Anomalous Fragmentation of Hydrated Clusters of DNA Base Adenine in UV Photoionization

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A multiphoton ionization study was carried out on hydrated clusters of DNA base adenine in supersonic molecular beams. Resonant two-photon ionization at 266 nm showed that the relative ion intensity between the hydrated clusters $A_m(H_2O)_n$ and the unhydrated ones was anomalously small, particularly for $m = 1$, in contrast to the case of electron impact ionization. The ratio was of the order of 10^{-2} for $m = 1$, 10^{-1} for $m = 2$, but about 1 for $m \geq 3$. One-photon excitation to the first electronically excited state was found to be responsible for the extensive fragmentation of adenine monomer hydrates $A_1(H_2O)_n$. The water acts as a proton-donating solvent whose hydrogen bonding with the solute becomes weakened in the $n\pi^*$ excited state, thereby giving the excited state its repulsive nature. Hydrates of adenine complex $A_m(H_2O)_n$ ($m \geq 2$) tend to better survive the rupture of the water cage at higher m, probably because the energy transfer between adenine molecules is not sufficiently fast. The fragmentation was found to be less extensive at higher levels of excitation with a much weaker $n\pi^*$ character. Change of solvent to those of less proton-donating or even proton-accepting character systematically reduced the tendency of fragmentation.

1. Introduction

The four bases of DNA are chromophores of DNA, and their complementary pairing provides means to store and replicate genetic information in the double helix structure of DNA. The role of water molecules surrounding nucleic acid in an aqueous environment is crucial in determining the structure and function of this important molecule.[1] Interaction between water and nucleic acid is mainly responsible for stabilization of macromolecular structures. For example, the electrostatic repulsion between phosphate groups is often affected by the high dielectric constant of water. The degree of hydration of DNA also plays a key role in its conformational change among its various polymorphic forms.

Despite the importance of DNA bases, molecular beam studies are rather limited. Some spectroscopic studies include electronic spectra of uracil, thymine, and guanine,[2] photoelectron spectroscopy of uracil, thymine, cytosine anions, and their water complexes,[3,4] Rydberg electron-transfer spectroscopy of uracil, thymine, and their *N*-methylated derivatives,[5] and femtosecond analysis of tautomerization dynamics in model base pairs (7-azaindole dimers).[6] Other studies also include laser separation of geometrical isomers of DNA base pairs,[7] base pair formation of free nucleobases and mononucleosides,[8] and hydration of nucleic acid bases and threshold ionization potential measurement using electron impact ionization.[9] Matrix isolation FT-IR studies and theoretical calculations were also carried out on hydrated clusters of base molecules modeling adenine, cytosine, and isocytosine tautomers,[10] and the heat of formation for DNA base−water complexes was estimated in a theoretical study.[11]

Study of weakly bound clusters in a supersonic molecular beam fills the gap between isolated molecular state and the statistically averaged bulk state of matter. The object of such studies is to track the evolution from an individual molecule to bulk with increasing cluster size. In particular, the solvation study of hydrogen-bonded clusters has been an active area of research in recent years because of growing interest in chemistry occurring in aqueous medium.

In this study, we investigated hydrated clusters of adenine using the resonant 2-photon ionization (R2PI) technique. A striking anomaly was found in the mass spectrum, where virtually no adenine monomer hydrates could be observed despite an intense search. Such near complete loss of ion signals for hydrated clusters was also observed in the past by Wanna et al. for the case of pyrazine and pyrimidine.[12] They attributed this anomaly to the increased rate of internal energy relaxation upon solvation, which makes it difficult for resonant ionization to occur. In this case, the clusters were believed to be still there, but just not ionized to be detected. On the other hand, they also suggested the possibility of actual loss of clusters because of a dissociative $n\pi^*$ excited state for the hydrated clusters of these molecules.

In our study, we came to prove that the near loss of ion signals for hydrated clusters was due more to extensive fragmentation at the electronically excited state than other mechanisms suggested so far or considered likely. Such fragmentation results from the increased repulsive nature of the *inter*molecular potential in the $n\pi^*$ excited state upon solvation by proton-donating solvents such as water. A strong vibronic coupling between the $n\pi^*$ and the $\pi\pi^*$ excited states was assumed to be at play. The effect of excitation energy and the proton-donating or proton-accepting capability of solvent was systematically studied. The partial survival of hydrated clusters of adenine dimer or larger complexes of adenine was explained by the slow rate of energy transfer between adenine molecules.

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2. Experimental Section

The experiments were carried out in a molecular beam machine with a time-of-flight mass spectrometer (TOF-MS) using R2PI or electron impact as the ionization method. The apparatus consisted of three chambers. The source and the buffer chambers were respectively pumped by a 10-in. and a 6-in. diffusion pump, while the detector chamber housing the TOF-MS was pumped by a 2-in. diffusion pump and a liquid nitrogen trap. With a skimmer of 1 mm hole size situated between the source and buffer chambers, typical operating pressures were 2×10^{-5}, 3×10^{-6}, and 5×10^{-7} Torr in the source, buffer, and detector chambers, respectively, when the pulsed nozzle was operated at 10 Hz under the helium pressure of 2 atm.

A commercial inline filter (Nupro, SS-4F-7) located just behind the pulsed valve was used as a source oven without its porous filter element installed. The oven and the pulsed valve were independently heated by resistive heating. Generally, the oven temperature was maintained at 240−250 °C in order to keep the vapor pressure of adenine at a few hundred milliTorr without the danger of pyrolysis, and the nozzle temperature at 255 °C. To run the nozzle at such temperatures, the coil assembly of a commercial solenoid valve (General Valve Series 9, hole size 0.5 mm) was rewound using Teflon insulated copper wire (Omega Engineering Inc., TFCP-005). A homemade pulsed nozzle driver controlled the pulse width (typically 500−700 μs) of the nozzle and was triggered by a pulse/delay generator (Stanford Research System Inc., DG 535). Since the adenine vapor is highly condensable, the skimmer tends to get clogged easily over a short period of time. This was prevented by resistively heating the skimmer base plate above 100 °C. Water vapor was introduced into the source when producing hydrated clusters by attaching a water bottle directly to the sample oven through a $^1/_4$ in. stainless steel tube to increase the flow rate of water and also to prevent condensation of water vapor in the gas line.

The TOF-MS was of the Wiley−McLaren type[13] and had a 1.1-m flight length. The molecular beam axis, TOF-MS axis, and laser propagation direction were all mutually orthogonal. The TOF-MS had an einzel lens to focus all ions, irrespective of their mass, onto the ion detector. This enabled us to obtain a mass spectrum over a wide mass range without much variation in ion detection efficiency. Laser pulses for excitation and ionization were provided by either the direct harmonic (355 or 266 nm) of an Nd:YAG laser (Spectra Physics, GCR-16S; 6 ns pulse width) or a frequency doubled output of a pulsed dye laser (Spectra Physics, PDL-3) pumped by the second or third harmonic of the Nd:YAG laser. Output of the dye laser was frequency-doubled by a homemade frequency doubler using a BBO crystal. The frequency was tuned by tilting the crystal angle according to the predetermined wavelength vs angle curve. A pulse/delay generator was used to synchronize laser firing with pulsed nozzle opening. The maximum mass resolution $M/\Delta M$ of our TOF-MS with a tightly focused light is estimated to be ca. 300.

A fast microchannel plate (Galileo Electro-Optics Corp., FTD 2003) was used as an ion detector. The output of the microchannel plate detector was fed into a preamplifier (EG & G Ortec, 9305) or amplified directly by a NIM module amplifier (EG & G Ortec, 574). The amplified signal was digitized and stored by a 400 MHz digital storage oscilloscope (LeCroy, 9310A). Adenine was purchased from Aldrich Chemical Co. and used without further purification.

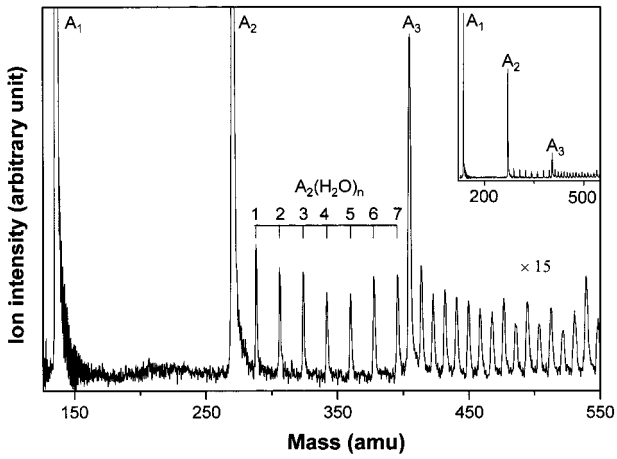

Figure 1. One-color R2PI mass spectrum of hydrated adenine clusters, $A_m(H_2O)_n$, with 266 nm photons. Virtually no ion signal was detected for hydrated clusters of the adenine monomer. The numbers represent the number of water molecules attached to the adenine dimer. The inset shows overall relative ion intensities.

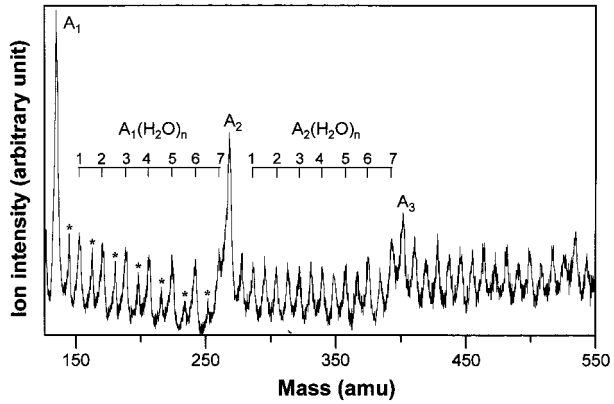

Figure 2. Electron impact ionization mass spectrum of hydrated adenine clusters at 40 eV of electron energy. The relative intensities between hydrated clusters and bare ones are much larger than in the case of R2PI. Peaks denoted by an asterisk represent neat water clusters ionized by electron impact.

3. Results

Near-Absence of Adenine Monomer Hydrate Signal in Mass Spectra. Figure 1 shows an R2PI TOF mass spectrum of hydrated adenine clusters generated by jet expansion under a backing pressure of 2 atm helium and 100 Torr water. The fourth harmonic of the Nd:YAG laser (266 nm = 4.66 eV) was used to ionize the clusters at a laser fluence of ca. 2 MW/cm^2. The spectrum in the inset shows overall relative peak intensities. Upon close examination, we found that the unhydrated "monomer" peak consists of a molecular adenine ion (A^+) peak as well as a protonated adenine ion (AH^+) peak. The protonated ion peak was also observed in the electron impact ionization study of adenine− and thymine−water complexes[9] and in the field ionization study of hydrated clusters of DNA base derivatives.[14] The protonated ion is readily formed due to the efficient dissociative photoionization and the high proton affinity of DNA bases in general.[15]

Perhaps the most striking feature of the spectrum in Figure 1 is the near complete absence of adenine monomer hydrates $A_1(H_2O)_n^+$. This is in stark contrast with the mass spectrum obtained by the electron impact ionization method, as shown in Figure 2. The ratio of ion intensities between the hydrated clusters $A_m(H_2O)_n$ and the unhydrated ones A_m in the photoionization spectrum is of the order of 10^{-2} or less for $m = 1$,

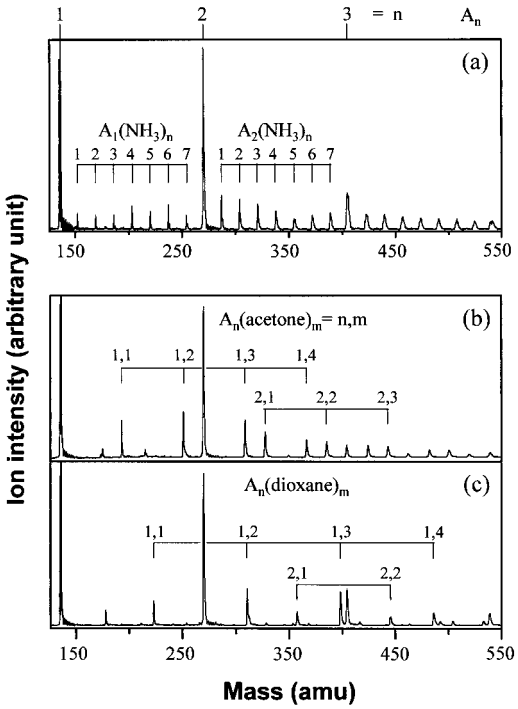

Figure 3. (a) One-color R2PI mass spectrum of ammoniated adenine clusters, $A_m(NH_3)_n$. The partial pressure of ammonia was ca. 10% of the total pressure. In contrast to the case of water as solvent, solvated adenine monomers show significant intensities. $A_1(NH_3)_{n+8}^+$ happens to have a mass only 1 amu larger than $A_2(NH_3)_n^+$, but appears indistinguishable here because of the insufficient mass resolution. (b) The same spectrum with acetone as solvent, and (c) with dioxane as solvent. The value of *n* and *m*, respectively, represents the number of adenine and the solvent molecule. Both show even larger ion intensities for the solvated adenine monomers than the solvated dimers, in stark contrast to the cases of water or $CHCl_3$.

10^{-1} for $m = 2$, and about 1 for $m \geq 3$. By comparison, the same ratio from the electron impact ionization spectrum never falls below 10^{-1} even for $m = 1$ (Figure 2). Although the relative intensities do vary somewhat depending upon the experimental conditions of photoionization, the intensity of the monomer hydrates $A_1(H_2O)_n$ always remains about 1 order of magnitude or 2 smaller than that of the hydrates of adenine complexes $A_m(H_2O)_n$ ($m \geq 2$). The large ion intensities of adenine monomer hydrates obtained by direct ionization with electron impact suggest that there seem to be a large number of these clusters initially produced. Therefore, the striking loss of ion signals as in Figure 1 by R2PI is believed to result from a loss in the ionization step or in the excitation step. Possibilities include reduced ionization efficiency of the hydrated clusters or actual loss of clusters by processes such as dissociation. Whatever the cause, the loss becomes less severe in the hydrated clusters of adenine complexes, and progressively so as they become larger in size.

Solvent Dependence of Cluster Ion Intensity Distribution. Various solvents were employed to determine whether there exists any dependence of cluster ion intensity distributions on the type of solvent. First of all, solvation by ammonia gave a rather different result. Figure 3a shows that the ammoniated clusters of adenine monomer, $A_1(NH_3)_n^+$, were detected with a comparable intensity to those of the adenine dimer, $A_2(NH_3)_n^+$. Complexes of adenine solvated by various other kinds of solvent molecules were also generated and the mass distributions were investigated. For example, with a solvent such as acetone or dioxane, $A_1(solvent)_n^+$ had even a larger intensity than $A_2(solvent)_n^+$ (Figure 3b,c). In the case of $CHCl_3$, however, the cluster mass spectrum turned out to be much like the case of water (not shown). This suggests that the near loss of hydrated adenine monomers is likely due to the specific nature of the base−water interaction, and in particular, to the hydrogen bonding. We would like to point out that for solvent molecules acting as proton donor in hydrogen bonding, such as $CHCl_3$ or H_2O, little ion signals are detected for $A_1(solvent)_n^+$, while for those acting as proton acceptor, such as acetone, dioxane, and NH_3, no such anomaly was observed. Table 1 gives some physical constants for the solvents used in this study, and summarizes their role in hydrogen bonding. Note that both H_2O and NH_3 are amphoteric, i.e. can act as both proton donor or acceptor, in hydrogen bonding, but the latter is a far stronger proton acceptor than the former, as seen by the much larger gas phase basicity. There appears to be a correlation between whether a given solvent generally acts as proton donor in hydrogen bonding and whether the cluster of adenine monomer with that solvent is observed in this study.

TABLE 1: Physical Properties of $CHCl_3$, H_2O, NH_3, Acetone, and Dioxane

solvent	dipole moment (D)	gas phase basicity[a] (kcal/mol)	role in H bonding	A_1:(solvent)$_n$ obsd
$CHCl_3$	1.04		proton donor	N
H_2O	1.85	159	proton donor (and acceptor)	N
NH_3	1.47	195.6	proton (donor and) acceptor	Y
acetone	2.88	188.9	proton acceptor	Y
dioxane	0	186	proton acceptor	Y

[a] Reference 34.

Excitation Wavelength Dependence of Cluster Ion Intensity Distributions. To see if the loss of hydrated adenine monomer depends on the excitation energy, we changed the wavelength of the laser over the range of 210−290 nm (5.90−4.28 eV). Since the vertical and adiabatic ionization energies of adenine are 8.48[16] and 7.8 eV,[17] respectively, the 2-photon energy of 290 nm (2×4.28 eV $= 8.56$ eV) is still large enough to ionize the molecule by one-color R2PI. The ion signal becomes too weak to be detected below 290 nm because the first electronically excited state lies above this energy. The extensive loss of ion signals for $A_1(H_2O)_n$ was observed at wavelengths between 250 and 290 nm (4.96−4.28 eV), but it became gradually less severe at wavelengths shorter than 250 nm. Eventually, we found comparable ion intensities between $A_1(H_2O)_n$ and $A_2(H_2O)_n$ at wavelengths between 210 and 230 nm (5.90−5.39 eV), as shown in Figure 4a. We also tried to use an even higher excitation energy by employing a $(2 + 1)$ resonance-enhanced multiphoton ionization scheme using the 355 nm (3.50 eV) photons. At the nominal excitation wavelength of 177.5 nm (2×3.50 eV $= 7.00$ eV) in this case, the ion intensity of $A_1(H_2O)_n$ became even larger than that of $A_2(H_2O)_n$ (Figure 4b). In summary, the wavelength range for the near complete loss of hydrated adenine monomer appears to be mainly confined between 250 and 290 nm. The electronic state reached by one-photon excitation at these wavelengths seems to result in very efficient loss of the ion signals for $A_1(H_2O)_n$. There are a few likely candidates for such a process, as will be discussed later in more detail, but the most likely one is fragmentation of the cluster in the excited state.

Femtosecond Excitation/Ionization of Hydrated Adenine Clusters. The above conclusion is further supported by the following investigation using a femtosecond laser system. A 267 nm (4.64 eV) femtosecond laser pulse generated from a

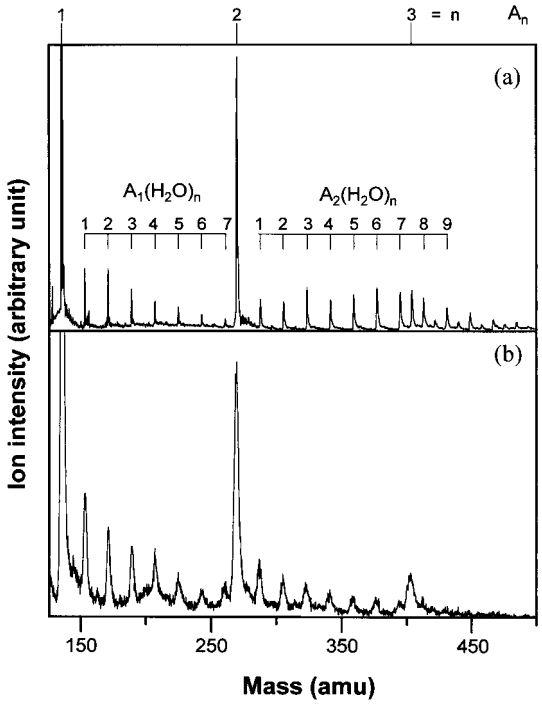

Figure 4. Mass spectrum of hydrated adenine clusters, (a) with 220 nm photons in R2PI and (b) with 355 nm photons in (2 + 1) multiphoton ionization scheme. The intensities of hydrated monomers are comparable to, or even larger than, those of hydrated dimers.

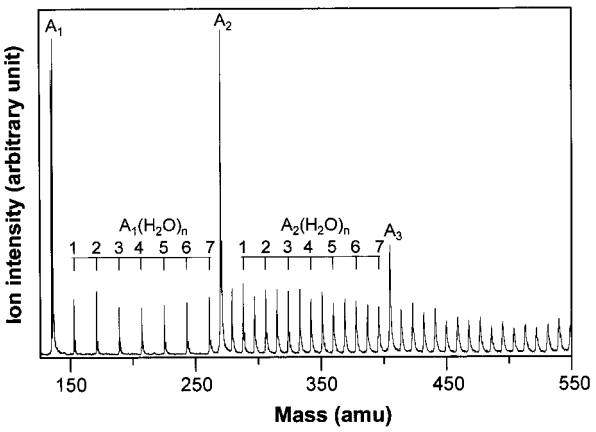

Figure 5. One-color R2PI mass spectrum of hydrated adenine clusters obtained by a 400 fs laser pulse at 267 nm. The relative ion intensities between hydrated adenines and bare ones are now generally comparable to those obtained by electron impact ionization (Figure 2).

regeneratively amplified Ti:sapphire femtosecond laser system was used in the same R2PI scheme as before. As Figure 5 shows, however, no conspicuous loss of $A_1(H_2O)_n$ was observed. Furthermore, the relative ion intensity between hydrated clusters $A_m(H_2O)_n$ and unhydrated ones A_m is now quite comparable to that observed by the electron impact ionization. It appears that the fragmentation process in question occurs on a time scale comparable to, or even somewhat longer than, the laser pulse width (~400 fs) in the excited state so that a significant fraction of clusters becomes ionized without having sufficient time to undergo fragmentation. With a nanosecond pulse, however, ionization of intact clusters is rare since extensive fragmentation takes place before the cluster becomes ionized by the second photon. This leads to near complete loss of ion signals for hydrated adenine monomer $A_1(H_2O)_n^+$ ions. Hydrated clusters of adenine complexes $A_m(H_2O)_n$ ($m \geq 2$) appear to better survive the fragmentation as m becomes larger.

4. Discussion

The ultimate question is what causes the loss of ion signal for the adenine monomer hydrates. Wanna et al.[12] suggested two possibilities for their inability to detect hydrated pyrimidine cluster ions in the mass spectrum. One is the possibility that the internal conversion (IC) or the intersystem crossing (ISC) rate becomes faster as the molecule becomes more hydrated since it comes to have more internal degrees of freedom, which shortens the lifetime of the electronically excited state. This will force the molecular system to undergo rapid relaxation so that the second photon cannot bring the system to the ionization level. The other possibility is that the potential energy surface of the $n-\pi^*$ transition of the hydrated cluster is repulsive.

As for the first possibility, if the faster IC or ISC rate was the reason for the small ion intensity of $A_1(H_2O)_n$ when adenine gets hydrated, the rate of relaxation in adenine dimer hydrates would be even faster than in adenine monomer hydrates, since the former has more internal degrees of freedom. This follows from general observation that the main factor governing the IC or ISC rate is the density of states coupled to the initially prepared level.[18] Upon forming a cluster, low-frequency intermolecular vibrations greatly increase the density of states, and hence more efficient IC or ISC generally results. Therefore, in this model, the decay rate of the dimer hydrates must be faster than that of the monomer hydrates, which implies that the dimer hydrates would be even harder to ionize than the monomer hydrates. Of course, this is quite the opposite of what is actually observed in our experiment.

With regard to the second possibility, we note that the following body of information can provide a clue. First of all, it was established by Brealey and Kasha[19] that the hydrogen bonding plays a role in the blue shift of the $n-\pi^*$ transition. Pimentel[20] pointed out the importance of the Franck−Condon principle applied to the intermolecular potential curve of the hydrogen bond. Krishna and Goodman[21] found that the hydrogen bond for pyrazine and pyrimidine either does not exist or is very weak in the triplet $n\pi^*$ states. Baba et al.[22] also reported that the hydrogen bonding of diazine in methanol or water is dissociative in the singlet $n\pi^*$ states. As for the hydrated adenine clusters, Del Bene[23] postulated that the hydrogen bond is destabilized by the $n-\pi^*$ transition, although she predicted rather small destabilization in the $n-\pi^*$ bands of the adenine−water complex.

In the $n-\pi^*$ transition, one of the localized nonbonding electrons goes into the delocalized antibonding π^* orbital so that the dipole moment of the excited state is greatly reduced. For example, the dipole moment of pyridazine and pyrimidine was found to be decreased by 2.84 and 2.72 D, respectively, upon $n-\pi^*$ transition.[22] With such reduction in dipole moment, the solute−solvent interaction generally becomes weaker. In addition, with the migration of electronic charge away from the nonbonding orbital, the effective bond order for the hydrogen bond becomes reduced as well. Therefore, the hydrogen-bonded configuration in the ground-state cannot be retained in the excited state. As a result, a Franck−Condon transition will bring the system to an unstable configuration in the excited state and lead to dissociation.[20] In other words, a decreased intermolecular bond order in the $n\pi^*$ excited state in effect brings in a more repulsive character to the potential energy surface. A Franck−Condon transition will then force the system to dissociate, as schematically represented in Figure 6.

The above argument goes parallel with the well-known trend of frequency shift vs the role of solvent in hydrogen bonding.[20] In an $n-\pi^*$ transition, reduction in dipole moment and

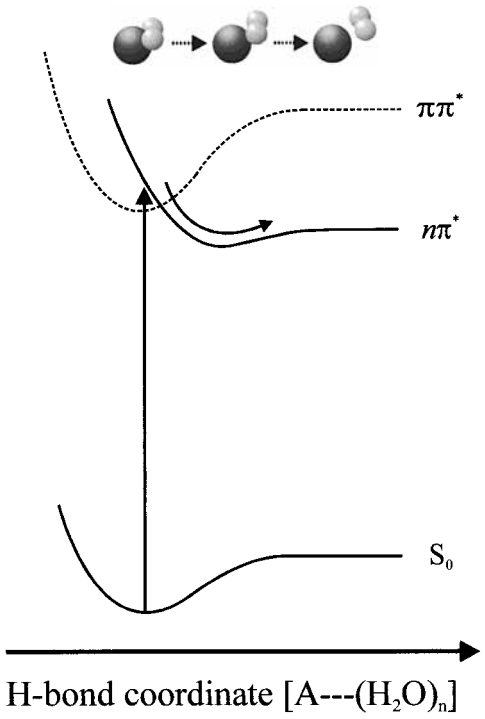

Figure 6. Schematic diagram of the potential energy surface along the adenine−water coordinate. The first absorption band of adenine consists of strong $\pi-\pi^*$ transitions and a weak $n-\pi^*$ transition. Upon hydration, however, the $n-\pi^*$ transition becomes blue-shifted and the $\pi-\pi^*$ transitions red-shifted. A strong vibronic coupling results, and the intrinsically weak $n-\pi^*$ transition now derives its oscillator strength from the strong $\pi-\pi^*$ transition. On the other hand, a proton donor such as water suffers reduction in intermolecular bond order by transitions of the $n-\pi^*$ type because of the removal of electronic charge from the nonbonding orbital. Therefore, the potential energy surface becomes more repulsive in the excited state, with a shallower minimum displaced farther out. A Franck−Condon transition will then bring the system to the repulsive wall in the excited state, which will lead to dissociation.

weakening of the hydrogen bond result in a blue shift of the transition when the solute molecule is solvated by proton donors. When solvated by proton acceptors, however, the molecule is little affected in its optical transition because its hydrogen-donating capability is virtually unaffected by the $n-\pi^*$ transition. On the other hand, in a $\pi-\pi^*$ transition, the solvation effect of proton donors is to cause a red shift, although the effect is not as great as in the $n-\pi^*$ transition case.

The observed dependence of the present anomaly on various solvent types seems to indicate that the electronic transition responsible for such an outcome is of the $n-\pi^*$ type: proton donors such as $CHCl_3$ or H_2O leading to a "blue shift" and thus dissociation, while no such loss being observed for proton accepting solvents such as acetone, dioxane, and NH_3. This is somewhat surprising since the first absorption band of bare adenine at 252 nm (4.92 eV)[24] is known to comprise two perpendicularly polarized π-π^* transitions.[25] But it is also suggested that this band contains a weak n-π^* transition as well.[26,27] Due to a small oscillator strength of the n-π^* transition, however, only a few experimental observations have been made so far in solution[26] or in a single crystal.[27] Furthermore, the relative location of the lowest $n\pi^*$ and $\pi\pi^*$ state is still quite uncertain, despite extensive theoretical studies. Lipiński,[28] Hug and Tinoco,[29] and Broo[30] found the $n\pi^*$ state to be located between the two lowest $\pi\pi^*$ states, but Danilov et al.[31] suggested that the $n\pi^*$ state should be the lowest singlet state

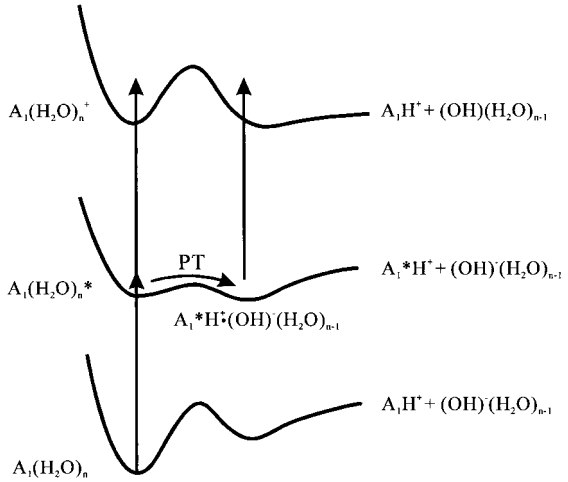

Figure 7. Schematic diagram of the potential energy surfaces for proton transfer in the excited state. One-photon excitation followed by proton transfer (denoted by the arrow marked with "PT") brings the system to an ion pair state. Photodetachment of the electron from the anionic moiety by the second photon yields protonated adenine or its complexes.

of adenine. On the other hand, they all agree that the energy difference between the $n\pi^*$ and the $\pi\pi^*$ states is small (0.1 ∼ 0.2 eV). With such a small energy difference, significant vibronic coupling is expected between the $n\pi^*$ and the $\pi\pi^*$ states. The intrinsically weak oscillator strength of the $n-\pi^*$ transition may be significantly increased by the vibronic coupling with the strongly allowed $\pi-\pi^*$ transition. Broo[30] suggested that the vibronic coupling in the lowest excited state of adenine gave rise to different emission properties between the two isomers of adenine and 2-aminopurine. Such vibronic coupling should be at play in hydrated clusters of adenine as well. In this case, we also note that the effect of hydration would be to blue shift the $n-\pi^*$ transition and to red shift the $\pi-\pi^*$ transition, as schematically shown in Figure 6. Therefore, if in fact the $n\pi^*$ state were indeed the lowest state, then the effect of hydration would be to cause an even larger degree of vibronic coupling by reducing the energy gap between these states.

The near complete loss of ion signal for $A_1(H_2O)_n^+$ appears to be a property related mainly to the lowest absorption band of adenine. We note that excitation to higher electronic states with shorter wavelengths does not lead to such results. As seen in Figure 4a, excitation to the second absorption band (centered around 207 nm, 5.99 eV)[24] of adenine yields comparable intensities between $A_1(H_2O)_n^+$ and $A_2(H_2O)_n^+$. Excitation to an even higher level by 2-photon absorption at 355 nm gives even significantly larger ion intensities for $A_1(H_2O)_n^+$ than for $A_2(H_2O)_n^+$ (Figure 4b). These results are apparently due to the fact that the high-lying levels have significantly different electronic characters, presumably with a much weaker $n\pi^*$ component.

As still another candidate for the ion loss mechanism, we examined the possibility of proton transfer from water to the base in its excited state. As schematically shown in Figure 7, such proton transfer produces an ion pair, and an electron is photodetached from the anion moiety by the second photon in its "ionization" step. This would not only cause extensive loss of hydrated clusters but also produce a lot of protonated bases, in apparent accord with our mass spectra. The problem with this model is, however, the thermodynamics of proton transfer is unfavorable in the case of hydrated adenine. We calculated an endothermicity of about 70 kcal/mol (3 eV) from a simple estimation method commonly used.[32] Such large endothermicity is virtually insurmountable even without a barrier.

There are a few apparent problems with our proposed mechanism: (1) It may explain why and how the cluster loses a water solvent, but it does not explain why it loses all the water. (2) It does not explain why hydrates of adenine complexes seem to better survive the fragmentation. We address these issues briefly. First of all, the total loss of water can be easily explained if the water−water interaction is large and comparable to the adenine−water interaction at favorable solvation sites. In this case, there is a high probability that breakage of an intermolecular bond at merely a site or two leads to extensive loss of the water cage. On the other hand, if each additional water occupies successively the most favorable site, such total loss of water would not likely occur. We have done some preliminary ab initio calculations on a model hydrated adenine cluster $A_1(H_2O)_2$, which indicates that the two water molecules bind cooperatively to adenine, rather than find their own individual binding sites. A recent semiempirical calculation also showed that up to three water molecules bind cooperatively to neutral adenine, in contrast to the case of adenine anion.[33]

The second issue of adenine complex hydrates better surviving the fragmentation can be related to the rate of electronic energy transfer within the cluster system. Let's assume that one of the adenine molecules in the cluster is locally excited. Then the water molecules directly attached to that particular adenine will suffer from dissociation, but others will mostly remain intact. There are two pathways to ionize this cluster. One is to directly ionize the newly produced unhydrated adenine moiety and the other is to ionize the other adenine after electronic energy transfer from the originally excited one. If the electronic energy is transferred from the initially excited adenine to the other adenine in a hydrated dimer, for example, the water bound to the latter will be also dissociated, and A_2^+ will mostly result. Since the experimentally observed ion intensities are generally much smaller than those obtained by electron impact ionization, we believe that the energy transfer is quite rapid, with the consequence of a considerable amount of dissociation. On the other hand, the ion intensity of the $A_m(H_2O)_n^+$ ($m \geq 2$) species is still quite significant, particularly when compared to that of $A_1(H_2O)_n^+$, implying that the energy transfer is not fast enough to destroy all water cages entirely. A time-resolved study in the ultrafast time scale will reveal the difference in the dissociation dynamics between adenine monomer hydrates and the dimer hydrates more clearly. The work is under progress in our laboratory with a femtosecond laser system.

5. Conclusion

We performed a photoionization study on the DNA base adenine and its solvated clusters with various solvent molecules. Anomalously small ion intensities for the adenine monomer hydrates were observed in the TOF mass spectrum, which was attributed to the dissociative nature of the $n-\pi^*$ transition to the first excited level in hydrated adenine. Solvents of proton donor character were found to lead to such anomaly, since they induce a "blue shift" of the transition, with a decreased bond order for the intermolecular bond. The culprit for the near complete loss of ion signals was found not to be rapid internal conversion or intersystem crossing, as previously known, but the increase in the repulsive nature of the excited state. It was deduced that the first excited state of adenine monomer hydrates has a significant $n\pi^*$ character through efficient vibronic coupling with the $\pi\pi^*$ state. The rate of intracluster electronic energy transfer among adenine moieties was suggested to affect the occurrence of the observed anomaly in the hydrates of adenine complexes.

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