Photodissociation and Spectroscopic Study of Cold Protonated Dipeptides

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A photodissociation spectrometer, containing a spray ionization source and a temperature-variable multipole ion trap, has been constructed to examine the structure and reactivity of gas phase biological molecular ions at various temperatures. Ultraviolet (UV) and infrared (IR) photodissociation spectra of protonated alanyltryptophan (Ala-TrpH^+) and tryptophanylglycine (Trp-GlyH^+) have been measured. In UV spectra, the S_1-S_0 band origin of Ala-TrpH^+ exhibits a significant red shift with respect to those of protonated tryptophan (TrpH^+) and Trp-GlyH^+. This red shift is ascribed to the stabilization of the excited state due to the strong interaction between the NH_3^+ group and indole ring. We also discuss the temperature effect on the structure and reactivity for these peptides. In addition to the UV photodissociation spectra of the dipeptides, IR spectra of the complex of Ala-TrpH^+ with methanol are measured. IR photodissociation spectra of solvated ions show that Ala-TrpH^+-methanol has the closed structure, which is consistent with the large spectral shift in UV spectrum of bare dipeptide.

1. Introduction

Because of its high absorption intensity in the ultraviolet region, tryptophan (Trp) and its derivatives have been used as probes investigating structure and reaction of various proteins. Thus, a large number of studies on photophysical/chemical properties of tryptophan and its derivatives have been carried out.[1−5] However, they have not yet been revealed completely even for tryptophan itself. Because, in general, amino acids are in their ionic form in solution and/or biological environment, it is important to investigate photophysical/chemical properties of ionic species such as protonated amino acids. It is well-known that the properties of amino acids largely depend on a local environment, such as position of charged group, configuration of side chains, relative orientation of solvent molecule(s), and so on.[6−8] Spectroscopic and dynamical studies in the gas phase are expected to provide very detailed information about structure and reaction of amino acids. Thus, the number of spectroscopic and/or dynamical studies on amino acids and other biological molecules in the gas phase is increasing rapidly.

Lioe *et al.* investigated the gas phase reaction of protonated tryptophan (TrpH^+).[9] They examined collision-induced dissociation (CID) process and found that a NH_3-loss pathway is the main process in the CID. They also obtained the most stable form of TrpH^+, where a proton is attached to the amino group, based on a density functional theory (DFT) calculation. Aribi *et al.* also investigated the CID process of TrpH^+ and examined consecutive processes following the NH_3 loss.[10] Photoinduced dissociation (PID) processes were examined by Kang *et al.*[11] They irradiate a 266 nm femtosecond laser pulse to TrpH^+ and measured a mass spectrum of the photofragments. In addition to the NH_3-loss pathway as in the CID reaction, they found a hydrogen atom loss pathway. They attributed the latter pathway to the dissociative $\pi\sigma^*$ electronic state. The lifetime of the electronically excited TrpH^+ was determined to be 380 fs.

In the case of neutral species, spectroscopic studies in the supersonic jets have succeeded in getting detailed structural information in various systems including biological molecules. On the contrary, it has been difficult to control a temperature condition of ionic species. However, a technique to cool gas phase ions is now available by means of collisions with cooled buffer gases in a cold ion trap.[12] Nolting *et al.* succeeded in measuring an electronic spectrum of TrpH^+ in the ultraviolet region at 140 K.[13] They determined the S_1-S_0 origin to be 284.5 ± 0.5 nm. Recently, Boyarkin *et al.* measured the electronic transition of TrpH^+ in the 22-pole trap cooled at 6 K.[15] They found that the S_1-S_0 origin band is very broad of 347 cm^{-1} width even in this cooled condition. The broad feature is attributed to the very short lifetime in the S_1 state. Kamariotis *et al.* also measured infrared (IR) spectra of protonated valine solvated with water molecules and discussed structure of them.[16]

We have been investigating reaction and structure of biological molecules and their solvated clusters produced by an electrospray ionization method.[17] We examined metastable decay processes of hydrated TrpH^+ and estimated binding energies.[18] In the present study, we have constructed a photodissociation spectrometer containing a spray ionization source and a temperature-variable multipole ion trap, and have carried out spectroscopic studies of protonated dipeptides involving tryptophan moiety. In the case of dipeptide system, a flexible conformation of the side chain relative to the indole ring should cause some effects to the photophysical/chemical properties. Thus, we investigate the electronic transition and the following photodissociation process of protonated alanyltryptophan (Ala-TrpH^+), tryptophanylglicine (Trp-GlyH^+) in the cold trap at ∼20 K. In addition, infrared spectra of solvated clusters of TrpH^+ and Ala-TrpH^+ with methanol are measured to examine the effect of the solvation to the conformation of the TrpH^+/Ala-TrpH^+.

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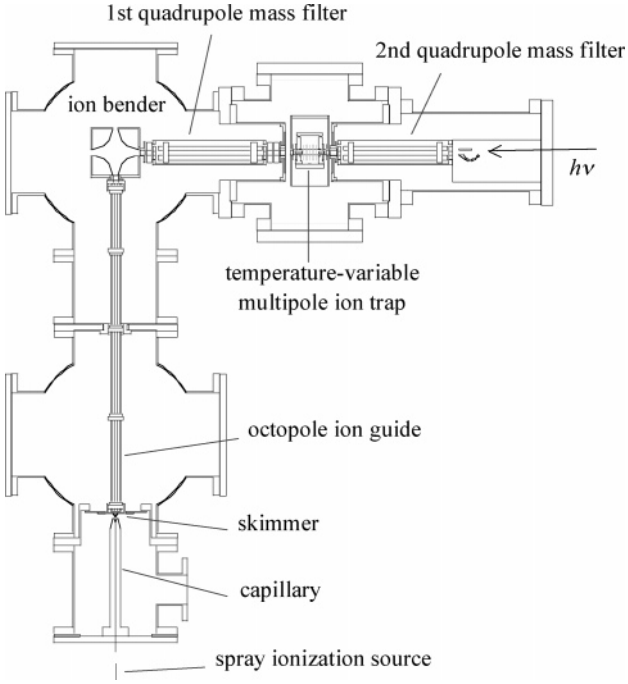

Figure 1. Schematic drawings of the experimental apparatus.

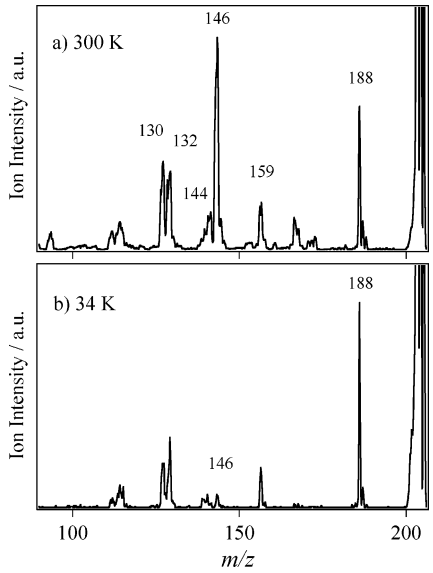

Figure 2. Photodissociation mass spectra of $TrpH^+$ at (a) 300 K and (b) 34 K. The excitation energy is 35 078 cm^{-1}.

2. Experimental Section

A tandem mass spectrometer for photodissociation spectroscopy of temperature-controlled gas phase biological molecule and cluster ions has been constructed. The apparatus consists of five differentially evacuated chambers as shown in Figure 1: an electrospray interface, an octopole ion guide, a quadrupole mass filter for mass selection of precursor ions, a temperature-variable multipole ion trap,[12] and a quadrupole mass filter for mass analysis of fragment ions. The sample, tryptophan, tryptophanylglycine, or alanyltryptophan, is diluted to concentration of 100 μM in a mixture of water and methanol (50/50) with 0.5% acetic acid. Protonated peptides are generated by electrospray[19] or sonic spray[20] ionization method and transferred to the gas phase through a metal capillary (length 260 mm, i.d. 0.6 mm) and a skimmer (i.d. 0.8 mm). The ions guided by an octopole (length 633 mm, i.d. 12 mm, rod diameter 4 mm, 7.2 MHz) are deflected 90° by an electrostatic quadrupole ion bender into the first quadrupole mass filter. The mass-selected ions are decelerated and refocused by a stack of electrostatic ion lenses into a temperature-variable multipole ion trap (10−350 K). The ions are stored and thermalized by multiple collisions with He buffer gas in the trap for ~80 ms, and then irradiated with a photodissociation laser. The product ions are extracted from the trap and analyzed by the second quadrupole mass filter. The ion signals are detected using an electron multiplier with a conversion dynode operated in pulse counting mode, and fed into a gated photon counter (Stanford Research Systems, SR400) after being amplified by a preamplifier (Stanford Research Systems, SR445).

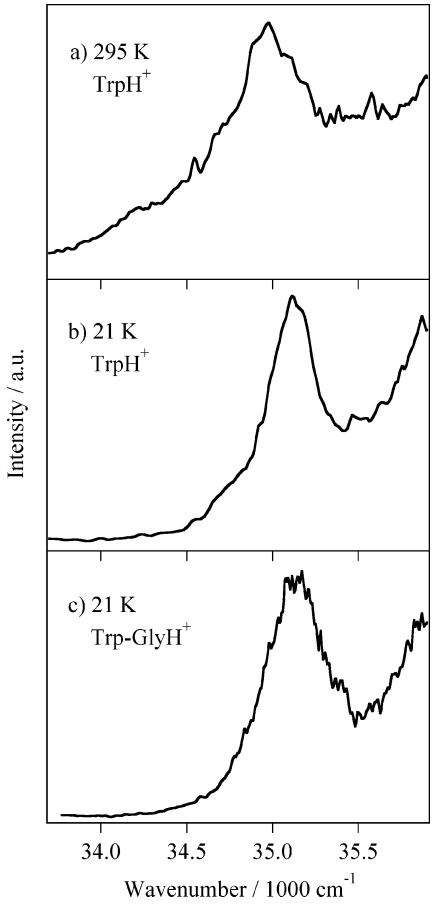

Figure 3. UV photodissociation spectra of (a) $TrpH^+$ at 295 K, (b) $TrpH^+$ at 21 K, and (c) Trp-$GlyH^+$ at 21 K.

The multipole ion trap consists of 22 stainless steel rods with a diameter of 1 mm and a length of 36 mm equally spaced on an inscribed radius $r_0 = 5$ mm. The whole unit is housed in a copper box mounted on the second stage of a closed cycle cryocooler (Iwatani, D510) capable of 2 W of cooling power at 20 K; the lowest achievable temperature is 10 K. For temperature-variable experiments, the trap is heated to 10−350 K using a heating wire winded around a trap holder. The temperature is measured by two thermocouples (Chromel-AuFe0.07%) attached directly to the body of the trap. The surrounding radiation shield is in thermal contact with the first cooling stage at a temperature of approximately 50 K. In the axial direction the ions are trapped by DC voltage applied to cylindrical entrance and exit electrodes. A RF amplitude (zero-to-peak) of $V_0 = 80$ V from a home-built RF power source[21] is applied to the rods of the trap at fixed frequency $\Omega/2\pi = 10$ MHz.

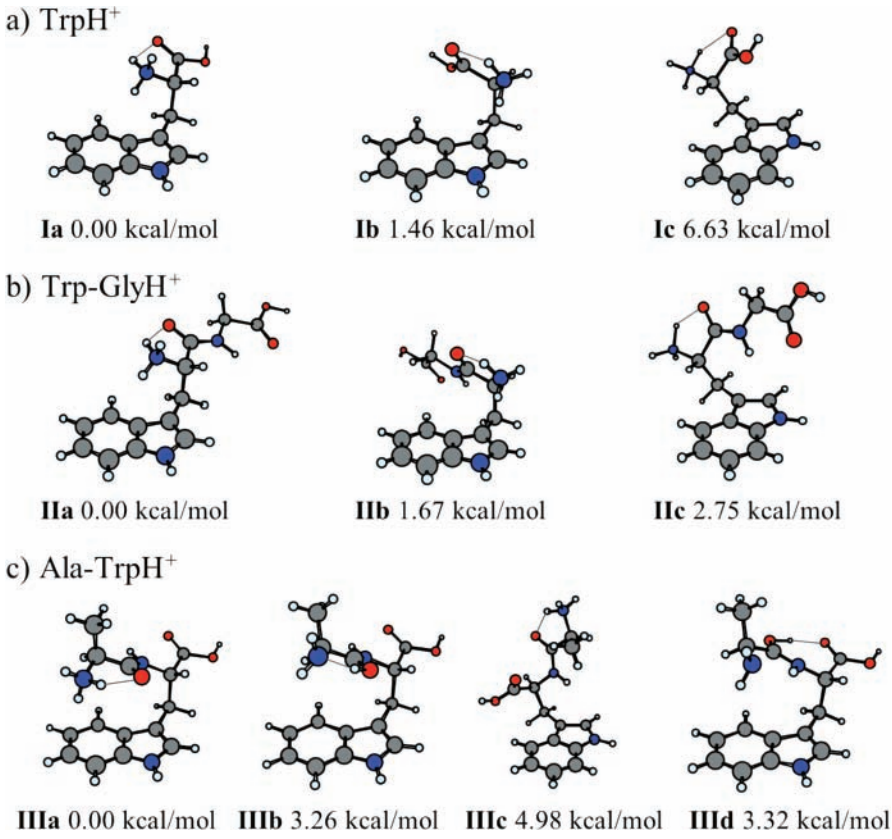

Figure 4. Selected sets of optimized structures of (a) $TrpH^+$, (b) $Trp\text{-}GlyH^+$, and (c) $Ala\text{-}TrpH^+$ at the B3LYP/6-31++G(d,p) level. Relative binding energies (kcal/mol) with ZPC are given under each structure.

UV laser pulses are generated by frequency doubling of a Nd:YAG (Continuum, NY61) pumped dye laser (Continuum, ND60) using a KDP crystal. IR laser pulses are produced by an optical parametric oscillator/amplifer (OPO/OPA) system (Laser Vision) pumped by the fundamental output of another Nd:YAG laser (Spectra Physics, GCR-250). Typical laser fluence is ca. 2 mJ/cm^2.

Geometry optimizations and vibration analyses are carried out by a DFT calculation at B3LYP/6-31++G(d,p) level using GAUSSIAN03.[22] The calculated vibration frequencies are scaled with a factor of 0.956.[16]

3. Results and Discussion

3.1. UV Photodissociation Spectroscopy. Before reporting the present results on dipeptide species, we briefly report the results on the $TrpH^+$ to show a performance of our experimental apparatus. Photodissociation mass spectrum of $TrpH^+$ (*m*/*z* 205) at 300 K is shown in Figure 2a. The excitation wavenumber is fixed to 35 078 cm^{-1}, which is the S_1-S_0 band origin of $TrpH^+$. The fragment ions observed are *m*/*z* 188, 159, 146, 144, 132, and 130, as observed in the previous CID[9,10] and PID[11,23] of $TrpH^+$. The fragment ion *m*/*z* 188 is the product of the NH_3 loss, which has been observed as the primary fragmentation pathway of $TrpH^+$ in low-energy (~1 eV) CID experiments. In the photodissociation mass spectrum of $TrpH^+$ cooled to 34 K shown in Figure 2b, the main product is *m*/*z* 188 (the NH_3 loss), and a branching ratio of the fragment ion *m*/*z* 146 corresponding to the loss of (NH_3 + CH_2CO) relative to the *m*/*z* 188 decreases drastically compared with the case at 300 K. These observations indicate that the *m*/*z* 146 is formed by the sequential reaction *via* the NH_3 loss, which is suppressed by cooling of $TrpH^+$.

Figure 3 shows UV photodissociation spectra of $TrpH^+$ at (a) 295 K and (b) 21 K obtained by monitoring the fragment ion *m*/*z* 188 as a function of excitation energy. The broad band at 35 100 cm^{-1} is attributed to the S_1-S_0 band origin of $TrpH^+$. When the ion trap is cooled from 295 to 21 K, the hot bands below 34 500 cm^{-1} disappear and the band at 35 100 cm^{-1} becomes narrower. Such spectral changes, disappearing of the hot bands and the narrowing of the width of the origin band, are consistent with those measured at 6 K by Boyarkin *et al.*[15] The absorption of $TrpH^+$ is in the same energy range as the S_1-S_0 transition of neutral Trp.[24] The result shows that the protonation does not affect on the electronic structure as pointed out previously.[13]

We also calculate the structure of $TrpH^+$ using a DFT method at B3LYP/6-31++G(d,p) level and the three most stable conformers are shown in Figure 4a. For all conformers, the protonation site is the amino group and one of the H atom of the NH_3^+ group points to the CO moiety of the carboxyl group. These conformers originate by an internal rotation of the amino acid group with respect to the indole ring. These results are consistent with the theoretical calculation by Grégoire *et al.*[14] As shown in Figure 4a, the NH_3^+ group points to the middle of the indole ring; however, the distance between the H atom of NH_3^+ group and indole ring is substantially large even for the most stable conformer **Ia**. With this conformation, the hydrogen bonding interaction may be too weak to affect on the S_1 (L_b) excited state of indole ring as observed in Figure 3b.

The broad feature of the S_1-S_0 transition that exhibits no resolved vibronic features at low temperature implies a coexistence of several stable conformers and/or a short excited state lifetime of $TrpH^+$. In relation to this finding, Kang *et al.* carried out a femtosecond pump−probe experiments for the excited state of room-temperature $TrpH^+$ following 266 nm excitation and determined the decay time of 380 fs.[11] Moreover, Mercier *et al.* have carried out the photodissociation experiments for hydrated $TrpH^+$ cooled at about 10 K and found that the addition

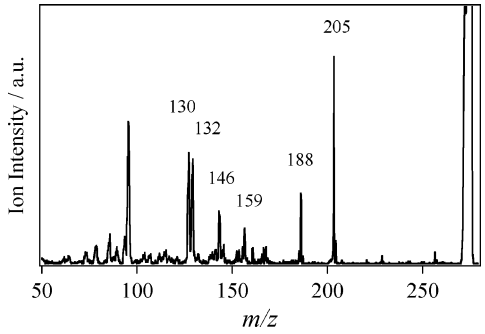

Figure 5. Photodissociation mass spectrum of Ala-TrpH$^+$ at 300 K. The excitation energy is 35 078 cm^{-1}.

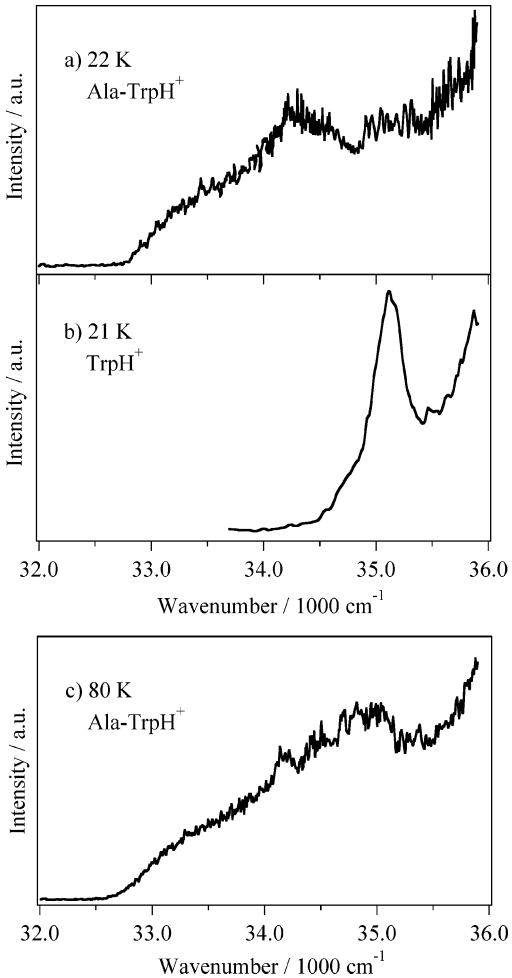

Figure 6. UV photodissociation spectra of (a) Ala-TrpH$^+$ at 22 K, (b) TrpH$^+$ at 21 K, and (c) Ala-TrpH$^+$ at 80 K.

of two water molecules sufficiently lengthens the excited state life time; a fully vibrationally resolved electronic spectrum has been obtained.[25] The results by the latter authors clearly indicate that the broad feature originates from the short-lifetime due to the interaction of the relevant $\pi\pi^*$ state with a dissociative state such as a $\pi\sigma^*$ state.

To get insight into the photophysical/chemical properties of peptides, the structure and reactivity of Trp-GlyH$^+$ are investigated by UV photodissociation spectroscopy. In a photodissociation mass spectrum of cooled Trp-GlyH$^+$ at 35 078 cm^{-1}, the fragmentation is dominated by the NH_3 loss (*m*/*z* 245) and the fragment ions *m*/*z* 159, 144, 132, and 130 are observed as in the case of TrpH$^+$. Figure 3c shows a UV photodissociation spectrum of Trp-GlyH$^+$ at 21 K obtained by monitoring the fragment ion *m*/*z* 245. The spectral feature of Trp-GlyH$^+$ is very

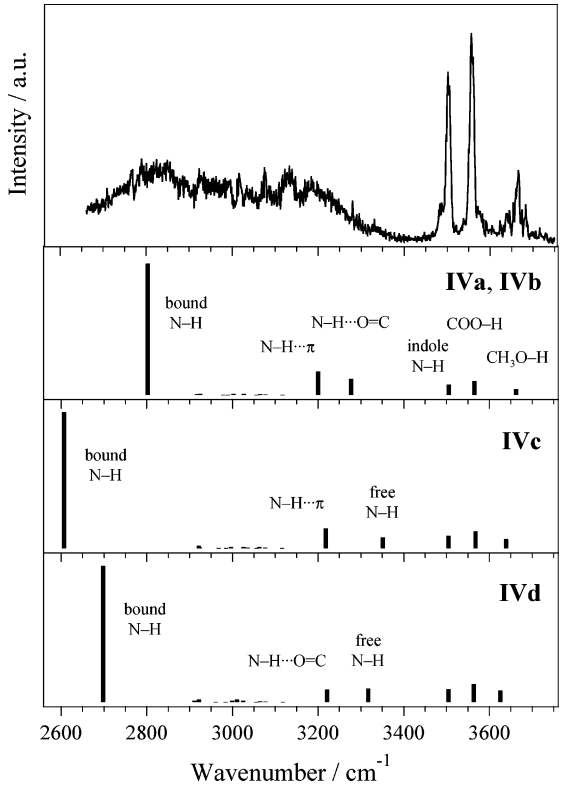

Figure 7. IR photodissociation spectra of TrpH$^+$(CH_3OH) at room temperature compared with theoretical IR spectra for the structures shown in Figure 8a.

similar to that of TrpH$^+$. The broad peak at 35 100 cm^{-1} is attributed to the S_1-S_0 band origin of Trp-GlyH$^+$, as in the case of TrpH$^+$. Figure 4b shows the optimized structures of Trp-GlyH$^+$ obtained in the present study. In these conformers, the protonation site is the amino group and the NH_3^+ group points to the CO moiety of the amide group. In the case of the two lowest-energy conformers **IIa** and **IIb**, the glycine moiety is located far from the indole ring. Thus, the electronic transition of these conformers should be similar to that of TrpH$^+$. In agreement with the theoretical predictions, the spectrum and dissociation process of Trp-GlyH$^+$ are similar to those of TrpH$^+$.

As mentioned above, the protonation site of Trp-GlyH$^+$ is N-terminal of Trp and as a result, its most stable conformer has the structure where the glycine moiety is opposite side of the indole ring. To gain more information on the relation between the conformational structure and hydrogen bonding of indole ring, we also examine the dipeptide such as Ala-TrpH$^+$, in which Ala is peptide bonded to N-terminal of Trp and has the larger proton affinity (214.8 kcal/mol) than that of Gly (212.0 kcal/mol).[26] A photodissociation mass spectrum of Ala-TrpH$^+$ (*m*/*z* 276) at 300 K is shown in Figure 5. The fragment ion *m*/*z* 205 is the main product, and the NH_3 loss is hardly observed, which is the primary fragmentation pathway of TrpH$^+$ and Trp-GlyH$^+$. To examine the dissociation process of Ala-TrpH$^+$, a photodissociation experiment for *m*/*z* 205 formed by CID of Ala-TrpH$^+$ is conducted. The fragment ions observed in the experiment are identical to those of TrpH$^+$. Therefore, the *m*/*z* 205 formed by the photodissociation of Ala-TrpH$^+$ is assigned to TrpH$^+$, which indicates a peptide bond cleavage. The other fragment ions *m*/*z* 188, 159, 146, 132, and 130 observed in Figure 5 are the products of the sequential reaction *via* the peptide bond cleavage that forms TrpH$^+$ (*m*/*z* 205).

In Figure 6a, the photodissociation spectrum of Ala-TrpH$^+$ at 22 K obtained by monitoring the fragment ion *m*/*z* 205 is

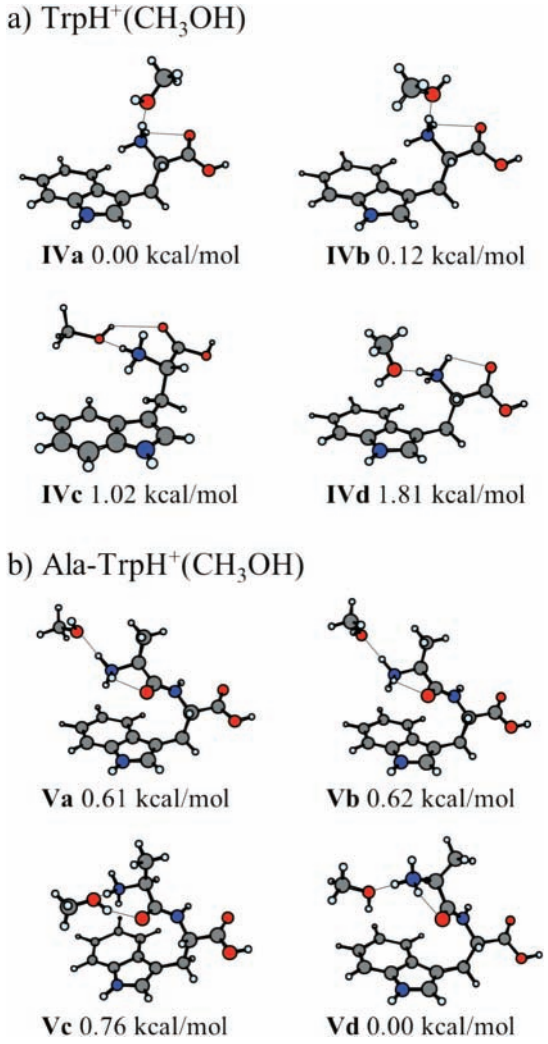

Figure 8. Selected sets of optimized structures of (a) $TrpH^+(CH_3OH)$ and (b) Ala-$TrpH^+(CH_3OH)$ at the B3LYP/6-31++G(d,p) level. Relative binding energies (kcal/mol) with ZPC are given under each structure.

presented with that of $TrpH^+$ for comparison. The broad band shifts to the red by ~2000 cm^{-1} with respect to the S_1-S_0 origin of $TrpH^+$ and Trp-$GlyH^+$. Selected sets of the optimized structures of Ala-$TrpH^+$ obtained in the present study are shown in Figure 4c. The protonation sites are the amino nitrogen, **IIIa** and **IIIc**, and the amide oxygen, **IIIb** and **IIId**, respectively. A conformer where the protonation site is on the amide nitrogen lies about 20 kcal/mol higher in energy. For the lowest-energy structure **IIIa** in which the protonation site is the amino group, one of the H atom of the NH_3^+ group points to the CO moiety of the amide group and another H atom bounds to the π cloud of indole by the hydrogen bond. The NH_3^+ group is close to the π cloud of indole in **IIIa**, whereas the distance between the NH_3^+ group and the indole ring is rather far in the case of $TrpH^+$ and Trp-$GlyH^+$, where the geometrical restriction by a $C_\alpha-C_\beta$ bond exists. For example, the distance between the hydrogen of the NH_3^+ group pointing to the indole ring and the center of the six-membered ring in the indole of the **IIIa** conformer of Ala-$TrpH^+$ is 2.5 Å, which is much shorter than that of the **Ia** conformer of $TrpH^+$ (3.1 Å). A chromophore of the electronic transition of $TrpH^+$, Trp-$GlyH^+$, and Ala-$TrpH^+$ is the indole moiety. Indole has two low-lying $\pi\pi^*$ excited states denoted L_b and L_a. The L_a origin lies about 1400 cm^{-1} above the L_b origin in the gas phase.[27] In polar solvents the L_a state is strongly stabilized and becomes the lowest excited state, whereas the

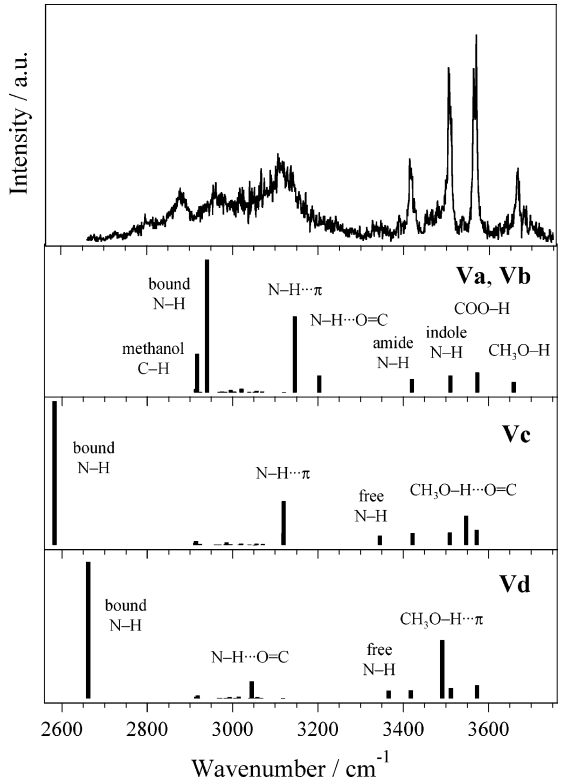

Figure 9. IR photodissociation spectra of Ala-$TrpH^+(CH_3OH)$ at room temperature compared with theoretical IR spectra for the structures shown in Figure 8b.

L_b state is the lowest-energy state in the gas phase and in nonpolar solvents.[28] It is generally considered that the larger dipole moment of the L_a state leads to larger stabilization in polar solvents. Thus, the significant red shift in the UV spectrum of Ala-$TrpH^+$ should be related to the structure in which the NH_3^+ group interacts with the π cloud of indole strongly. This interpretation is supported by our preliminary time-dependent (TD) DFT calculation with B3LYP/6-31++G(d,p) level. The lowest unoccupied molecular orbital obtained by the calculation consists of the π orbital of the indole ring and also orbitals at the NH_3^+ group. This indicates the interaction between the NH_3^+ group and the π cloud of indole in the electronic excited state. The L_a excited state of Ala-$TrpH^+$ is found to be located much lower in energy than the L_b states. The stabilization of the L_a state should originate from this interaction between the NH_3^+ group and the π cloud of indole. It is revealed that the geometrical structure plays an important role in electronic structure of peptides in addition to the protonation site. Very recently, Stearns *et al.* performed IR-UV double resonance spectroscopy of protonated alanyltyrosine (Ala-$TyrH^+$) in a cold ion trap.[29] They found that the electronic band origin of Ala-$TyrH^+$ is shifted 650 cm^{-1} to the red of the lowest-energy band origin of protonated tyrosinylalanine (Tyr-$AlaH^+$) resulting from the stronger interaction between the NH_3^+ group and the π cloud of phenol side chain in Ala-$TyrH^+$. The geometrical structure of Ala-$TyrH^+$ revealed from IR-UV depletion spectrum is similar to the **IIIa** conformer of Ala-$TrpH^+$, which supports the argument for Ala-$TrpH^+$.

We also investigate the effect of temperature on the electronic spectrum to examine a conformational change. A UV photodissociation spectrum of Ala-$TrpH^+$ at 80 K is shown in Figure 6c. With an increase of temperature, the hot band appears at around 32 600 cm^{-1} and the band intensity at 35 000 cm^{-1} is enhanced. The enhanced band is in the same energy range as the 0−0 transitions of $TrpH^+$ and Trp-$GlyH^+$. Thus, it is

indicated that the band intensity enhancement at 35 000 cm^{-1} is ascribed to the increase of isomers in which the interaction between the NH_3^+ group and the π cloud of indole is weaker than that in the lowest-energy structure **IIIa**. The candidates are the extended conformer **IIIc** in which the NH_3^+ group points away from the indole ring and the isomer **IIIb** formed in the proton transfer reaction of **IIIa**. A definitive assignment of the band at 35 000 cm^{-1} has not yet been made; however, the spectral change in Figure 6c is ascribed to the thermally induced conformational change. To examine detailed structure and reactivity of Ala-$TrpH^+$, it is necessary to perform IR and UV spectroscopies at various temperatures.

3.2. IR Photodissociation Spectroscopy. We have discussed the spectral changes related to the fundamental properties of amino acid and dipeptides in the previous sections. To understand these changes in more detail, the geometrical structures of $TrpH^+(CH_3OH)$ and Ala-$TrpH^+(CH_3OH)$ are examined by IR photodissociation spectroscopy. An IR photodissociation spectrum of $TrpH^+(CH_3OH)$ at room temperature is shown in Figure 7. The spectrum is measured in the region of 2650−3750 cm^{-1} by monitoring $TrpH^+$ formed by an evaporation of a methanol molecule. Three sharp bands at 3666, 3556, and 3502 cm^{-1}, and broad bands below 3350 cm^{-1} are observed. In optimized structures shown in Figure 8a, a methanol molecule bounds to the NH_3^+ group. In the **IVa** and **IVb** conformers, three hydrogen atoms in the NH_3^+ group are bound to the oxygen of a methanol molecule, the CO moiety of the carboxyl group, and the π cloud of indole, respectively. A difference between these conformers comes simply from a different orientation of a methanol molecule. The theoretical IR spectra of **IVa** and **IVb** are almost the same with each other. Thus, only the theoretical IR spectrum of the **IVa** conformer is shown in Figure 7. Both of **IVc** and **IVd** conformers have a free ammonium N−H stretch, and a methanol molecule in these conformers bounds to the NH_3^+ group. In the case of the **IVc** conformer, the hydrogen of the methanol molecule bounds to the CO moiety of the carboxyl group, whereas it bounds to the π cloud of indole in the case of the **IVd** conformer. By comparison with theoretical IR spectra, the three bands at 3666, 3556, and 3502 cm^{-1} are respectively assigned to the free methanol O−H stretch, the free COO−H stretch, and the free indole N−H stretch of **IVa** and **IVb**. The bands corresponding to the free ammonium N−H stretch and the hydrogen-bonded methanol O−H stretches, which are expected to appear in the case of **IVc** and **IVd**, are hardly observed in the experimental IR spectrum, indicating that they are not the dominant isomers. These observations suggest that the broad feature below 3350 cm^{-1} is attributed to superimposition of several bands corresponding to the bound ammonium N−H stretches, which are bonded to the CO moiety of the carboxyl group, the π cloud of indole, and a methanol molecule, respectively.

IR photodissociation spectrum of Ala-$TrpH^+(CH_3OH)$ at room temperature is shown in Figure 9. Four sharp bands at 3667, 3569, 3505, and 3414 cm^{-1}, a weak band at around 3186 cm^{-1}, and three broad bands at 3109, 2961, and 2878 cm^{-1} are observed. In optimized structures shown in Figure 8b, a methanol molecule bounds to the NH_3^+ group as in the case of $TrpH^+(CH_3OH)$. In the case of **Va** and **Vb** conformers, which differ by an orientation of a methanol molecule, the NH_3^+ group is bound to a methanol molecule, the CO moiety of the amide group, and the π cloud of indole, respectively. In the case of **Vc** and **Vd** conformers, which have a free ammonium N−H stretch, a methanol molecule bounds to the NH_3^+ group. The hydrogen of the methanol molecule bounds to the CO moiety of the amide group in the **Vc**, whereas it bounds to the π cloud of indole in **Vd**. Theoretical IR spectra of **Va** and **Vb** are found to reproduce well the experimental IR spectrum of Ala-$TrpH^+(CH_3OH)$. The four bands at 3667, 3569, 3505, and 3414 cm^{-1} are assigned to the free methanol O−H stretch, the free COO−H stretch, the free indole N−H stretch, and the free amide N−H stretch, respectively. The three broad bands at around 3186, 3109, and 2961 cm^{-1} are assigned to the bound ammonium N−H stretches, which are bonded to the CO moiety of the amide group, the π cloud of indole, and a methanol molecule, respectively. The band at 2878 cm^{-1} is assigned to the methanol C−H stretches. The bands corresponding to the hydrogen-bonded methanol O−H stretch and the free ammonium N−H stretch are hardly observed in the experimental IR spectrum, indicating that **Vc** and **Vd** are minor isomers. Our results imply that the interaction between the NH_3^+ group and the indole ring in Ala-$TrpH^+$ is stronger than the hydrogen bond with methanol so that the solvation of single methanol does not change the conformation of the side chain with respect to the indole ring. It is expected that an increase of the number of the methanol may change the conformation of the Ala-$TrpH^+$. It is worth noticing that the above arguments support the fact that the strong interaction of the proton attached to the N terminal of alanine and indole ring induces the large spectral shift in the UV spectrum in Figure 6.

4. Conclusions

We have constructed a temperature-variable photodissociation spectrometer to examine the structure and reactivity of gas phase biological molecular ions at various temperatures. UV photodissociation spectra of Ala-$TrpH^+$ exhibit the significant red shift with respect to the S_1-S_0 transition of $TrpH^+$ and Trp-$GlyH^+$. The spectral change has been ascribed to the stabilization of the L_a excited state due to the strong interaction between the NH_3^+ group and the π cloud of indole resulting from the flexible geometrical structure. We have also observed the thermally induced conformational change in Ala-$TrpH^+$. IR photodissociation spectra of solvated ions show that Ala-$TrpH^+(CH_3OH)$ has the closed structure, which is consistent with the large spectral shift in UV spectrum of bare dipeptide.

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