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Infrared Multiple Photon Dissociation Spectra of Proline and Glycine Proton-Bound Homodimers. Evidence for Zwitterionic Structure

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Protonation and proton transfer are very common in biological processes,[1] and many studies involving proton transfer in amino acids have been carried out.[2] Proton transfer from the carboxyl group of an amino acid to its amino group results in the zwitterionic structure and such structures play an extremely important role in the biochemical function of amino acids, peptides, and proteins.[3] Amino acids are well-known to exist as zwitterions in the solid state and aqueous solution, but in the gas phase their structures are in the canonical form. Interaction with other molecules or ions is thus crucial to stabilization of the zwitterionic form of amino acids.[4] Recently, limited direct IR spectroscopic evidence has shown the existence of zwitterionic structure in the gas phase.[5]

In the present work, the structures of Gly and Pro proton-bound homodimers have been investigated using infrared multiple photon dissociation (IRMPD) spectroscopy. This is a very useful tool to elucidate clearly the structures of both ions and ionic clusters in the gas phase in combination with theoretical calculations.[5,6] Experiments have been carried out using the free electron laser (FEL) at CLIO coupled to an electrospray ionization-ion trap mass spectrometer (Bruker Esquire3000+). The FEL facility has been described in detail previously.[6b] The IRMPD spectrum of $(Gly)_2H^+$ is shown in Figure 1. The dominant photodissociation channel of the dimer during IRMPD is loss of a neutral amino acid with the protonated amino acid as the abundant fragment ion. To identify the structure, the calculated vibrational spectra of the three most stable isomers are included in Figure 1; the structures and energies are shown in the Supporting Information, Figure S1 and Table S1. These spectra have been determined at the B3LYP/6-311+G(d, p) level,[7] with frequencies scaled by a factor of 0.985. The calculated band frequencies and intensities were convoluted assuming a Lorentzian profile with a 50 cm^{-1} full-width at half-maximum.

For glycine, GG-CS01 is the most stable isomer[8] in which the protonated amino group forms intramolecular and intermolecular hydrogen bonds with the carbonyl oxygens in both protonated Gly and neutral Gly. The calculated binding energy between protonated Gly and neutral Gly is 29.7 kcal mol^{-1}, based on the most stable protonated Gly and neutral Gly, at the MP2(full)/6-311++G(2d, 2p)// B3LYP/6-311+G(d, p) level, and the entropy change is −34.2 cal mol^{-1} K^{-1} at the B3LYP/6-311 G(d, p) level. While GG-CS02 had previously been considered to be the most stable isomer,[9,5b] in fact, the relative energetics imply that GG-CS01 is more stable than GG-CS02 by 1.3 kcal mol^{-1} at 298 K at the level of theory employed here. In addition, the entropy change for its formation is also 1.5 cal mol^{-1} K^{-1} less favorable. GG-ZW01 is very similar in structure to GG-CS01, with the exception that an intramolecular proton transfer from the hydroxyl to the amino group occurs in the neutral glycine moiety such that its structure becomes zwitterionic. The binding energy of 28.0 kcal mol^{-1} is 1.7 kcal mol^{-1} less than that of the most stable isomer.

As can be seen in Figure 1, the experimental IRMPD spectrum matches very well with that calculated for GG-CS01. The sharp peak at 1191 cm^{-1} may be assigned to the bending vibration of the free OH of protonated Gly. For the other isomers, this band is also located at nearly the same position. The strong band at 1439 cm^{-1} is due to the same mode of the hydrogen-bonded OH group of neutral Gly. This is strong evidence that the dominant species is GG-CS01, because there is no strong IR active band in this range in GG-CS02. The hydroxyl group of the neutral Gly moiety in GG-CS02 is free, as is that in the protonated Gly, making their band positions very similar. This is confirmed by the presence of calculated bands at 1176 and 1168 cm^{-1} for the protonated Gly and neutral Gly moieties. The weak band at 1523 cm^{-1} is due to the umbrella vibration of the ammonium group. The strongest band at 1757 cm^{-1} and its shoulder at 1808 cm^{-1} correspond to the stretch vibrations of the two carbonyl groups, respectively. These are also consistent with the corresponding calculated values of 1737 and 1789 cm^{-1}. From the present IRMPD spectrum in the mid-infrared region GG-CS01 can easily be seen to be the most stable isomer. $(Gly)_2H^+$ has also recently been studied by IRMPD in the 3000−3800 cm^{-1} range.[5b] This experimental spectrum had only two peaks, which were used to assign the structure as one analogous to GG-CS02. According to our calculated spectra, the experimentally observed bands actually fit better with GG-CS01 than GG-CS02 in the H-stretching region, as shown in Figure S2. According to the calculated energies, presuming a Boltzmann distribution of possible isomers, the amounts of GG-CS01, GG-CS02, and GG-ZW01 are approximately 94, 5, and 1%, respectively, as shown in Table S1. From the experimental and calculated results, it is obvious that the dominant species is the most stable isomer, GG-CS01. However, the presence of other species cannot be excluded.

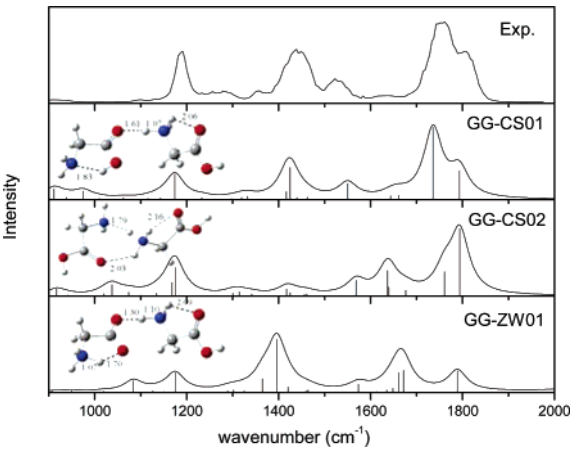

Figure 1. IRMPD spectrum of $(Gly)_2H^+$ and calculated spectra of the three most stable isomers (GG-CS01, GG-CS02, and GG-ZW01).

As a secondary amine, proline is unique among the natural code amino acids, which results in more facile formation of a zwitterionic structure.[10] The IRMPD spectrum of $(Pro)_2H^+$ is shown in Figure 2, together with the calculated spectra of the most stable forms of

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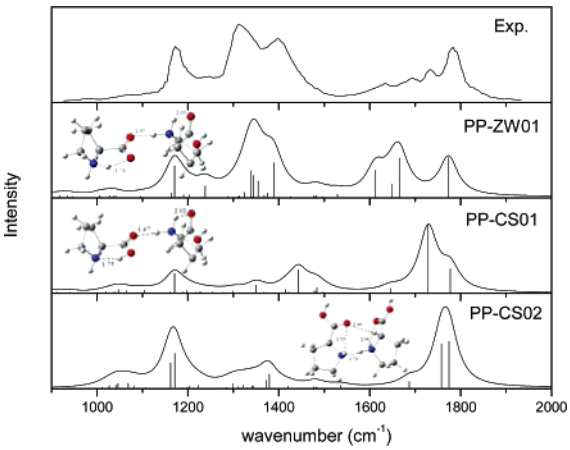

Figure 2. IRMPD spectrum of $(Pro)_2H^+$ and calculated spectra of the three structurally distinct isomers (PP-ZW01, PP-CS01, and PP-CS02).

three structurally distinct isomers. Each of the proton-bound proline isomers consists of a protonated and a neutral proline; however, it is of interest to know whether the neutral proline adopts a zwitterionic structure. PP-CS01 is the most stable nonzwitterionic isomer in which the proton is bound to the amine moiety that then forms intramolecular and intermolecular hydrogen bonds with the carbonyl oxygens in both the protonated and neutral proline moieties. Proline ring-puckering isomers exist (PP-CS01a, b, c, structures in Figure S3 and energies given in Table S1). However the species shown are the most stable by about 0.5 kcal mol^{-1}. Each isomer of a series has nearly the same IR spectrum (Figure S4). Proton transfer from the carboxyl group to the secondary amine results in the new, most stable isomer, PP-ZW01, which contains a zwitterionic Pro. The energy of PP-ZW01 at 298 K is 2.0 kcal mol^{-1} lower than that of PP-CS01 at the B3LYP/6-311+G(d, p) level and 2.9 kcal mol^{-1} lower from the single-point calculations. PP-CS02, the next most stable structural type, has an energy much higher than that of PP-ZW01. Many other isomers have been calculated, and the structures of some relatively stable isomers and the calculated spectra are given in Figures S3 and S4.

In the experimental spectrum, most of the bands are in good agreement with those of the calculated spectrum of PP-ZW01. The band at 1173 cm^{-1} corresponds to the bending vibration of the free OH, which is present in the calculated spectra of all isomers, including those for $(Gly)_2H^+$. The strongest band, at 1309 cm^{-1}, may be assigned to the combination of the NH_2 and CH_2 twisting motions in the protonated and zwitterionic proline moieties. The band at 1396 cm^{-1} is the symmetric stretching vibration of the carboxylate group, which is characteristic of all zwitterionic amino acids and can be considered to be diagnostic of a zwitterionic structure.[5a,11] The broad, weak feature at 1636 cm^{-1} can be assigned as the scissors modes of NH_2 in both protonated Pro and zwitterionic Pro. The asymmetric stretch of the carboxylate group of zwitterionic proline appears at 1692 cm^{-1}, while the carbonyl stretch of protonated proline occurs at 1783 cm^{-1}. In contrast to the experimental spectrum, the calculated intensity of the band in zwitterionic Pro is stronger than that in protonated Pro. This discrepancy between the predicted and observed ratios of these two features is not surprising, and does not necessarily invalidate the assignment of the dominant structure as PP-ZW01, since it is well-known that, for a variety of reasons, peak ratios in IRMPD spectra often deviate significantly from the calculated one-photon absorption spectra.[6]

The band at 1733 cm^{-1} cannot be assigned to any vibrational mode of PP-ZW01. However it corresponds very well to the strongest vibration of PP-CS01, which is the stretching vibration of the carbonyl group of neutral proline in PP-CS01. The calculated IR intensity of this band is about two times stronger than the strongest peak of PP-ZW01 in this range. This indicates that PP-CS01 is also present under the experimental conditions used. While the predicted approximate population ratio of PP-ZW01 and PP-CS01 of 95:5 cannot be accurately determined on the basis of IRMPD intensities, it appears that the PP-ZW01 conformer is most abundant, in accordance with the calculations.

In conclusion, a zwitterionic structure of proline has been confirmed to exist in $(Pro)_2H^+$ in the gas phase by IRMPD spectroscopy, together with the theoretical calculations. A new most stable isomer of $(Gly)_2H^+$ has also been demonstrated to be the dominant species.

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Supporting Information Available: Complete refs 6a, 6b, and 7; Figures 1S−4S, Table S1. This material is available free of charge via the Internet at http://pubs.acs.org.

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