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Hydration of DNA base cations in the gas phase

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Abstract

Hydration of the adenine and thymine cations was studied in the gas phase. Metastable fragmentation of the cluster ions was used to draw information on the relative strengths of the binding energy of each additional solvent molecule. Both cations of adenine and thymine exhibited a well-defined hydration shell structure, with the first hydration shell complete with four water molecules. On the other hand, the hydration shell structure for the cation dimer of adenine was less evident, but appeared to require seven or eight water molecules for the first hydration shell. An ab initio calculation was carried out at the Hartree–Fock level to address some of these issues. (Int J Mass Spectrom 219 (2002) 11–21)

Keywords: Hydration; DNA base; Hydration shell; Adenine; Thymine; Cluster ion; Metastable ion

1. Introduction

Hydration is a general phenomenon in chemistry. It governs structure, energetics, and kinetics of molecules and chemical processes. Most biological processes occur in aqueous media, and therefore hydration plays a very important role in the chemistry of molecules of biological importance. The effect of hydration can be examined on a microscopic, molecular level by investigating how a certain property of a molecule changes as it becomes progressively hydrated by stepwise addition of the individual water molecule. The combined technique of molecular beam and laser spectroscopy is particularly suited for this purpose because clusters of various sizes and compositions can be easily produced and studied in size-specific manner.

It has long been known that purines and pyrimidines could form charge transfer complexes with organic compounds [1,2]. The electron and hole transfer in DNA has also become a topic of intense research in recent years to understand the long-range process of DNA damage and repair [3]. In these cases, the base molecules generally behave as an electron donor [2]. Because the ionization energy of the electron donor is a crucial quantity in modeling a charge transfer reaction, it is necessary to learn how the ionization energy of DNA bases varies as they become gradually hydrated in order to understand charge transfer processes involving DNA bases on a microscopic level. A molecular beam study of the DNA bases has been carried out with the aim to determine the ionization energy of these bases as a function of hydration [4]. The appearance potential measured in the electron impact ionization of the hydrated DNA bases revealed that a single water molecule decreases the ionization energy of adenine and thymine by 0.5 and 0.3 eV,

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respectively. Following this earlier study, we attempted to use photoionization to measure more accurate values for the ionization energy of the DNA bases. In the case of adenine, however, we found that virtually all hydrated adenine monomer clusters disappear from the photoionization mass spectrum because of a facile decomposition of the adenine–water complex [5]. Such remarkable loss of the solvent structure was due to the n–π^* character of the transition that leads to an increased repulsive nature of the potential energy surface along the solute–solvent coordinate when the solvent acts as a proton donor.

The hydration shell structure of DNA bases is another important aspect not just for the charge transfer reaction but also more generally for various biological processes in the cell. It has been reported that the water molecules surrounding a DNA and protein were an important contributor to the specific affinity of the protein–DNA interaction [6]. In the charge transfer reaction, reorientation of the water molecules around a DNA base upon formation of the charged species should be included in the consideration of the activation barrier for such a reaction [7]. In general, the water molecules in the first hydration shell interact with the solute more strongly than those in the outer shells. When the solute is an ion, the interaction is even stronger, and the hydration shell obtains a more rigid structure. This could strongly affect the rate of various processes such as energy transfer, relaxation, and solvent reorganization. Therefore, information on hydration shell structure for neutral and cationic solutes is often of crucial importance to understand dynamical processes involving DNA bases.

In the present study, we investigated the hydration shell structure for the cations of adenine and thymine using the reflectron time-of-flight mass spectrometry. The lower vapor pressure of guanine and cytosine along with the strong tendency of the latter for thermal dehydrogenation did not allow us to study these molecules with our thermal pulsed nozzle source. It was found that both adenine and thymine exhibited a strongly bound first solvation shell with four water molecules, followed by a less tightly bound second shell. In the hydration of the dimer of adenine, however, the shell structure was found to be much less pronounced. These findings are discussed in comparison with the results of some computational studies.

2. Experimental methods

The details of our experimental apparatus have been described elsewhere [5,8–10]. The apparatus is a typical molecular beam machine with a reflectron time-of-flight mass spectrometer (RTOF-MS). It consists of three differentially pumped chambers: the source chamber pumped by a 10-in. diffusion pump, the buffer chamber by a 6-in. diffusion pump, and the detector chamber by a 2-in. diffusion pump and a liquid nitrogen trap. Typical operating pressures were 2×10^{-5}, 3×10^{-6}, and 5×10^{-7} Torr in the source, buffer, and detector chambers, respectively.

The DNA sample was purchased from Aldrich Chemical Company and used without further purification. The sample in a powder form was heated in a metal oven up to 240 °C, and the beam was expanded through a high temperature pulsed nozzle with water vapor and a helium carrier gas at a backing pressure of a few atm. The beam was sampled by a skimmer 1 cm downstream and then two-photon ionized by the fourth harmonic of an Nd:YAG laser.

The ion was initially accelerated to 3 keV in a double electrostatic field. It travels in the first drift tube of 1.1-m length and then reflected into the second drift tube before being detected by a microchannel plate detector. The RTOF-MS had a mass resolution of $M/\Delta M \sim 900$. The signal from microchannel plate detector was first amplified, and then digitized and stored by a 400-MHz digital storage oscilloscope.

3. Results and discussion

3.1. Clusters of neat adenine

Fig. 1 is a resonant two-photon ionization (R2PI) TOF mass spectrum of adenine clusters obtained under the expansion with a helium gas at 1.6 atm. The fourth

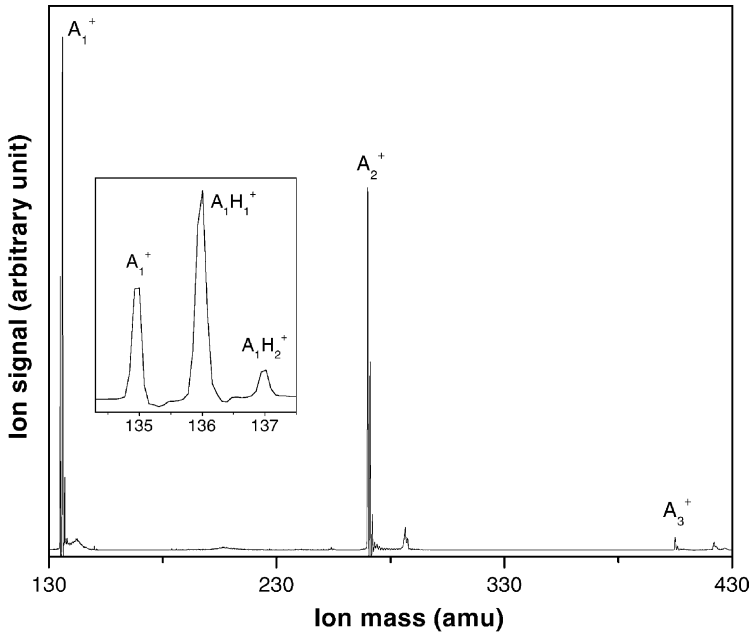

Fig. 1. The R2PI mass spectrum of neat adenine clusters. The region around the monomer peak is expanded and shown in the inset. The intensity ratio between unprotonated and protonated peaks is reversed in the dimer and larger clusters.

harmonic of Nd:YAG laser (266 nm = 4.66 eV) was used to excite and ionize the clusters at a laser fluence of 1.3 MW/cm^2. The inset in the figure with an expanded view around the adenine monomer peak shows that protonated forms of adenine $A_1H_1^+$ and $A_1H_2^+$ are produced in addition to the molecular ion of adenine A_1^+. In fact, the splittings of all the major peaks in Fig. 1 are due to the formation of $A_iH_j^+$ ($j = 0$–2) for all i. The relative intensity of the protonated peak drops rapidly as we go from the adenine monomer to the dimer, and then to the trimer and so on. In the expansion of neat adenine such as this, the only possible hydrogen source is the other adenine molecules within the cluster. We observed that the relative intensity of $A_1H_1^+$ to A_1^+ increased with a higher backing pressure, which indicates that the formation of the protonated species is aided by the generation of a larger amount of the clusters. Photofragmentation is expected to occur rather extensively and in parallel with photoionization, as in the case of electron impact ionization [4]. The protons produced in photofragmentation are readily picked up by these molecules, which are after all bases with a large proton affinity [11].

We believe that a good part of the $A_1H_2^+$ peak intensity in the inset of Fig. 1 is due to the genuine $A_1H_2^+$ itself because the ^{13}C isotope contribution of $A_1H_1^+$ to the intensity of the $A_1H_2^+$ peak is only 5.5% of the $A_1H_1^+$ peak intensity. The relative intensity of this peak to the $A_1H_1^+$ peak changed as the backing pressure or the time delay between the laser excitation and the opening of the pulsed nozzle were varied.

Fig. 2 is a mass spectrum of larger adenine clusters obtained under the same conditions as in Fig. 1, but employing the reflectron function of the mass spectrometer with different voltage settings. The smaller peaks next to the major ion peaks represent daughter ions resulting from fragmentation of metastable parent ions in the first field-free region of the RTOF-MS.

Since the adiabatic ionization energy of adenine is 7.8 eV [12], the two-photon ionization at 266 nm (4.66 × 2 = 9.32 eV) will deposit an excess

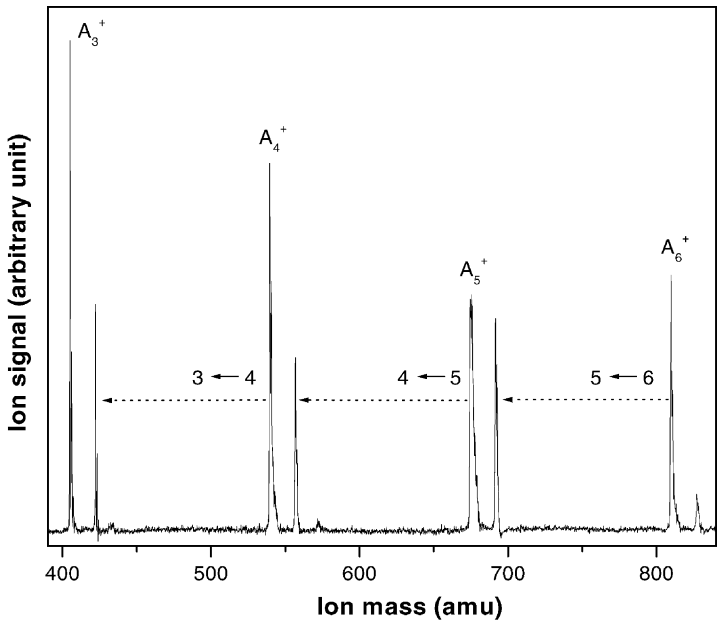

Fig. 2. The R2PI reflectron mass spectrum to show the fragmentation pattern. The smaller satellite peaks next to the main peaks represent fragmentation products of the adenine clusters, mostly from a single molecule loss denoted by the sign, $(m-1) \leftarrow m$.

energy of 1.5 eV to the adenine monomer ion, and an even more excess energy to larger adenine cluster ions due to their lower ionization energies. With this excess energy, extensive fragmentation of adenine cluster ions is likely to occur in the ionization region. Because of the statistical nature of energy dissipation, however, some cluster ions (and their fragments) may retain residual excess energy beyond the ionization region. This leads to further fragmentation of these metastable cluster ions in the field-free region. When these ions decompose into a daughter ion and a neutral fragment, the daughter ion carries with it a kinetic energy of $E_d = (M_d/M_p)E_p$, where M_d and M_p are the masses of the daughter and parent ions, respectively, and E_p is the parent ion energy [13]. Because of the difference in the kinetic energy, the daughter and parent ions penetrate into the reflectron over different distances to obtain different flight times, and thus become separately detected. Fig. 2 shows that only one neutral adenine monomer is dissociated from their metastable parent cluster ions.

3.2. Clusters of hydrated adenine monomer

We also investigated metastable decay of hydrated adenine cluster ions. Fig. 3 is a mass spectrum of hydrated adenine clusters under a helium expansion condition at 1.1 atm. The mass spectrum was obtained by R2PI at 277 nm with a laser fluence of 1 MW/cm^2. The vapor pressure of water was about 100 Torr. The intensity of hydrated adenine monomer clusters was anomalously small compared to that of the bare adenine, as already found to be due to the extensive loss of water cage in the photoexcited electronic state [5].

Fig. 4 shows the metastable fragmentation of hydrated adenine cluster ions in RTOF-MS. We note that the fragmentation occurs predominantly through the loss of one neutral water molecule. Although the daughter ions that result from the loss of two neutral water molecules could be also detected with the trajectory-normalization method [13], their intensity was an order of magnitude smaller. The fact that the metastable cluster ions of hydrated adenine tend to

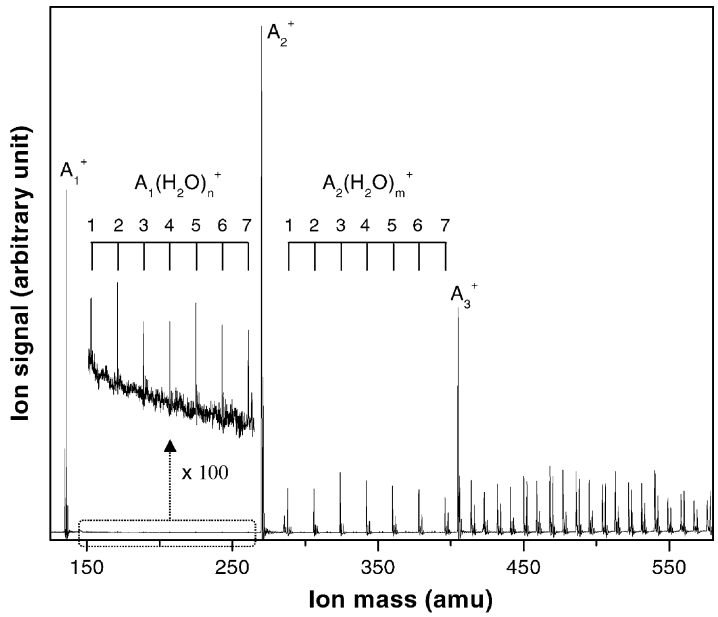

Fig. 3. The R2PI mass spectrum of hydrated adenine. Note that the intensity of the monomer hydrates is anomalously small relative to that of the unhydrated peak [5].

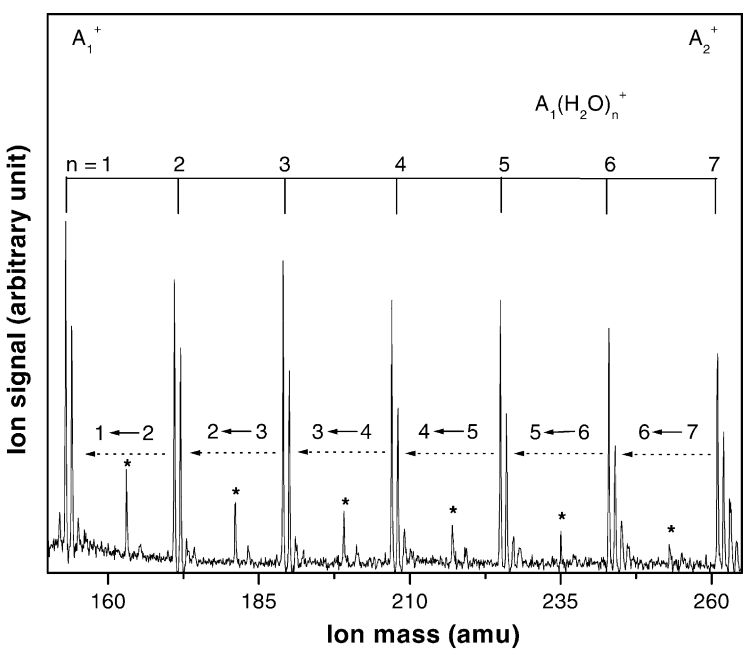

Fig. 4. The R2PI reflectron mass spectrum of hydrated adenine monomer to show the fragmentation pattern. As in the case of pure adenine clusters, the cluster ion fragmentation is dominated by a single molecule loss. The asterisk represents protonated neat water clusters, starting from $H^+(H_2O)_9$ at 163 amu.

lose only a single solvent molecule is in stark contrast to the near complete loss of solvent molecules in the fragmentation of neutral hydrated adenine clusters at the electronically excited state [5]. The culprit for the latter was that the water–water interaction was comparable in strength with the adenine–water interaction. As a result, the water molecules form a globule of "ice", which as a whole binds to the adenine at a single binding site. The repulsive nature of the n–π* transition in the presence of proton-donating solvents then dissociates the adenine–"ice" complex along the solute–solvent coordinate, and a near complete loss of solvents occurs in a single fission event. In the present case of an ionic solute, however, the interaction between the solute (the adenine cation) and solvent is much stronger than the solvent–solvent interaction, and thus each solvent molecule binds individually rather than cooperatively to the adenine cation. Therefore, the cation of adenine is expected to be much more evenly solvated than its neutral form even at the low temperature of our experimental conditions. In the metastable decay of the cluster ion, the water molecule in the least stable binding site will dissociate most easily, and the decay will be largely a sequential dissociation process rather than a concerted, single step fission as in the case of the neutral hydrated adenine clusters.

In order to quantify the tendency of the cluster ion to give up a single solvent molecule, we defined the fragmentation ratio $\beta = S_{n-1}/(S_{n-1} + S_n)$, where S_{n-1} is the intensity of the daughter ion containing $n-1$ water molecules, while S_n represents that of the parent ion containing n water molecules. Fig. 5 shows the plot of β versus n in the case of the cluster ions of hydrated adenine monomer. The fragmentation ratio increases gradually as n becomes larger, which reflects the progressive decrease in the binding energy of each additional water to the cluster ion. Quite remarkable is the fact that the slope of this plot shows an unmistakable break at $n = 4$. This means that fragmentation becomes much easier at $n = 5$ abruptly, and follows that trend in larger clusters. It is likely that such

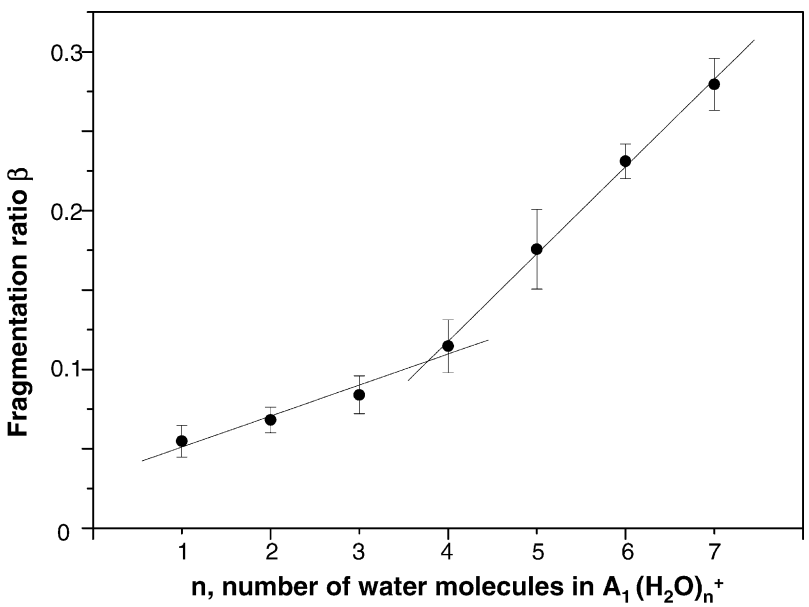

Fig. 5. The plot of the fragmentation ratio β vs. the number of water molecules n in the adenine monomer cation hydrates, $A_1(H_2O)_n^+$. Note that β increases gradually up to $n = 4$, but much faster afterwards. This indicates that the rather solid structure for the cluster ion up to $n = 4$ becomes much more loose from $n = 5$, with a tendency for easier fragmentation. A first hydration shell with four water molecules is proposed for the cation of adenine.

a sharp change is brought about by the formation of a more loosely bound second solvation shell after the completion of the first solvation shell. In the pioneering work of gas phase solvation by Kebarle et al. on $H^+(H_2O)_n$ [14], crowding of the first solvation shell or transition to an outer shell was suggested to occur when a considerable decrease in the hydration step enthalpy $\Delta H_{n-1,n}$ was observed that correlated with the binding energy of each additional water to the cluster ion. Likewise, we propose from our result that it take four water molecules to form a tightly bound first solvation shell around the adenine cation.

3.3. Clusters of hydrated adenine dimer

In a similar way, we investigated the metastable fragmentation behavior of the hydrated clusters of the adenine dimer. Fig. 6 shows the RTOF mass spectrum, which again reveals that the predominant dissociation channel is through the single molecule loss. The fragmentation ratio is plotted in Fig. 7 against the number of water molecules. Contrary to the case of the adenine monomer cation, the fragmentation ratio for the dimer cation does not show a sharp change in its slope, indicating that the solvation shell structure is less well-defined. This is probably because the solute has now many binding sites, and some of them may be comparable in energy with some sites in the second solvation shell. In fact, the small change occurring at $n = 7$–8 appears to be "inverted", i.e., it shows a lower gradient for the larger clusters. This indicates that the decrease in the stepwise solvation energy in the second solvation shell is relatively smaller than in the first solvation shell. Therefore, it is quite likely that the least stable binding sites in the latter are comparable in energy with the most stable binding sites in the former. The rather erratic scatter of β near $n = 7$–8 in Fig. 7 may reflect such "confusion" and the blurring of the solvation shell structure.

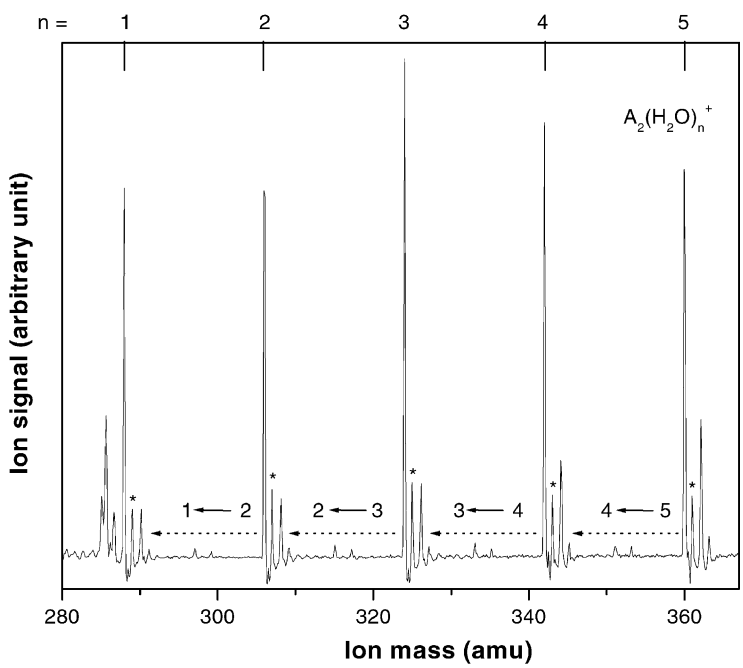

Fig. 6. The R2PI reflectron mass spectrum of hydrated adenine dimer to show the fragmentation pattern. The asterisk represents mostly protonated clusters of hydrated adenine dimer, $A_2H_1(H_2O)_n{}^+$, while a small fraction (ca. 5%) of it is due to the protonated neat water clusters, $H^+(H_2O)_m$, with $m = n + 15$.

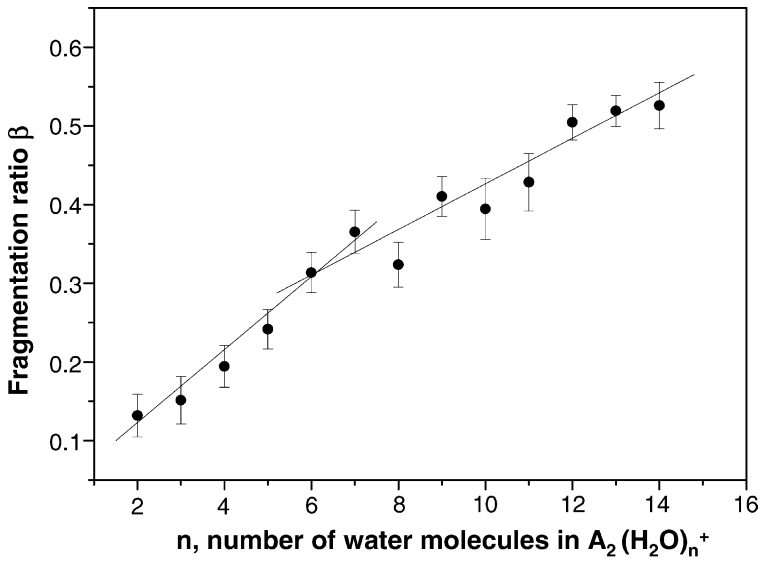

Fig. 7. The plot of the fragmentation ratio β vs. the number of water molecules n in the hydrated adenine dimer cations, $A_2(H_2O)_n^+$. In contrast to the case of the hydrated adenine monomer cations $A_1(H_2O)_n^+$, β shows no sharp break in its rate of increase, or even an inverted rate of increase (as represented by the guiding lines).

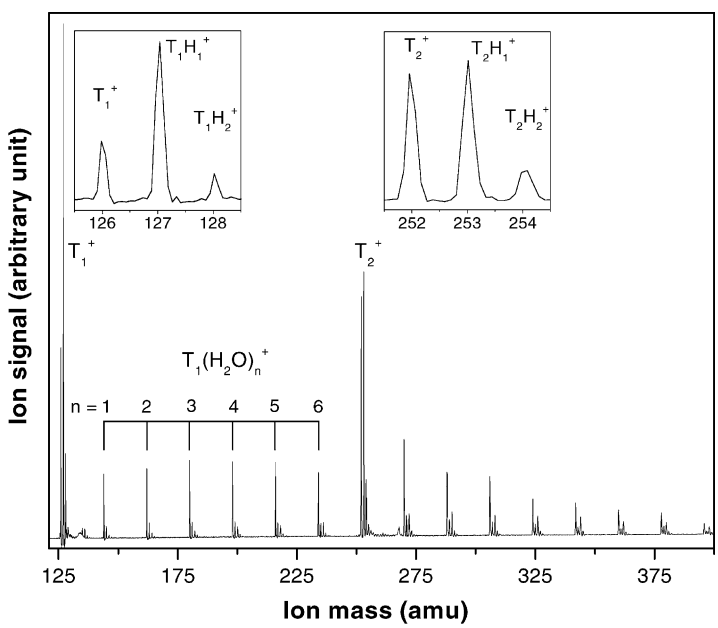

Fig. 8. The R2PI mass spectrum of hydrated thymine clusters.

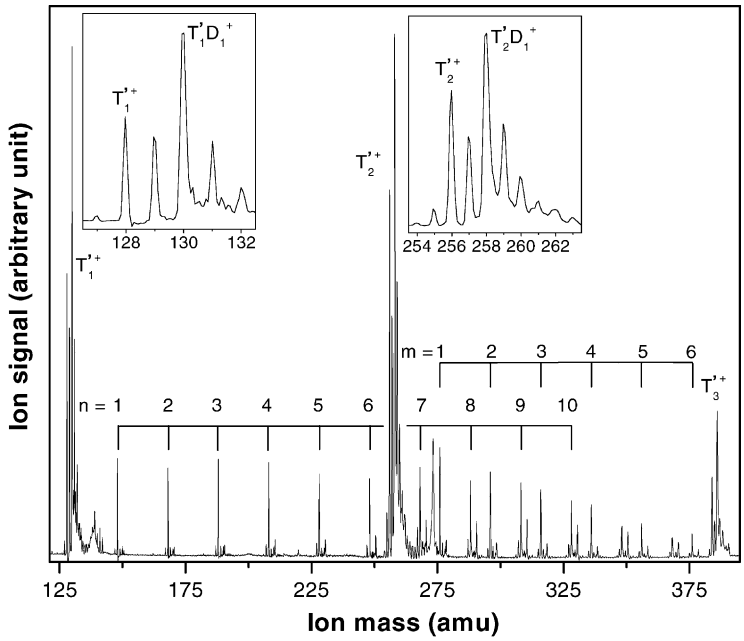

Fig. 9. The R2PI mass spectrum of thymine clusters hydrated by D_2O. Efficient H/D exchange turns most thymine into a doubly deuterated form, denoted by T'. The H/D exchange is expected to involve the hydrogen atoms at the N1 and N3 positions of thymine.

3.4. Clusters of hydrated thymine monomer

Fig. 8 is an RTOF mass spectrum of hydrated thymine clusters formed in expansion with helium at 2.3 atm, obtained by R2PI at 266 nm with a laser fluence of 0.7 MW/cm^2. Since the mass of seven water molecules is identical with that of a thymine molecule, it is impossible to distinguish the peak of $T_m(H_2O)_n{}^+$ from that of $T_{m+1}(H_2O)_{n-7}{}^+$ ($n \geq 7$). To avoid this problem, we used D_2O as solvent, and its mass spectrum is shown in Fig. 9. By comparing the masses of the hydrated thymine cluster ions between Figs. 8 and 9, we found that two hydrogen atoms are substituted with deuterium atoms when we use D_2O as solvent. Such substitution reaction is expected to take place within the molecular beam source. These two hydrogen atoms are most likely the ones at the N1 and N3 positions of thymine.

Following a similar procedure as before, we determined the fragmentation ratio of the hydrated thymine clusters from their RTOF mass spectrum (not shown). It is plotted in Fig. 10 as a function of the number of the water molecules in the cluster. We note that the slope shows a sharp increase at $n = 4$, much like the case of the hydrated adenine monomer cluster ions. The hydration shell structure of the thymine monomer cation appears to be well-defined, and the first solvation shell seems to be completed with four water molecules around the solute ion.

3.5. Comparison with computational results

There have been extensive theoretical calculations on hydrated adenine and thymine clusters in the neutral state, and more recently, in the anionic state as well. To the extent of our knowledge, however, there is no such study on the cations of the hydrated adenine clusters. Pullman et al. [15], Ohta et al. [16], and Del Bene [17] calculated optimized structures of the adenine–water and thymine–water complexes, and suggested that the first hydration shell is formed by four water molecules for adenine and

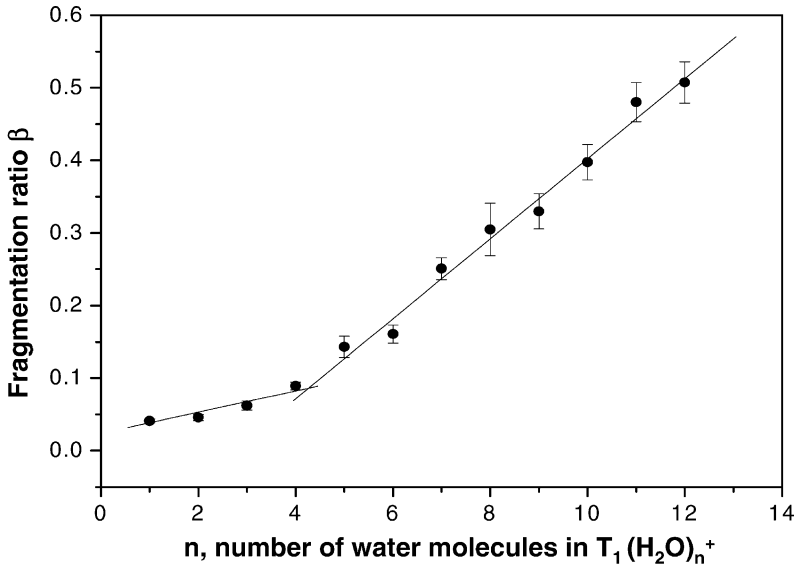

Fig. 10. The plot of the fragmentation ratio β vs. the number of water molecules n in the thymine monomer cation hydrates, $T_1(H_2O)_n^+$. Note that β increases gradually up to $n = 4$, but much faster afterwards. As in the case of hydrated adenine, this indicates that the first solvation shell may be composed of four water molecules for the cation of thymine.

three for thymine. Recently, Jalbout and Adamowicz [18] performed ab initio calculations on the neutral and anionic adenine–water clusters and found that three water molecules are needed to complete the first hydration shell for both the neutral and the anion. On the other hand, Zhanpeisov and Leszczynski [19] reported that six water molecules make the first hydration shell in the adenine–water complex.

Despite all these results, however, it should be noted that the geometry and energetics of the clusters in the cationic state can be very different from those in the neutral state due to the strong charge–dipole or charge-induced dipole interactions. Périquet et al. [20] reported two different sets of optimized structures for adenine–water complexes calculated by means of a semi-empirical model. They showed that water binds to adenine cooperatively up to three water molecules in the neutral state. On the contrary, in the anion, each water molecule occupies the most favorable hydrogen bonding site available, and a hydration shell structure is developed. Zhanpeisov and Leszczynski [21] also performed ab initio calculations for the neutral and the one-electron oxidized formamidine–formamide complexes with several water molecules. They pointed out that the one-electron oxidized cation–radical complexes display a relative stability opposite in order from that predicted for the neutral systems.

We carried out ab initio calculations at the Hartree–Fock level for the cation of hydrated adenine monomer, which yielded some results of relevance. The ground state energy of $A_1(H_2O)_4^+$ was found to be much lower for the hydration shell type structure than for the cooperative binding type structure. The energy difference between the two structures ranged from 20 kcal/mol when using the HF 6-31G basis set to 7 kcal/mol with the HF 6-311++G** basis set. It indicates that the charge–dipole force does indeed play a dominant role in the solvation of the cation. On the other hand, a cursory MP2 level calculation with the 6-31G basis set yielded an energy difference of only 2 kcal/mol. It appears that more refined calculations are needed to properly address the energetics and structures of the hydrated clusters of adenine cation.

4. Conclusions

Metastable decay of cluster ions in the reflectron time-of-flight mass spectrometry was employed to investigate the relative strengths of the binding energy of each additional solvent molecule in the hydrated cation clusters of adenine and thymine. Both molecules exhibited a well-defined hydration shell structure. The first hydration shell was found to be complete with four water molecules in both cases. On the other hand, the cation dimer of adenine did not reveal a clear sign of the solvation shell structure, although the rather ill-defined first solvation shell appears to be formed with 7–8 water molecules. Such difference seems to stem from the increased number of the binding sites for the dimer cation, which blurs the distinction between the first and second solvation shells in terms of energy. In the absence of any theoretical calculations for the cation clusters of hydrated adenine, we carried out a cursory ab initio calculation at the Hartree–Fock level. It showed a significant energy gain for the complexes with an enclosing type of hydration shell structure over those with a more cooperative binding character among solvents.

Acknowledgements

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References

[1] B. Pullman, A. Pullman, Quantum Chemistry, Academic Press, New York, 1963.
[2] B. Pullman, J. Chem. Phys. 43 (1965) S233.
[3] S.O. Kelly, J.K. Barton, Science 283 (1999) 375.
[4] S.K. Kim, W. Lee, D.R. Herschbach, J. Phys. Chem. 100 (1996) 7933.
[5] N.J. Kim, H. Kang, G. Jeong, Y.S. Kim, K.T. Lee, S.K. Kim, J. Phys. Chem. A 104 (2000) 6552.
[6] X. Qu, J.B. Chaires, J. Am. Chem. Soc. 121 (1999) 2649, and references therein.
[7] R.A. Marcus, N. Sutin, Biochim. Biophys. Acta 811 (1985) 265.
[8] N.J. Kim, G. Jeong, Y.S. Kim, J. Sung, S.K. Kim, Y.D. Park, J. Chem. Phys. 113 (2000) 10051.
[9] N.J. Kim, H. Kang, G. Jeong, Y.S. Kim, K.T. Lee, S.K. Kim, Proc. Natl. Acad. Sci. U.S.A. 98 (2001) 4841.
[10] N.J. Kim, H. Kang, G. Jeong, Y.S. Kim, K.T. Lee, S.K. Kim, J. Chem. Phys. 115 (2001) 7002.
[11] M. Meot-Ner, J. Am. Chem. Soc. 101 (1979) 2396.
[12] S.G. Lias, J.E. Bartmess, J.F. Liebman, J.L. Holmes, R.D. Levine, W.G. Mallard, J. Phys. Chem. Ref. Data 17 (1988) (Suppl. 1).
[13] S. Wei, W.B. Tzeng, A.W. Castleman Jr., J. Chem. Phys. 92 (1990) 332.
[14] P. Kebarle, S.K. Searles, A. Zolla, J. Scarborough, M. Arshadi, J. Am. Chem. Soc. 89 (1967) 6393.
[15] B. Pullman, S. Miertus, D. Perahia, Theoret. Chim. Acta 50 (1979) 317.
[16] Y. Ohta, H. Tanaka, Y. Baba, A. Kagemoto, K. Nishimoto, J. Phys. Chem. 90 (1986) 4438.
[17] J. Del Bene, J. Chem. Phys. 76 (1982) 1058.
[18] A.F. Jalbout, L. Adamowicz, J. Phys. Chem. A 105 (2001) 1033.
[19] N.U. Zhanpeisov, J. Leszczynski, Struct. Chem. 12 (2001) 121.
[20] V. Périquet, A. Moreau, S. Carles, J.P. Schermann, C. Desfrançois, J. Electron. Spectrosc. 106 (2000) 141.
[21] N.U. Zhanpeisov, J. Leszczynski, J. Phys. Chem. A 103 (1999) 8317.