the electronic properties of the DNA molecules themselves.[4] This stems in part from the impetus of using DNA in molecular electronics. Molecular shape, molecular conformation, and molecular charge distribution play crucial roles in the selectivity and function of DNA. Hence, there is an intrinsic fundamental interest in the study of these biomolecules and their bonding properties with water, the solvent that is required for life.

Ionization of individual DNA bases complexed with water was pioneered in the Herschbach group.[5] Electron impact ionization was utilized to measure the appearance energies of thymine and adenine complexed with water. Schlag and co-workers performed anion spectroscopy of uracil, thymine, and the amino-oxy and amino-hydroxy tautomers of cytosine complexed to water.[6] Subsequently, photoionization with one and two color lasers was employed by the groups of de Vries, Mons, and Kim to study the microhydration of the DNA bases.[7−12] IR-UV double resonance spectroscopy was employed by the de Vries group to examine guanine−water[7] and guanine−guanine−water[8] clusters. A number of structures were identified. Piuzzi and co-workers, utilizing resonant two photon ionization (R2PI), laser-induced fluorescence, and hole burning spectroscopy, identified three forms of guanine−water complexes in the gas phase.[9] Kim and co-workers explored the hydration of DNA base cations (adenine and thymine) with R2PI and used the metastable fragmentation of the cluster ions to derive the strength of the binding energy of water−DNA base complexes.[10] Their results suggested that the first hydration shell for adenine and thymine cations is completed with four water molecules; the cation dimer of adenine requires 7−8 water molecules to complete the solvation. In a separate study, these authors observed that virtually all hydrated adenine monomers disappeared from the photoionization mass spectrum because of a facile decomposition of the adenine−water complex.[11] This phenomenon was attributed to the n-π^* character of the transition accessed in this two-photon ionization process, which leads to an increased repulsive nature of the potential energy surface along the solute−solvent rearrangement coordinate when the solvent acts as a proton donor. The hydrated monomers were readily discernible with electron impact ionization and with

† Part of the "Roger E. Miller Memorial Issue".

* To whom correspondence should be addressed. Tel: 510-486-6355. Fax: 510-486-5311. E-mail: MAhmed@lbl.gov.

‡ Lawrence Berkeley National Laboratory.

§ University of California.

10.1021/jp0705929 CCC: $37.00
Published on Web 04/10/2007

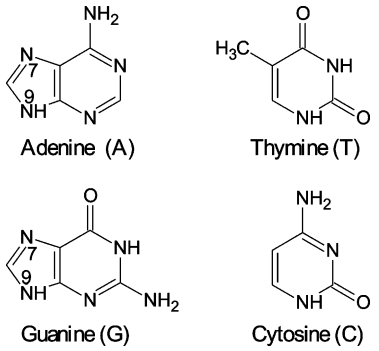

Figure 1. Canonical structures of four DNA bases denoted by A, T, C, and G for adenine, thymine, cytosine, and guanine. The numbers 7 and 9 in the case of guanine and adenine denote the possible position for H to form either a 7H or a 9H tautomer. In all cases, the keto forms are shown. To form enol structures, an H atom migrates from an NH group to the nearest O atom to form an enol.

femtosecond ionization, suggesting that the decomposition occurs within nanosecond time scales.[11] Time-resolved photoionization spectroscopy shows that low-lying stabilized $\pi\sigma^*$ states play an important role in the excited-state relaxation of partially or fully solvated adenine.[12]

In contrast to the paucity of experimental results, there are substantial theoretical efforts being devoted to studying the ionization properties of DNA bases[13,14] and the effect of water on these properties.[15] While most of the studies consider the relative energies and structures of the various tautomers of the DNA bases, recently there have been a number of papers detailing the ionization properties of the DNA bases and their complexes with water.[16] Of particular mention among these are the results from Hobza and co-workers who performed detailed ab initio calculations of the DNA bases and their tautomers, in a microhydrated environment and in aqueous solution.[15,17] There have also been a number of papers where ab initio methods have been employed to evaluate the ionization energy thresholds of the DNA bases in the gas phase and in aqueous solution. Close et al.[16] determined the ionization energy thresholds of thymine and thymine keto−enol tautomers with 1−3 water molecules placed in the first hydration shell and compared these results with the experimental determinations of Kim et al.[5]

To date, these elaborate calculations have been benchmarked to the electron impact ionization data of Kim et al.[5] for the hydrated bases and to some very early photoelectron spectroscopy work for the bases themselves.[18] Very recently, Kim and co-workers published high-resolution vacuum-ultraviolet (VUV) mass-analyzed threshold ionization (MATI) spectra of jet-cooled thymine.[19] The authors derive the adiabatic ionization energy for thymine to be 8.9178 eV and report the vibrational spectra of the resulting cation. This number is contrasted to the available data in the literature, which are scattered in the range of 8.82−9.18 eV.[19] Recently, there has been a photoionization mass spectrometry (PIMS) study of adenine, thymine, and uracil utilizing synchrotron radiation.[20]

In this work, we have carried out a systematic investigation of the PIMS of the four DNA bases (guanine, cytosine, adenine, and thymine) and their complexes with water utilizing tunable VUV radiation coupled to a jet-cooled thermal vaporization source. The canonical structures of the four DNA bases are shown in Figure 1. Single-photon ionization (SPI), where absorption to intermediate levels is not required, shows much promise as a method of efficient ionization for fragile systems. With the relatively low photon fluences having sufficient photon energy to ionize a neutral molecule in a single step, photofragmentation resulting from multiple photon absorption can be effectively eliminated. Analysis of the photoionization efficiency (PIE) curves allows the extraction of appearance energies. The DNA bases can adopt a variety of tautomeric forms, and microhydration with water could also lead to a number of different structures. The PIE curves measured in this work are convolved over a range of these possible tautomers and structures and the measured appearance energies are discussed in the text keeping this in mind.

Experimental Section

The experiments have been performed on a molecular beam apparatus coupled to a 3 m VUV monochromator on the Chemical Dynamics Beamline at the Advanced Light Source.[21] This apparatus has been described recently,[22] and here, we illustrate the continuous nozzle source used to produce the DNA bases and their complexes with water in a supersonic expansion.

To date, most photoionization experiments with DNA bases and their complexes with water have utilized pulsed valve technology as these couple well to the low repetition rate lasers that are typically used in these experiments. In our experiment, we implemented a simple variant of the design of Kim et al.[5] to produce continuous beams of the thermally labile DNA bases and their complexes with water. The nozzle consisted of a 0.953 cm diameter disk (1 mm thick) with a 100 μm diameter center hole welded on to one end of a closed stainless steel tube of 0.953 cm OD and 15.24 cm long. This front end of the stainless steel tube contained the DNA bases and could be heated to between 298 and 700 K with a cartridge heater. The temperature of the tube was monitored with a thermocouple attached between the heater and the tube. To produce the water complexes, Ar carrier gas at 1 bar was passed over a water reservoir held at room temperature (2.986 kPa) and directed into the stainless steel nozzle piece near the 100 μm orifice. The temperatures utilized for generating the DNA bases were 650−680 K for guanine, 600−630 K for cytosine, and 600−640 K for adenine and thymine. A separate experiment in which a nozzle extension based on a channel was put after the nozzle gave rise to cytosine clusters up to $n = 5$ and water clusters up to $n = 10$ for each cytosine species.

The skimmed molecular beam was interrogated in the ionization region of a commercial reflectron mass spectrometer (R. M. Jordan) by tunable, monochromatized synchrotron undulator VUV radiation. The photoionization region was situated 11 cm from the nozzle. As the synchrotron light is quasi-continuous (500 Mhz), a start pulse for the TOF ion packet was provided by pulsing the electrical fields of the ion optics. In general, the ion optics were biased in such a fashion that all positive ions were accelerated away from the detector until the start pulse occurred. Ion signals from the microchannel plate detector were collected with a multichannel-scalar card (FAST Comtec 7886). Time-of-flight spectra were recorded for the photoionization energy range between 7.8 and 10.5 eV. The typical step size of the VUV photon energy used for these experiments was 50 meV, and the data collection time at each of these energy steps was 240 s.

The PIE curves of the DNA bases and their complexes with water were obtained by adding all of the ion counts in the mass peak at each photon energy, normalized by the photon flux. The synchrotron VUV photon flux was measured by a Si photodiode (IRD, SXUV-100).

Results and Discussion

In Figure 2, the TOF mass spectra (resolution $m/\Delta m = 400$ in the m/z 150 region) of the DNA bases along with their water

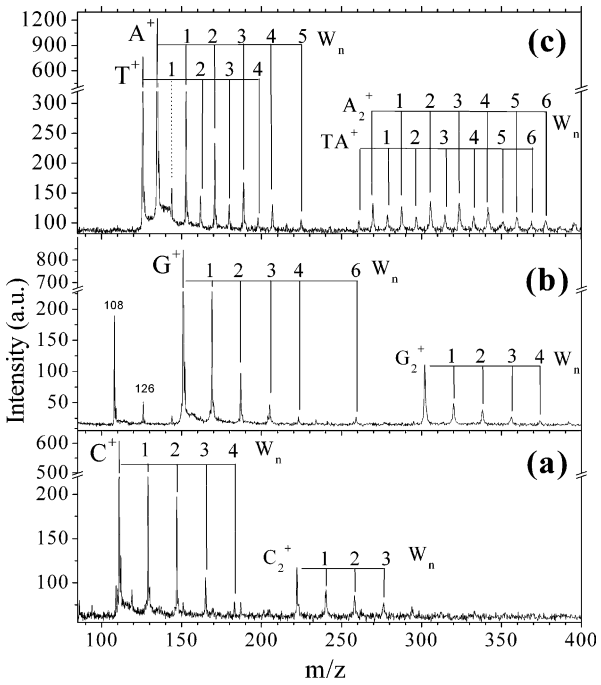

Figure 2. Mass spectra of four DNA bases with water clusters at 10 eV photon energy: (a) cytosine, (b) guanine, and (c) adenine and thymine. The break in the intensity scale is introduced to better describe the cluster distribution relative to the monomer peak.

complexes are displayed, recorded at a photon energy of 10 eV. Dimers for all of the DNA bases along with their corresponding water clusters are also observed in the mass spectrum as can be seen in Figure 2. The signal levels of the dimers are lower compared to the bare monomers and their water complexes; hence, extraction of ionization energy onsets for the dimers proved difficult, and these will not be covered in this paper. The fact that we observe complexes of the DNA bases with water and also that dimers can be produced in our supersonic expansion suggests that significant cooling is being provided in the jet. While we do not have an experimental measurement of our molecular beam temperature, our beam conditions can be compared to those of Amirav et al.[23] for tetracene seeded in Ar. They suggest that in their supersonic expansion, the temperature of a large and heavy molecule is around $\approx$7 K for rotations and $<$50 K for vibrations. The results of Amirav et al.[22] were obtained with a 150 μm diameter nozzle and an Ar backing pressure of 23.33 kPa and, when compared to our conditions of 100 μm diameter nozzle and an Ar backing pressure of 101.32 kPa, would suggest better cooling conditions for our supersonic expansion. A true determination of the temperature of our molecular beam will have to await the availability of a means to probe the internal energies of the DNA bases and their water clusters with sufficient resolution to map out their rotational and vibrational states.

Figure 2a displays the time-of-flight mass spectra for cytosine monomer and its complexes with water clusters recorded using a heater temperature of 610 $\pm$ 5 K. To the best of our knowledge, these are the first measurements for cytosine−water clusters in the gas phase using photoionization. Kim et al.[5] did report a mass spectrum for cytosine−water clusters utilizing electron impact. However, they went on to add that their mass spectrum showed evidence for chemical transformation. They detected very small amounts of *m*/*z* 111 (cytosine parent) and its clusters with water. The prominent peak in their spectra occurred at *m*/*z* 109, which indicates dehydrogenation, and additional peaks were at *m*/*z* 110, 112, and 113, suggesting that cytosine converted to uracil in the presence of water and thermal heating. We did not observe any such conversions or dehydrogenation in our experiments. This probably arises from the lower heating required in this experiment to generate stable mass spectra.

Figure 2b displays the time-of-flight mass spectra for guanine monomer and complexes with water clusters recorded also at a heater temperature of 650 $\pm$ 10 K. For this particular set of experiments, guanine and cytosine were heated together in the nozzle. However, no guanine−cytosine complexes were observed in the mass spectrum. This arises in all likelihood because the cytosine is evaporated away at lower temperatures than those required for guanine. This is confirmed via the fact that the same mixture at 610 K gives rise to only cytosine and cytosine−water clusters as observed in Figure 2a. Various groups have generated guanine and its hydrates in the gas phase with laser desorption coupled to supersonic jet cooling in pulsed beams. Recently, the Miller group[24] prepared gas-phase tautomers of guanine within the environment of a cold superfluid helium droplet via thermal vaporization of guanine powder. However, to the best of our knowledge, our experiment is the first time that a thermal vaporization source has given rise to detectable levels of guanine molecules in a traditional molecular beam. In contrast to the other molecules examined in this work, fragments of guanine at *m*/*z* 108 and 126 are also detected. The former arises from HNCO elimination while the latter is a complex of the *m*/*z* 108 fragment with water. The presence of the ion at *m*/*z* 126 suggests that fragmentation of guanine takes place in the nozzle itself, due to the thermal degradation.

The ratios of the integrated mass spectral peak intensities from Figure 2b for G:GW:GW_2:GW_3 are 1.00: 0.44:0.17:0.14, respectively. This stands in sharp contrast to the results obtained by laser desorption and R2PI photoionization methods. Recently, Saigusa et al.[25] reported one color R2PI at 296.5 nm for hydrated clusters of guanine where the resulting mass spectrum showed almost complete absence of hydrated clusters of the guanine monomer. An examination of another one color R2PI experiment performed by the de Vries group also shows considerable reduction in the ability to detect hydrates of the guanine monomer.[7] They observe a ratio of 1.00:0.14 for G:GW when the guanine−water complex is at a strong 2 photon resonance. In the SPI results reported here, we readily observe up to GW_3, which suggests that accession through electronically excited states in these R2PI experiments leads to extensive fragmentation similar to that observed for the hydrated adenine monomer.

Figure 2c displays the time-of-flight mass spectra for thymine and adenine complexed with water clusters recorded at a heater temperature of 620 $\pm$ 10 K. The dominant peaks are for the thymine and adenine monomer, followed by clusters of these bases with water up to $n = 4$. Kim et al., utilizing nanosecond one color R2PI with 266 nm photons, observed an absence of hydrated clusters of the adenine monomer.[11] These clusters were readily observable in the electron impact spectrum and with femtosecond one color R2PI with 267 nm photons. Our own observation of hydrated adenine monomers recorded by SPI would suggest that the assertion made by Kim et al. that one-photon excitation to the first electronically excited state leads to extensive fragmentation is essentially correct.[11] Such a fragmentation results from the increased repulsive nature of the n-π^* excited state with water upon solvation.

Figure 3 shows the PIE curve for thymine (*m*/*z* 126) and TW_n ($n = 1-3$) recorded between 7.8 and 9.8 eV. Typically, in PIMS, a least-squares fit to the change of slope and extrapolation of this fit allows for the determination of the ionization energy

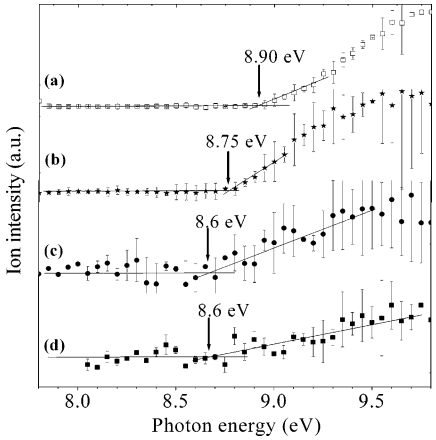

Figure 3. PIE curves recorded for (a) thymine (T), (b) thymine−H_2O (TW), (c) thymine−$(H_2O)_2$ (TW_2), and (d) thymine−$(H_2O)_3$ (TW_3). The appearance energies are rounded off to the most significant digits within experimental error and are denoted by an arrow in each spectrum.

for a particular species. This approach works well when there are relatively good Franck−Condon factors in the ionization process, in which there is a minimal change in molecular geometry in going from the neutral to the cation. In this work, we have chosen to report the appearance energies as the intercept of the onset with the baseline. For thymine, this approach for data points between 8.9 and 9.3 eV obtains an appearance energy of 8.90 (±0.05) eV. This value agrees extremely well with a recent high-resolution VUV MATI experiment of jet-cooled thymine, in which the adiabatic IE is reported to be 8.9178 eV.[19] It is also within the collective error limits for another SPI measurement of 8.82 (±0.03) eV reported recently.[20] The discrepancy of 0.08 eV between the two SPI measurements probably arises from insufficient cooling in the thermal evaporation source of ref 20, leading to a lower ionization onset due to hot band effects. This agreement with recent experimental results provides confidence in the absolute calibration scales for the photon energy and also suggests that our molecular beam is sufficiently cold to eliminate hot bands that typically plague ionization energy determinations using PIE curves.

From Figure 3b−d, the appearance energies for TW, TW_2, and TW_3 are 8.75 (±0.05), 8.6 (±0.1), and 8.6 (±0.1) eV, respectively. The signal-to-noise ratio for TW_2 and TW_3 is poor, making it difficult to extract unambiguously the appearance energies for these complexes. The shapes of the PIE curves are relatively smooth and rise in a linear manner. This pattern is followed by all of the water complexes of the DNA bases. Within the range of our data, it appears that the appearance energies for thymine−water complexes stabilize around 8.6 eV with increasing numbers of water.

Our absolute reported energies are discrepant with those reported by Kim et al.[5] who utilized electron impact; however, the decreasing trend in ionization energies with the addition of water to thymine is reproduced well. Their reported values for T, TW, TW_2, and TW_3 are 9.15, 8.85, 8.65, and 8.50 eV, respectively. While their absolute accuracy is ±0.15 eV, they quote a relative accuracy of ±0.05 eV. The theoretical calculations of Close et al.[16] at the B3LYP and P3 levels of theory suggest that there is a microhydration effect on the ionization energies for thymine. Their calculations suggest that the first water molecule decreases the ionization energy by about 0.1 eV, whereas the second and the third water molecules cause a decrease of 0.07 and 0.08 eV, respectively, for canonical structures of thymine (Figure 1) complexed with water. Calculations on microhydrated keto−enol tautomers of thymine

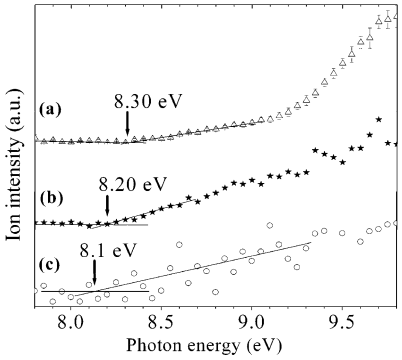

Figure 4. PIE curves recorded for (a) adenine (A), (b) adenine−H_2O (AW), and (c) adenine−$(H_2O)_2$ (AW_2). The appearance energies are rounded off to the most significant digits within experimental error and are denoted by an arrow in each spectrum.

(H from one of the NHs is transferred to one of the O groups) gave a better agreement with the results of Kim et al.;[5] however, it is observed that the keto−enol tautomers are considerably higher in energy than the canonical form and would most likely not be present in a jet-cooled experiment. Our experimental results are in better agreement with these calculations; however, caution should be exercised when comparing theoretical results with experiments at this level of theory. Because we do not have an assignment of the tautomeric composition of thymine and its hydrated clusters in our molecular beam and the calculations suggest that there are many low-lying tautomeric structures with different ionization energies, our comparison at best suggests that we are most likely accessing the canonical forms of thymine in our molecular beam.

The appearance energy for the adenine monomer is 8.30 (±0.05) eV, derived from the PIE curve shown in Figure 4a. An earlier photoionization measurement of 8.20 eV utilizing a thermal evaporation source without jet cooling may be due to a hot band contribution, which could give rise to the lower ionization energy onset.[20] Photoelectron spectroscopy measurements report a vertical and adiabatic IE of 8.48 and 8.26 eV, respectively.[26] Plutzer and Kleinermanns[27] performed a two-color R2PI measurement and reported an ionization threshold of 8.606 ± 0.006 eV as the upper limit for the 9H tautomer (Figure 1) of adenine. This is the canonical form. R2PI experiments, as well as the IR absorption spectra of adenine, suggest contributions from both 9H and 7H tautomers in the gas-phase spectrum of thermally desorbed molecules.[28] Theoretical calculations,[29] however, place the 7H isomer even higher in ionization energy when compared to the 9H tautomer. This would suggest that Plutzer and Kleinermanns did not access the adiabatic limit in their two-color R2PI experiment.

For the adenine:water (AW) complex (Figure 4b), we derive an appearance energy of 8.20 (±0.05) eV. The data for AW_2 are noisy (Figure 4c); however, we are reasonably confident that the appearance and ionization energy converge to 8.1 (±0.1) eV. The decrease in the ionization energies with increased hydration is very similar to that observed for thymine, mentioned previously. These results are, however, at both a qualitative and a quantitative variance with the only other published experimental result for the ionization energies of the hydrates of adenine. Kim et al.[5] obtained electron impact ionization data that derive values of 8.45, 7.95, 7.80, and 7.70 eV for A, AW, AW_2, and AW_3, respectively. The discrepancies probably arise from the different ionization sources—electron impact vs SPI—and from the extrapolation method utilized in the electron impact ionization studies. The SPI results reported in this work are

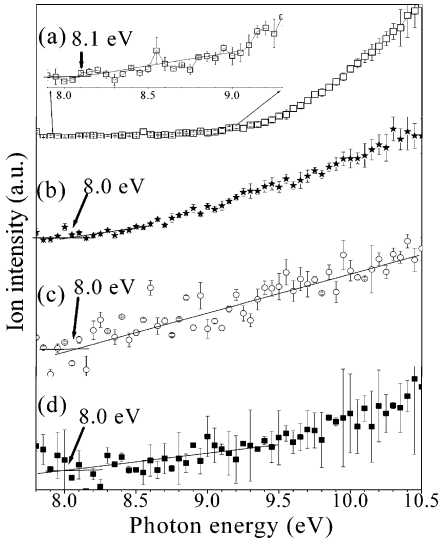

Figure 5. PIE curves recorded for (a) guanine (G), (b) guanine−H_2O (GW), (c) guanine−$(H_2O)_2$ (GW_2), and (d) guanine−$(H_2O)_3$ (GW_3). The expanded region between 8.0 and 9.2 eV for guanine is shown as an insert above panel a. The appearance energies are rounded off to the most significant digits within experimental error and are denoted by an arrow in each spectrum.

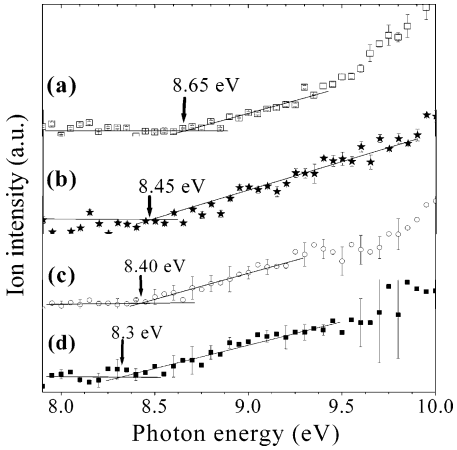

Figure 6. PIE curves recorded for (a) cytosine (C), (b) cytosine−H_2O (CW), (c) cytosine−$(H_2O)_2$ (CW_2), and (d) cytosine−$(H_2O)_3$ (CW_3). The appearance energies are rounded off to the most significant digits within experimental error and are denoted by an arrow in each spectrum.

probably more reliable given that the ionization energies of the unsolvated molecules agree very well with the literature values.

The extraction of the ionization energy for guanine is complicated by the possible existence of a number of tautomers in the molecular beam. The works of de Vries and of Mons utilizing R2PI suggest that as many as four tautomers populate the molecular beam.[30,31] The UV spectroscopy of free guanine is controlled by the 7/9 NH tautomerism (Figure 1). Both of the 7NH tautomers observed in the gas phase are red-shifted as compared to the 9NH molecules, and the origin transitions utilizing one-color R2PI[30] follow an origin transition order: 7NH enol (32864 cm^{-1}), 7NH keto (33269 cm^{-1}), 9NH keto (33910 cm^{-1}), and 9NH enol (34755 cm^{-1}). These correspond to the photoionization origins 0−0 at 8.15, 8.25, 8.41, and 8.62 eV, respectively. The PIE curve for guanine is shown in Figure 5a, and the onset part is shown in the insert for the range 8.0−9.2 eV. The onset is at 8.10 (±0.1) eV. The intensity in this region is very weak when compared to the main part of the PIE curve. Future experiments with better signal-to-noise and two color spectroscopy should allow for an unambiguous determination of the origin of these structures. The weakness of the signal intensity most probably arises from very poor Franck Condon factors. The onset observed in this work, and noting the values obtained by Mons et al.,[30] would suggest that a number of tautomers is generated in our molecular beam. It is of course entirely possible that the tautomers populated by thermal vaporization in the presence of water are entirely different from the populations generated in the laser desorption experiments performed in the R2PI experiments. Very recently, a report has been published where IR spectroscopy was performed on thermally vaporized guanine embedded in superfluid He droplets.[24] Again, four low-lying tautomers of guanine were identified by their IR spectrum. This recent determination suggests that the originally postulated keto tautomers of guanine seen in the two-photon experiments of Mons[30] and de Vries[31] are most likely imino tautomers.[32]

Recently, Elshakre[33] examined the six most stable tautomers of guanine in the neutral and cationic ground states at the MP2 level of theory. The molecular structures were optimized to show that all guanine tautomers are nonplanar in the ground neutral state. Upon ionization, it was predicted that guanine cations originate from the removal of one of the electron pairs on the terminal NH_2 group of the pyrimidine ring, and the other electron enhances the interaction between the nitrogen atom of the NH_2 and the immediate carbon atom neighbors upon hyperconjugation. Interestingly, these interactions force the molecule to become planar in the cationic state, and also, dipole moment analysis suggests that these tautomers suffer large geometry changes upon ionization. This could explain the lack of intensity in the ion signal at threshold. MATI experiments, so elegantly demonstrated in the work of Kim et al. on uracil[24] and thymine,[19] will provide for an unambiguous determination of the ionization energies of the different tautomers in guanine.

The PIE plots for GW, GW_2, and GW_3 are shown in Figure 5b−d, which allow for possible extraction of AEs of 8.0 (±0.1), 8.0 (±0.1), and 8.0 eV, respectively. For GW_3, the signal increases again below 8.0 eV, suggesting that the appearance energy is lower. This complication arises from the fact that the second harmonic of the undulator radiation becomes transparent to the gas filter, which is typically filled with Ar (IE = 15.78 eV). Therefore, any enhancement in signal below 7.9 eV could originate from ionization by second-order photons. This is definitely a problem with hydrated guanine since the appearance energies happen to lie right in this region.

For cytosine, Figure 6a, there is a very sharp rise in the ion signal at around 8.65 (±0.05) eV. Nir et al.,[34] utilizing one-color R2PI and two-color R2PI, postulated that the ionization energy of cytosine is between 8 and 9 eV. In the same work, they also reported that there were two tautomeric forms, one keto and one enol, prevalent in their molecular beam. In other work,[35] it has been shown that, in the gas phase, a mixture of four tautomer/conformer forms of cytosine may be produced upon thermal vaporization. A very recent photoelectron spectroscopy measurement posits the vertical ionization energy for cytosine to be around 8.89 eV;[26] the lower appearance energy at around 8.65 eV reported in this work is likely an adiabatic value. One possible explanation is that cytosine is formed with substantial internal energy and this would give rise to hot bands and consequently the lower ionization energy in comparison to the photoelectron measurements. However, we believe this is unlikely since in this work jet cooling was employed, in contrast to the photoelectron work, and the very good agreement of our results for thymine with literature values provides confidence in our value for cytosine.

TABLE 1: Appearance Energies of Four DNA Bases and Complexes with Water (All Energies in eV)

	monomer	monohydrate	dihydrate	trihydrate
thymine	8.90 ± 0.05	8.75 ± 0.05	8.6 ± 0.1	8.6 ± 0.1
adenine	8.30 ± 0.05	8.20 ± 0.05	8.1 ± 0.1	
guanine	8.1 ± 0.1	8.0 ± 0.1	8.0 ± 0.1	8.0
cytosine	8.65 ± 0.05	8.45 ± 0.05	8.4 ± 0.1	8.3 ± 0.1

The AEs for CW, CW_2, and CW_3 are 8.45 ($\pm$0.05), 8.40 ($\pm$0.05), and 8.3 ($\pm$0.1) eV, respectively, as reported in Figure 6b−d, which follows the general trend seen for all of the bases. As mentioned previously, to the best of our knowledge, there have been no previous measurements of the appearance energies for cytosine−water clusters.

In Table 1, the appearance energies are summarized to aid in detecting trends and to contrast the energy stabilization of the AEs upon hydration. It should be pointed out that while these are appearance energies, for certain systems, they do represent the adiabatic ionization energies for the bare bases. The physical shapes of the PIE curves affect the extraction of the ionization energies for the hydrates. For all of the water complexes, there is no sharp threshold for ionization; in other words, the PIE curves have a very gentle and curved rise. This has been recognized as a signature for very different equilibrium geometry when the system goes from the neutral to the cation that is formed after photoionization. The literature is replete with examples of folded conformations of flexible molecules and extensive hydrogen-bonded molecular systems where such PIE curves are seen.[30]

The effect of hydration on the DNA bases leads to a gradual lowering of the appearance energies from the monomer alone, as can be seen in Table 1. Qualitatively, this is in agreement with both the theoretical calculations[16] and the experimental[5] determinations of other workers, as has been discussed above in the case of thymine. While there have been a number of theoretical efforts to calculate the electronic properties of the microhydration of the DNA bases, the discussion has mostly revolved around the techniques used to extract the ionization energies. It is difficult to extract a detailed physical picture of the ionization process and the effect that water has on the ionization process from these works. In the case of a few water clusters, short-range H-bonding interactions between the water molecules and the DNA base could lead to a reorientation of the solvent water dipoles to effect a stabilization process of the radical cation that is formed upon photoionization. However, theoretical work[16] has shown that it is not trivial to find a correlation between the solvent water dipole and the ionization energy of the microhydrated DNA bases. Furthermore, a very recent paper suggests that earlier theoretical models do not emphasize water−water interactions but focus exclusively on water−DNA base interactions, which can lead to erroneous structures.[36]

Within a bulk environment, long-range polarization interactions have been suggested to play a significant role in the lowering of the first vertical and adiabatic ionization energies for the DNA bases in water. In aqueous solutions, electron ejection into solution has been observed from the purine and pyrimidine bases at a photon energy of 4.66 eV.[37] Photoelectron studies of DNA components in aqueous solution coupled to theoretical calculations also suggest that ionization takes place well below the gas-phase thresholds for the DNA bases.[38] Very recent theoretical calculations suggest that bulk water solvation leads to stabilization of the adiabatic radical cation that is formed upon photoionization in solution by about 2.12−2.79 eV when compared to gas-phase values.[39] With the incorporation of the hydrated electron stabilization energy (−1.3 eV), these theoretical calculations place the ab initio ionization energy thresholds of the DNA bases in aqueous solution to be 5.05, 4.91, 4.81, and 4.42 eV, for thymine, cytosine, adenine, and guanine, respectively.[39] Our measured appearance energies for the DNA bases microhydrated by small water clusters are much higher when compared to the solution-phase results, suggesting that a significant number of water molecules are required to provide an adequate model for photoinduced electron removal from DNA bases in solution. Our measurements are derived from PIMS measurements where ionization onsets are used to derive an appearance energy, while in the solution studies, it is the photoelectron ejection onset that is being measured. It is entirely possible with future measurements of DNA microhydration by larger water clusters that a convergence could appear between the photoionization and the photoelectron work. It would also be desirable to perform photoelectron spectroscopy on size-selected microhydrated DNA bases.

In this work, we have shown that thermal vaporization in conjunction with supersonic jet cooling allows for the formation of a microhydrated environment for the DNA bases. The gentle environment of thermal vaporization in comparison to laser desorption that is typically used to prepare these thermally labile species gives rise to unfragmented parent molecules of the DNA bases. Furthermore, SPI in which intermediate levels are not required and the ion state can be accessed directly provides for a convenient way to prepare stable cations that could be interrogated further with sophisticated two-color schemes. Variations of IR-UV double resonance spectroscopies, in particular mass selected, resonant ion-dip IR spectroscopy, and IR-photoinduced Rydberg ionization techniques demonstrated so elegantly by the groups of de Vries,[31] Zwier,[40] and most recently by Ng,[41] could be used to probe the structural dynamics of various conformers and their clusters, utilizing our simple and convenient thermal source. A caveat of course is that high repetition rate and/or cw laser systems will be necessary to take advantage of the continuous nature of the source. Attempts toward this have been demonstrated recently utilizing both IR[42] and visible[43] lasers coupled to a synchrotron.

Conclusion

In this work, we have carried out a systematic investigation of the PIMS of the four DNA bases (guanine, cytosine, adenine, and thymine) and their complexes with water, utilizing tunable VUV radiation coupled to a jet-cooled thermal vaporization source. PIE curves are recorded for the DNA bases, dimers, and the following water (W) clusters: G, GW_n (n = 1,2,3); CW_n (n = 1−3); A, AW_n (n = 1−2); and T, TW_n (n = 1−3). AEs are derived from the onset of these PIE curves (all energies in eV): G (8.1 ± 0.1), GW (8.0 ± 0.1), GW_2 (8.0 ± 0.1), and GW_3 (8.0); C (8.65 ± 0.05), CW (8.45 ± 0.05), CW_2 (8.4 ± 0.1), and CW_3 (8.3 ± 0.1); A (8.30 ± 0.05), AW (8.20 ± 0.05), and AW_2 (8.1 ± 0.1); T (8.90 ± 0.05); and TW (8.75 ± 0.05), TW_2 (8.6 ± 0.1), and TW_3 (8.6 ± 0.1). The AEs of the DNA bases decrease slightly with the addition of water molecules (up to three) but do not converge to values found for photoinduced electron removal from DNA bases in solution.

Acknowledgment. This paper is dedicated to the memory of Roger Miller, a great scientist and an inspiration to his field. This work was supported by the Director, Office of Energy Research, Office of Basic Energy Sciences, Chemical Sciences Division of the U.S. Department of Energy, under contract no. DE-AC02-05CH11231. M.A. acknowledges support from the

Laboratory Directed Research and Development (LDRD) program at LBNL.

References and Notes

(1) Weinkauf, R.; Schermann, J. P.; de Vries, M. S.; Kleinermanns, K. *Eur. Phys. J. D* **2002**, *20*, 309.
(2) Hall, D. B.; Holmlin, R. E.; Barton, J. K. *Nature* **1996**, *382*, 731.
(3) Giese, B. *Acc. Chem. Res.* **2000**, *33*, 631.
(4) Endres, R. G.; Cox, D. L.; Singh, R. R. P. *Rev. Mod. Phys.* **2004**, *76*, 195.
(5) Kim, S. K.; Lee, W.; Herschbach, D. R. *J. Phys. Chem.* **1996**, *100*, 7933.
(6) Schiedt, J.; Weinkauf, R.; Neumark, D. M.; Schlag, E. W. *Chem. Phys.* **1998**, *239*, 511.
(7) Crews, B.; Abo-Riziq, A.; Grace, L.; Callahan, M.; Kabelac, M.; Hobza, P.; de Vries, M. S. *Phys. Chem. Chem. Phys.* **2005**, *7*, 3015.
(8) Abo-Riziq, A.; Crews, B.; Grace, L.; de Vries, M. S. *J. Am. Chem. Soc.* **2005**, *127*, 2374.
(9) Piuzzi, F.; Mons, M.; Dimicoli, I.; Tardivel, B.; Zhao, Q. *Chem. Phys.* **2001**, *270*, 205.
(10) Kim, N. J.; Kim, Y. S.; Jeong, G.; Ahn, T. K.; Kim, S. K. *Int. J. Mass Spectrom.* **2002**, *219*, 11.
(11) Kim, N. J.; Kang, H.; Jeong, G.; Kim, Y. S.; Lee, K. T.; Kim, S. K. *J. Phys. Chem. A* **2000**, *104*, 6552.
(12) Kang, H.; Lee, K. T.; Kim, S. K. *Chem. Phys. Lett.* **2002**, *359*, 213.
(13) Cauet, E.; Dehareng, D.; Lievin, J. *J. Phys. Chem. A* **2006**, *110*, 9200.
(14) Roca-Sanjuan, D.; Rubio, M.; Merchan, M.; Serrano-Andres, L. *J. Chem. Phys.* **2006**, *125*, 084302.
(15) Hanus, M.; Ryjacek, F.; Kabelac, M.; Kubar, T.; Bogdan, T. V.; Trygubenko, S. A.; Hobza, P. *J. Am. Chem. Soc.* **2003**, *125*, 7678.
(16) Close, D. M.; Crespo-Hernandez, C. E.; Gorb, L.; Leszczynski, J. *J. Phys. Chem. A* **2006**, *110*, 7485.
(17) Hanus, M.; Kabelac, M.; Rejnek, J.; Ryjacek, F.; Hobza, P. *J. Phys. Chem. B* **2004**, *108*, 2087.
(18) Hush, N. S.; Cheung, A. S. *Chem. Phys. Lett.* **1975**, *34*, 11.
(19) Choi, K. W.; Lee, J. H.; Kim, S. K. *J. Am. Chem. Soc.* **2005**, *127*, 15674.
(20) Jochims, H. W.; Schwell, M.; Baumgartel, H.; Leach, S. *Chem. Phys.* **2005**, *314*, 263.
(21) Heimann, P. A.; Koike, M.; Hsu, C. W.; Blank, D.; Yang, X. M.; Suits, A. G.; Lee, Y. T.; Evans, M.; Ng, C. Y.; Flaim, C.; Padmore, H. A. *Rev. Sci. Instrum.* **1997**, *68*, 1945.
(22) Nicolas, C.; Shu, J. N.; Peterka, D. S.; Hochlaf, M.; Poisson, L.; Leone, S. R.; Ahmed, M. *J. Am. Chem. Soc.* **2006**, *128*, 220.
(23) Amirav, A.; Even, U.; Jortner, J. *Chem. Phys.* **1980**, *51*, 31.
(24) Choi, K. W.; Lee, J. H.; Kim, S. K. *Chem. Commun.* **2006**, 78.
(25) Saigusa, H.; Tomioka, A.; Katayama, T.; Iwase, E. *Chem. Phys. Lett.* **2006**, *418*, 119.
(26) Trofimov, A. B.; Schirmer, J.; Kobychev, V. B.; Potts, A. W.; Holland, D. M. P.; Karlsson, L. *J. Phys. B.: At., Mol. Opt. Phys.* **2006**, *39*, 305.
(27) Nir, E.; Plutzer, C.; Kleinermanns, K.; de Vries, M. *Eur. Phys. J. D* **2002**, *20*, 317.
(28) Plutzer, C.; Kleinermanns, K. *Phys. Chem. Chem. Phys.* **2002**, *4*, 4877.
(29) Guerra, C. F.; Bickelhaupt, F. M.; Saha, S.; Wang, F. *J. Phys. Chem. A* **2006**, *110*, 4012.
(30) Mons, M.; Dimicoli, I.; Piuzzi, F. *Int. Rev. Phys. Chem.* **2002**, *21*, 101. Chin, W.; Mons, M.; Dimicoli, I.; Piuzzi, F.; Tardivel, B.; Elhanine, M. *Eur. Phys. J. D* **2002**, *20*, 347.
(31) Nir, E.; Janzen, C.; Imhof, P.; Kleinermanns, K.; de Vries, M. S. *J. Chem. Phys.* **2001**, *115*, 4604.
(32) Mons, M.; Piuzzi, F.; Dimicoli, I.; Gorb, L.; Leszczynski, J. *J. Phys. Chem. A* **2006**, *110*, 10921.
(33) Elshakre, M. *Int. J. Quantum Chem.* **2005**, *104*, 1.
(34) Nir, E.; Muller, M.; Grace, L. I.; de Vries, M. S. *Chem. Phys. Lett.* **2002**, *355*, 59.
(35) Yang, Z. B.; Rodgers, M. T. *Phys. Chem. Chem. Phys.* **2004**, *6*, 2749.
(36) Danilov, V. I.; van Mourik, T.; Poltev, V. I. *Chem. Phys. Lett.* **2006**, *429*, 255.
(37) Crespo-Hernandez, C. E.; Arce, R. *Photochem. Photobiol.* **2002**, *76*, 259.
(38) Fernando, H.; Papadantonakis, G. A.; Kim, N. S.; LeBreton, P. R. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 5550.
(39) Crespo-Hernandez, C. E.; Arce, R.; Ishikawa, Y.; Gorb, L.; Leszczynski, J.; Close, D. M. *J. Phys. Chem. A* **2004**, *108*, 6373.
(40) Zwier, T. S. *J. Phys. Chem. A* **2006**, *110*, 4133.
(41) Ng, C. Y. *J. Electron Spectrosc. Relat. Phenom.* **2005**, *142*, 179.
(42) Qian, X. M.; Zhang, T.; Ng, C. Y.; Kung, A. H.; Ahmed, M. *Rev. Sci. Instrum.* **2003**, *74*, 2784.
(43) Plenge, J.; Nicolas, C.; Caster, A. G.; Ahmed, M.; Leone, S. R. *J. Chem. Phys.* **2006**, *125*, 133315.