Structures of Aliphatic Amino Acid Proton-Bound Dimers by Infrared Multiple Photon Dissociation Spectroscopy in the 700−2000 cm^{-1} Region

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Structural aspects of proton-bound dimers composed of amino acids with aliphatic side chains are investigated using infrared multiple photon dissociation (IRMPD) spectroscopy and electronic structure calculations. Features in the IRMPD spectra in the 700−2000 cm^{-1} range are due primarily to C═O stretching, NH_2 bending, and COH bending. It was possible to distinguish between isomeric structures by comparing the experimental IRMPD spectra and those predicted using B3LYP/6-31+G(d,p). It was possible, based on the calculations and IRMPD spectra, to assign the experimental spectrum of the glycine proton-bound dimer to a structure which was slightly different from that assigned by previous spectroscopic investigations and in agreement with recent thermochemical studies. Since all proton-bound dimers studied here, composed of the different amino acids, have very similar spectra, it is expected that they also have very similar lowest-energy structures including the mixed alanine/glycine proton-bound dimer. In fact, the spectra are so similar that it would be very challenging to distinguish, for example, the glycine proton-bound dimer from the alanine or valine proton-bound dimers in the 700−2000 cm^{-1} range. According to the calculated IR spectra it is shown that in the ∼2000−3200 cm^{-1} range differentiating between different structures as well as different proton-bound dimers may be possible. This is due mainly to differences in the asymmetric stretch of the binding proton which is predicted to occur in this region.

1. Introduction

In recent years there has been an enormous amount of work dedicated to studying the effects of noncovalent interactions such as normal and ionic hydrogen bonding on the structures of molecules which are of biological and pharmacological interest[1] as well as supermolecular structures[2−4] in aqueous solutions. The combination of experiment accompanied with ab initio calculations has provided a growing wealth of fundamental knowledge in this area.[5,6]

Of equal importance to knowing the structures of the aqueous phase molecules is understanding the role that solvent has in this structure. To this end, rotational, vibrational, and electronic spectra of molecules isolated in the gas phase[7,8] or vibrational spectra of species frozen in cryogenic matrices[9−11] have helped in determining the solvent-free structures. For amino acids such as glycine,[9,12,13] alanine,[10,14] valine,[11,15] and arginine,[16] non-zwitterion structures are observed to be the only structures present in the gas phase and isolated in solid argon matrixes, and theory predicts the non-zwitterion structure to be the lowest in energy.

Protonation reactions are fundamental in aqueous solutions containing compounds with heteroatoms such as amino acids. In fact, protonation of biological compounds is of a great interest as it is the initiating step in hydrolysis of amides, peptides, and proteins at biological pH.[17] Protonation obviously has an important effect on the electronic and dynamic structure of proteins as well as their conformational structure. Their biological activities are dependent upon three-dimensional structures, and therefore protonation plays an important role in the biological activity of proteins. Completely understanding the role of particular conformations in biological activity may be facilitated by studying the solvent-free fundamental building blocks and is possible because of existing modern ionization methods in mass spectrometry. Gas phase studies of proteins can be useful to determine the role of protons in stabilizing protein conformation and can also suggest the role of solvent on protein conformation. For example, ion mobility measurements have been used to study helix formation in the gas phase for a series of alanine/glycine-based peptides.[18] In this study, solvent-free peptide ions were produced by electrospray and their conformations determined using ion mobility mass spectrometry. The authors found a reduction in the amount of helix due to interaction of residues with polar side chains for the Ac-$A_3G_{12}KH^+$ peptide, possibly due to hydrogen bonding between the backbone and polar side chains that stabilize the nonhelical globular conformations.

Numerous studies have been performed on relative proton affinities of amino acids.[19−26] Temperature-dependent equilibrium constants and binding energies for amino acid proton-bound dimers, such as the glycine proton-bound dimer, have been recently studied using pulsed-ionization high-pressure mass spectrometry (PHPMS).[27] Comparing the experimental thermochemistries with those predicted by ab initio methods can provide evidence for structural aspects of these fundamental biological species.

Proton-bound dimers are also of great interest since they contain a principle intermolecular interaction—the ionic hydrogen bond—noncovalent interactions with strengths of up to about 150 kJ mol^{-1}. They have received much attention in the past mostly from thermochemical and computational studies.[28] Most studies are geared toward understanding the structural aspects

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of the proton-bound dimers as well as their strengths. Infrared spectroscopy is a more sensitive technique for studying the structures of any molecular or ionic species. Proton-bound dimers have been the topic of many recent infrared multiple photon dissociation (IRMPD) spectroscopic studies.[29−36] Dimers of amino acids which are bound by protons or metal ions are important from a biochemical point of view due to proton and metal ion mobility issues as well as the interest in the strong ionic hydrogen bonding or metal ion binding that exists in these clusters. Small proton-bound dimers are models for larger systems where strong ionic hydrogen-bonding exists, such as in proteins. A few IRMPD spectroscopic studies of protonated and solvated amino acids,[37] amino acid proton-bound dimers,[38] and complexes of amino acids with sodium ion[39] in the gas phase have appeared recently. Most recently using IRMPD spectroscopy and computational methods, Bush et al.[40] have observed gas phase zwitterionic arginine when it is bound to larger metal ions such as Na^+ and K^+. Here we report on the IRMPD spectra of amino acid proton-bound dimers with aliphatic side chains in the 700−2100 cm^{-1} range as well as the mixed alanine/glycine proton-bound dimer. The spectra are compared to those calculated by DFT methods and discussed in terms of the structures of the proton-bound dimers.

2. Methods

2.1. Experimental Details. The combination of a Bruker Esquire 3000 quadrupole ion trap mass spectrometer equipped with an electrospray source coupled to the mid-infrared free-electron laser (FEL) at Centre Laser Infrarouge d'Orsay (CLIO)[41,42] was used and has been described previously.[43] Solutions containing 0.05 M of glycine, 0.03 M of alanine, 0.03 M valine, and a mixture of glycine/alanine with 1:1 ratio were prepared in 18 Mohm (Millipore) water. The amino acids were obtained from Aldrich and have been used without further purification. The aqueous amino acid solutions were electrosprayed into a Bruker Esquire 3000 quadrupole ion trap mass spectrometer, and accumulation times varied between 150 and 300 ms. There was no organic phase or acid added to the solution. It was found that by adding acid or an organic layer to the solution, the ion intensity for our proton-bound dimers was depleted, and the protonated amino acids were the main species electrosprayed. The ions of interest, proton-bound dimers, were isolated by resonantly ejecting ions of all other masses. The mass spectrometer was modified[43] to include a Brewster-angle window through which passes the monochromatic radiation with a bandwidth of 0.3−0.5% of the spectral bandwidth. The FEL was scanned using <5 cm^{-1} increments, and the experimental IRMPD spectra in the 700−2100 cm^{-1} energy range were recorded. Ions were irradiated for between 200 and 500 ms with the CLIO FEL. If the particular wavelength of the FEL is resonant with a fundamental mode of the trapped ion, dissociation is observed. On the contrary if the FEL is tuned such that there is no absorption, no dissociation of the ion is observed. The IRMPD spectra reported are the extents of dissociation plotted against the wavenumber value of the radiation impinging on the ions from the FEL. The resulting IRMPD action spectra were not normalized for the power of the free electron laser.

2.2. Computational Details. The Gaussian 03 suite of programs was used.[44] Geometries were optimized and vibrational spectra were calculated using the B3LYP hybrid density functional method and the 6-31+G(d,p) basis sets. For comparison, the lowest-energy structure of the glycine proton-bound dimer was calculated also using 6-311++G(d,p) basis set. Many different structures of the proton-bound dimers were considered including zwitterionic structures. Here we show the structures and spectra of the lowest-energy proton-bound dimers. The calculated frequencies were scaled using a scale factor of 0.96.[45] The 298 K free energy differences relative to the lowest-energy isomer are reported for all proton-bound dimers using B3LYP/6-31+G(d,p). For comparison, single-point calculations were computed using the 6-311+G(2df,p) basis, and the thermochemistry and entropies were taken from the B3LYP/6-31+G(d,p) calculations (herein denoted B3LYP/ 6-311+G(2df,p)//B3LYP/6-31+G(d,p)). These latter calculations were done on the glycine proton-bound dimer and the alanine-glycine mixed proton-bound dimer.

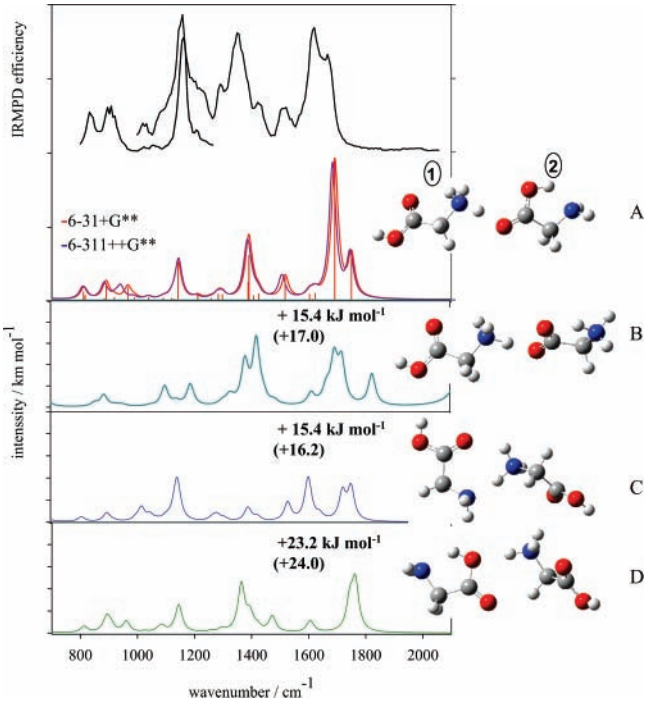


Figure 1. IRMPD spectrum of the glycine proton-bound dimer as well as the 6-31+(d,p) predicted spectra for the four lowest-energy structures. B3LYP/6-311+G(2df,p)//B3LYP/6-31+G(d,p) relative free energies are in parentheses.

3. Results and Discussion

3.1. Electrospray and IRMPD of Protonated Amino Acid Dimers. Electrospraying solutions of glycine, alanine, or valine mainly produced the protonated monomers although there was significant intensity for the proton-bound dimer as well as a little trimer in all cases. For the solution containing both glycine and alanine, the *m*/*z* 165 peak corresponding to the mixed proton-bound dimer was smaller than the *m*/*z* 151 and 179 peaks corresponding the glycine and alanine proton-bound dimers, respectively. The proton-bound dimer intensities were small, $<5 \times 10^5$ counts for the homogeneous proton-bound dimers and $\sim 10^4$ counts for the alanine/glycine proton-bound dimer. Upon irradiation of the isolated proton-bound dimers with the CLIO FEL the only dissociation observed was loss of neutral amino acid as expected. For the alanine/glycine proton-bound dimer, loss of neutral glycine was the only dissociation observed. The results for each amino acid proton-bound dimer are discussed separately.

3.2. Glycine Proton-Bound Dimer. The experimental IRMPD spectrum of the glycine proton-bound dimer is shown in Figure 1 along with the four lowest-energy glycine proton-bound dimer structures and their predicted spectra at the B3LYP/6-

TABLE 1: Experimental and Calculated IR Absorption Wavenumbers (in cm^{-1}) for Glycine Proton-Bound Dimers[a]

vibrational modes	exptl IRMPD (cm^{-1})	predicted spectrum (**A**)	neutral glycine[10]
CO stretch (g1)	1666 sh	1751	1779
CO stretch (g2)	1620	1692	–
NH_3 d-deform (g1)	1521	1624	1630
NH_2 bend (g2)		1604	
NH_3 s-deform	1435 sh	1517	–
COH bending/CH_2 bend (g2)	1356	1391	–
COH bend/CC stretch (g1)		1388	
CH_2 s-deform (g2)	1290 sh	1299	–
CH_2 s-deform (g1)		1284	
C−O str/COH bend (g1)	1158	1190	–
COH oop bend (g2)	1032	967	–
NH_2 wag/CH_3 d-deform (g2)	908	892	–
NH_2 wag/CC str (g2)	832	811	–

[a] sh = shoulder, g1 = glycine labeled 1 in Figure 1, g2 = glycine labeled 2 in Figure 1.

31+G(d,p) level and basis set. The differences in 298 K free energies are also shown compared to structure **A**. The first three of these structures are the same as those found by Raspopov and McMahon[27] and are similar in relative energies. They found structures **B** and **C** to be 12.8 and 12.7 kJ mol^{-1} higher in free energy (MPW1PW91/6-31+G*) than structure **A**. Structure **D** in Figure 1 was determined to be 23.2 kJ mol^{-1} higher in energy and has not been previously reported. The B3LYP/6-311+G-(2df,p)//B3LYP/6-31+G(d,p) calculated free energies also confirm the energetic ordering of the proton-bound dimers (see Figure 1). The symmetric double zwitterion structure,[27] by our calculations, is 42 kJ mol^{-1} higher in free energy than **A**. Also shown in Figure 1 is the computed IR spectrum using the 6-311++G(d,p) basis set, also scaled by 0.96, showing that there is no dependence on the basis set of the computed IR spectrum.

Comparison of the experimental IRMPD spectrum and the calculated IR spectra shows that there is much better agreement between the most stable structure, **A**, and the experimental IRMPD spectrum. This better agreement between the experimental spectrum and that predicted for structure **A** in itself does not absolutely confirm the structure of the proton-bound dimer to be that of **A**. Clearly there is a significant red-shift of the experimental spectrum with respect to the predicted spectrum. However, comparison of the experimental spectrum for the glycine proton-bound dimer with the alanine and valine proton-bound dimers (section 3.6) shows a clear similarity, and all of the proton-bound dimers studied here are most likely to have a similar structure about the central part of the proton-bound dimer.

It should be noted that based on our computed value for ΔG between structures **A**, **B**, and **C**, both **B** and **C** would be expected to contribute only about 0.2% to the mixture at 298 K. The C═O stretching bands at 1666 and 1620 cm^{-1} observed in the experimental IRMPD spectrum are in agreement with those calculated for structure **A** (see Table 1 and Figure 1). It is expected that the C═O stretch is red-shifted from the neutral position since the C═O bond is weakened due to the positive charge. The C═O group involved in the ionic hydrogen bond is expected to be more red-shifted since it is bound strongly to the proton. Other assignments based on comparison between the experimental spectrum, the predicted spectra, and from typical assignments of neutrals are made in Table 1. In this table, the frequencies for neutral glycine monomer are also shown from Stepanian and co-workers.[9] In their work different conformers of neutral glycine were observed based on matrix isolation infrared spectra.

Oh et al.[38] have recorded an IRMPD spectrum of electrosprayed glycine proton-bound dimers using an OPO laser in the 3050−3800 cm^{-1} range at 27 °C. They assigned the species responsible for the infrared spectrum to structure C in Figure 1. In the inset of Figure 2 is a comparison of the computed spectra for structures **A**, **B**, and **C** as well as the experimental spectrum of Oh et al.[38] Price et al.[46] also determined that structure **C** was the lowest-energy structure using MP2/3-21G-(d,p). However, neither group had considered structure **A** for the proton-bound dimer of glycine. We repeated the calculation on structure **A** using MP2/3-21G*[46] and found that it is lower by a similar amount, 14.1 kJ mol^{-1}. Structure **A** must not have been considered by these previous authors. It is apparent by inspecting the inset of Figure 1 that their experimental IRMPD[38] spectrum is at least as comparable to that predicted for structure **A**. In their spectrum the two observed features are the OH stretch (of g1) at about 3580 cm^{-1} and the NH stretch (of g1) at 3370 cm^{-1}. Also predicted to occur in this experimental region are strong bands at 3180 cm^{-1} (NH str of g1) and at 3040 cm^{-1}

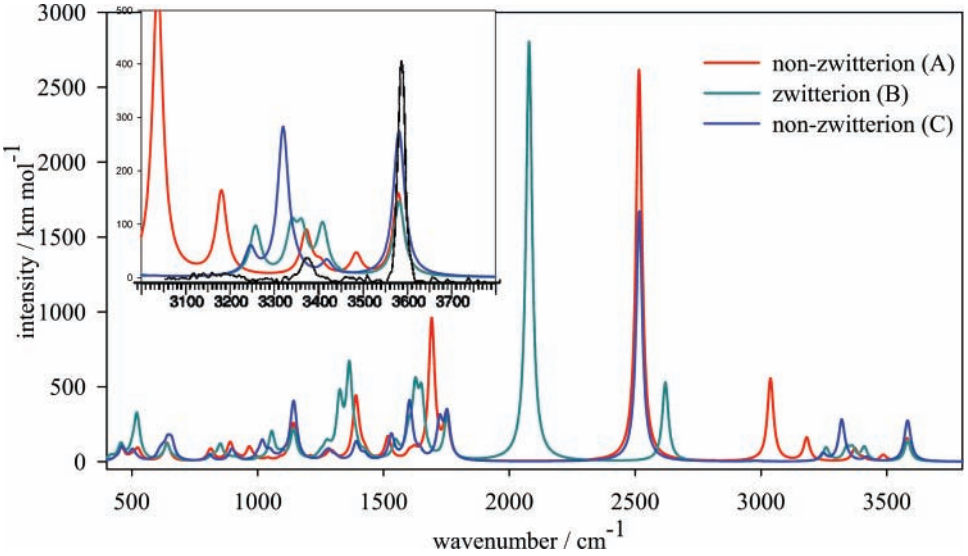


Figure 2. Calculated infrared spectra for the three lowest-energy structures for the glycine proton-bound dimer also showing the 2000−3800 cm^{-1} region where it may be more likely to be able to distinguish between isomeric structures. In the inset the experimental IRMPD spectrum from Oh et al.[38] is compared with the computed spectra.

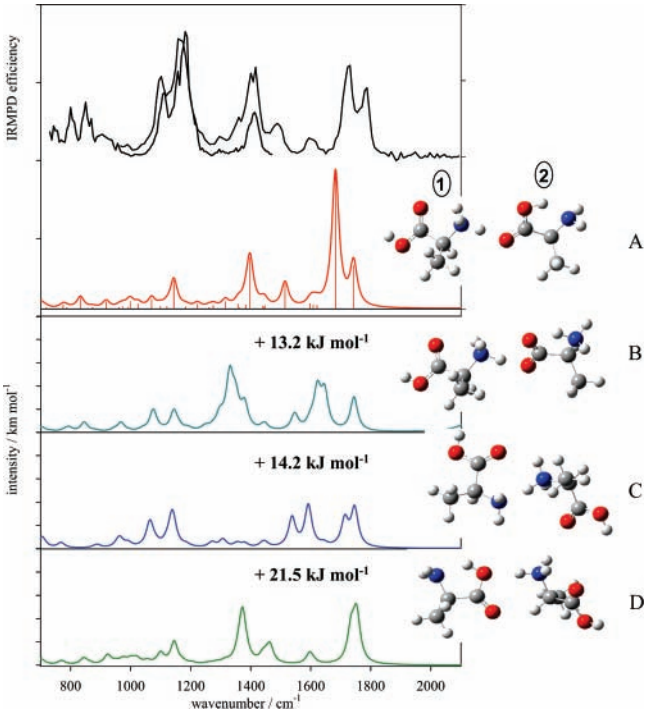


Figure 3. IRMPD spectrum of the alanine proton-bound dimer as well as the 6-31+(d,p) predicted spectra for the four lowest-energy structures.

TABLE 2: Experimental and Calculated IR Wavenumber Positions (in cm^{-1}) for Alanine Proton-Bound Dimers[a]

vibrational modes	exptl IRMPD (cm^{-1})	predicted spectrum (**A**)	neutral alanine[10]
CO stretch (a1)	1787	1742	1787
CO stretch (a2)	1730	1683	
NH_3 d-deform (a1)	1593	1609/1621	
NH_2 bend (a1)		1598	
NH_3 s-deform	1489	1515	1642
COH bending (a2)	1416 (sh)	1397	
C−O str/COH bend (a1)	1182	1144	
NH_3 rock (a1)	1113 (sh)	1120	1117, 1153
C−N str (a2)		1098	
NH_2 wag (a2)	850	832	852
	756		
	743		

[a] sh = shoulder, a1 = alanine labeled 1 in Figure 1, a2 = alanine labeled 2 in Figure 1.

(OH str of g2) which are probably not observed due to the weakened power from the OPO laser at the limits of its range. Note that this OH stretch predicted to occur at 3040 cm^{-1} is red-shifted from a normal OH stretch due to hydrogen bonding with the amino nitrogen. Based on the additional computational data, our infrared spectrum in the 800 to 2000 cm^{-1} range, and the recent equilibrium studies[27] it is clear that the structure of the glycine proton-bound dimer is most likely that of structure **A**.

Also shown in Figure 2 is the computed complete IR spectrum from 400 to 3800 cm^{-1}. It would be beneficial to have data in the 2000 to 3200 cm^{-1} range as this is probably the best region to be able to differentiate the probable structures of the amino acids. In this region, the asymmetric stretching vibration of the central binding proton between the two amino acids is expected to occur.

3.3. Alanine Proton-Bound Dimer. In Figure 3 are presented the four lowest-energy structures for the alanine proton-bound dimer which were predicted using B3LYP/6-31+G(d,p) as well as the difference in 298 K free energies compared to structure **A**. The structures are similar to those of the glycine proton-bound dimer where structure **A** is calculated to be the most stable. The predicted IR spectrum for structure **A** shows good

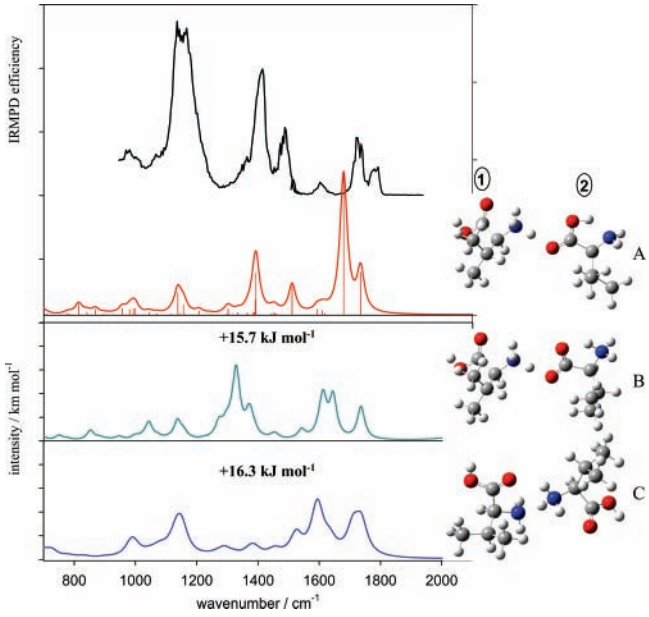


Figure 4. IRMPD spectrum of the valine proton-bound dimer as well as the 6-31+(d,p) predicted spectra for the three lowest-energy structures.

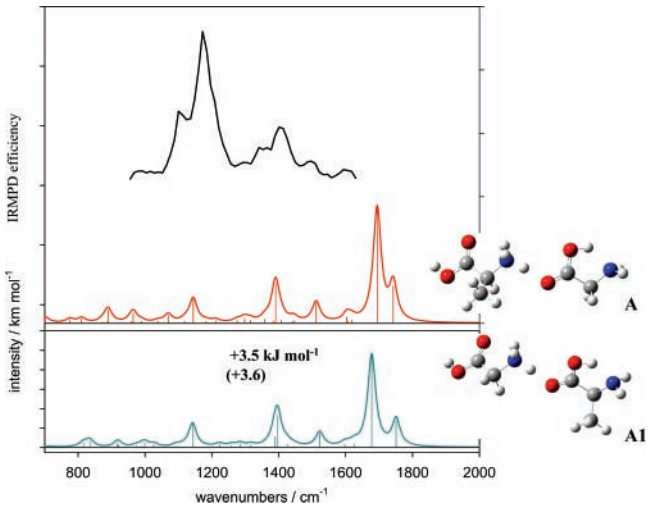


Figure 5. IRMPD spectrum of the heterogeneous alanine/glycine proton-bound dimer as well as the 6-31+(d,p) predicted spectra for the two lowest-energy structures. B3LYP/6-311+G(2df,p)//6-31+G-(d,p) relative free energies are in parentheses.

TABLE 3: Experimental and Calculated IR Absorption Frequencies (in cm^{-1}) for Valine Proton-Bound Dimers

vibrational modes	exptl IRMPD (cm^{-1})	predicted spectrum (**A**)	neutral valine[11]
CO stretch (v1)	1792	1735	1762
CO stretch (v2)	1721	1679	–
NH_3 d-deform (v1)	1602	1637	–
NH_2 bend (v2)		1594	
NH_3 s-deform	1488	1512	–
COH bend (v2)	1414	1392	–
COH bend/$CH_{2/3}$ deform (v1)	1166	1157	–
COH bend/NH_3 rock (v1)		1138	
OH oop (v2)	981	999/993	–
C−N str (v1)		980	

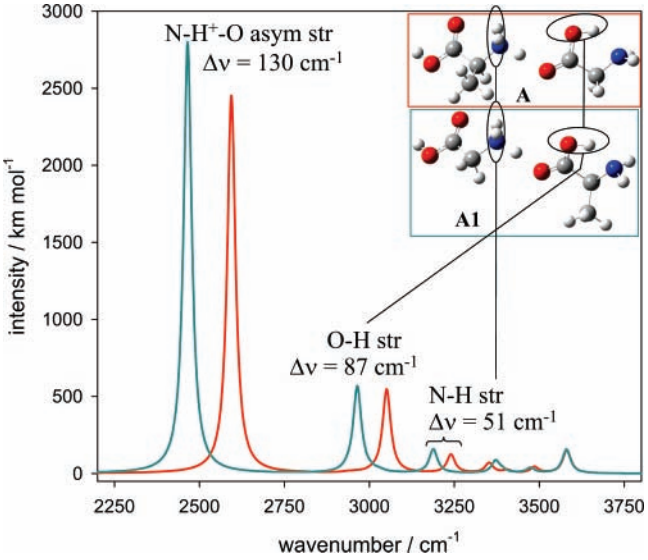
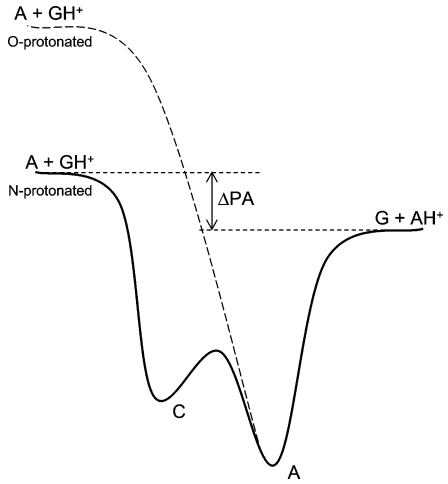


Figure 6. Calculated spectra for the two lowest-energy structures of the heterogeneous alanine/glycine proton-bound dimer in the 2200−3800 cm^{-1} region.

SCHEME 1



agreement with the experimental IRMPD spectrum in the 700−2000 cm^{-1} region (see Figure 3). Similar to the glycine proton-bound dimer, the C═O stretching absorptions in the alanine proton-bound dimer are slightly red-shifted compared to that of the neutral amino acid[10] (see Table 2).

It is puzzling that in the 1000−1300 cm^{-1} range the experimental intensities are consistently larger than predicted. The problem could be that the normal mode approximation neglects or underestimates coupling between the modes in this region and, perhaps, stretching motion associated with the proton. This larger than estimated intensity was also observed for the glycine proton-bound dimer (Figure 1) and the valine proton-bound dimer (Figure 5).

3.4. Valine Proton-Bound Dimer. The experimental IRMPD spectrum and calculated IR spectra for the lowest-energy structures of the valine proton-bound dimers are shown in Figure 4. The calculated most stable structure (structure **A**) is similar to the glycine and alanine proton-bound dimers discussed above. Absorptions for the C═O stretching at 1792 and 1721 cm^{-1} are observed in the experimental IRMPD spectrum which are in agreement with the calculated IR spectra at 1740 and 1679 cm^{-1}. For neutral valine the C═O stretching absorption is reported[11] to occur at 1762 cm^{-1}. In Table 3 a comparison of our calculated and experimental frequencies for the valine

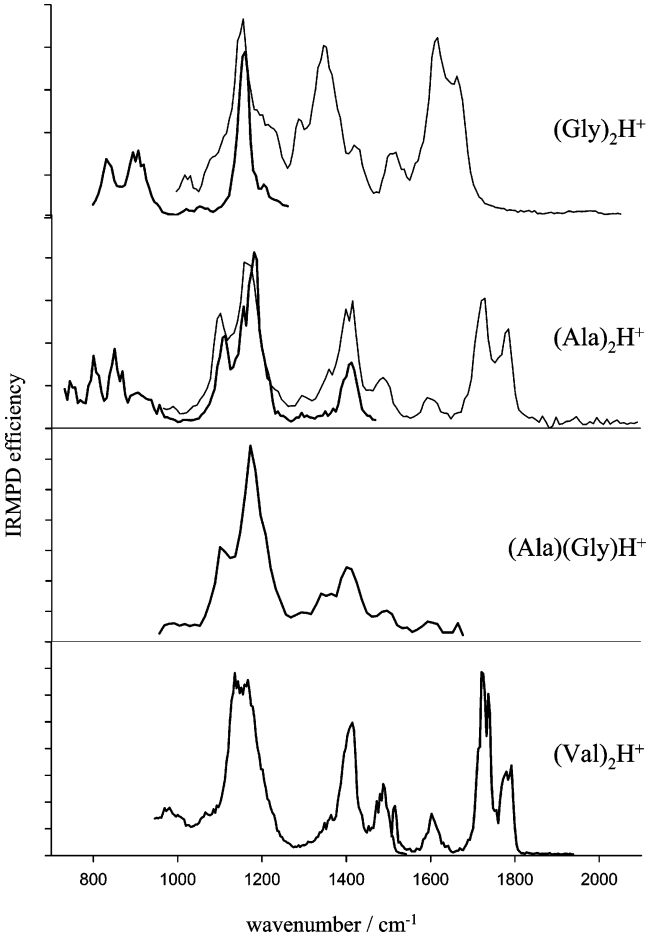


Figure 7. IRMPD spectra of all four proton-bound dimers in the 700−2000 cm^{-1} region which shows the similarity of the spectra in this region.

proton-bound dimer as well as experimental frequencies for neutral valine monomers are listed.

3.5. Alanine/Glycine Mixed Proton-Bound Dimer. For the mixed alanine/glycine protonated dimer, the IRMPD spectrum is only recorded at 950−1600 cm^{-1} due to time constraints on the FEL. The experimental IRMPD spectrum and the two lowest-energy structures, based on structure **A**, of the homogeneous glycine and alanine proton-bound dimers above are shown in Figure 5. In all, 10 alanine/glycine proton-bound dimer structures were optimized, and these two were found to be the lowest in energy and are similar to the lowest-energy structures for the homogeneous proton-bound dimers. It can be seen that the calculated IR spectra for both structures shown in Figure 4 are very similar and, based on this IRMPD spectrum, it is obviously impossible to differentiate between the two. In fact based on the predicted spectra, even if the experimental IRMPD spectrum in the full 700−2000 cm^{-1} range were available, it would not likely be possible to differentiate between these two species. From the calculated free energy difference, 3.5 kJ mol^{-1}, the ratio of these two species is predicted to be about 4:1, so structure **A1** would need to be considered in a mixture of the proton-bound dimers. By comparing the observed and predicted spectra as well as the experimental spectra for the homogeneous proton-bound dimers, the band observed at 1173 cm^{-1} is assigned to the C−O stretch/COH bend of amino acid 1 which is predicted to occur at 1144 cm^{-1} for both **A** and **A1**. The shoulder to the red at 1100 cm^{-1} is most likely due to the C−N str/CH_3 rock which is predicted to occur at about 1070 cm^{-1} for both isomers. The C−O stretch/COH bend for amino

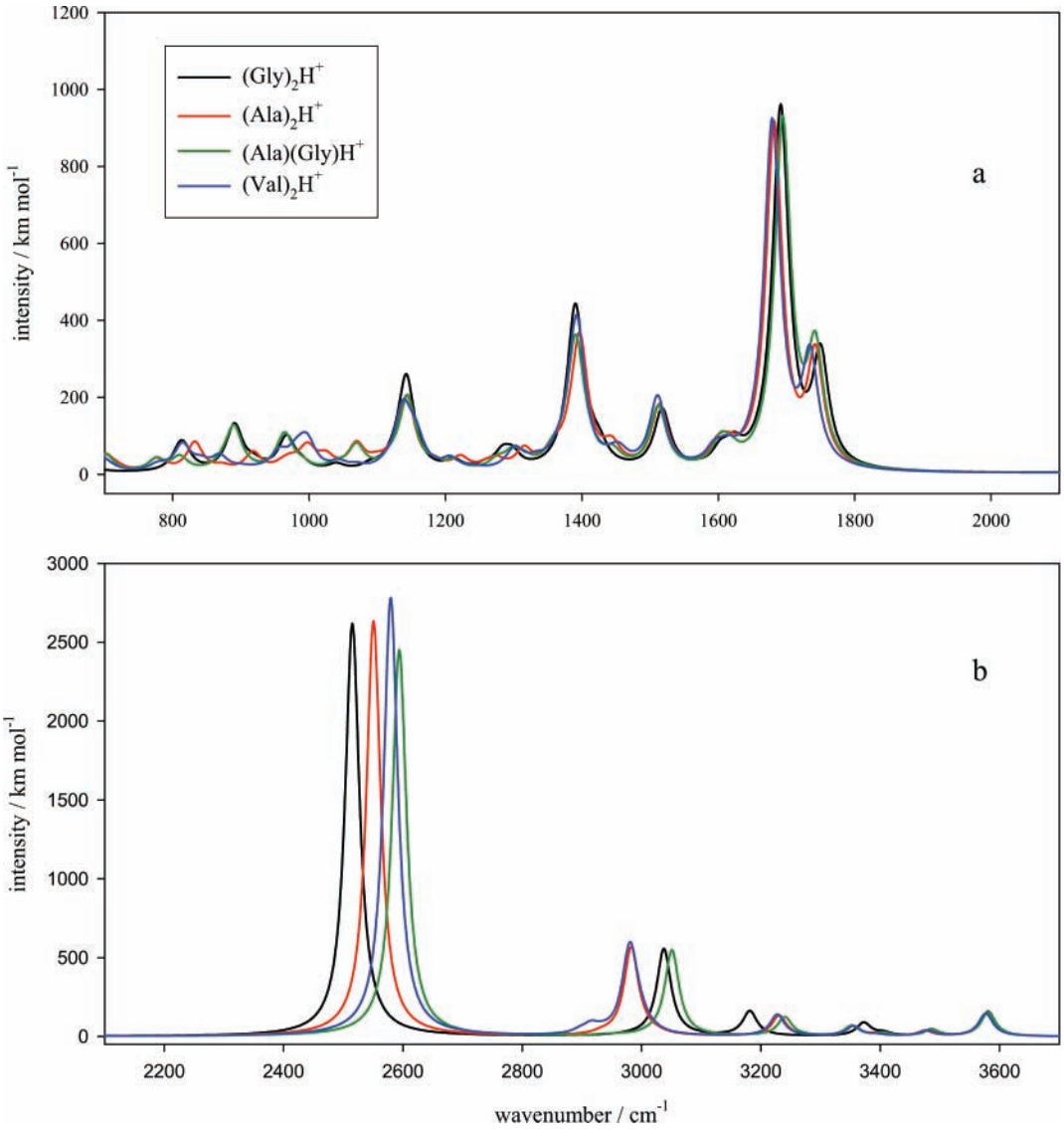


Figure 8. Calculated IR spectra for glycine, alanine, alanine/glycine, and valine proton-bound dimers showing that they are predicted to have very similar spectra in the 700−2000 cm^{-1} region (a) but that they might be discernible in the higher-energy region (b).

acid 2 in the proton-bound dimer is observed in the 1400 cm^{-1} region and is also predicted to occur at about 1400 cm^{-1} for both isomers.

As with the systems discussed above the best region of the infrared to differentiate between these two species is in the 2000−3100 cm^{-1} region. This is shown clearly in Figure 6 where the predicted spectra for these two isomeric structures are compared in the 2250−3750 cm^{-1} range. The asymmetric stretching vibration for the central proton is predicted to occur at 2595 and 2466 cm^{-1} for **A** and **A1**, respectively, and the hydrogen-bonded O−H stretch is predicted to occur at 3052 and 2965 cm^{-1} for **A** and **A1**, respectively. Similarly the N−H stretch is separated by some 50 cm^{-1}. Clearly in this region of the spectrum the two structures could be easily distinguished.

The lowest-energy structures of the proton-bound dimers presented here require some discussion. It is clear that the amino group is the most basic site of the amino acid, and based on this fact it might be expected that the proton-bound dimers of type **C** are the lowest in energy. On the basis of the experimental and computed spectra presented here, the preferred structure of the proton-bound dimers is the **A** type where the N-protonated amino acid is bound to the carbonyl oxygen of the second monomer. This can be explained qualitatively on the basis of strong electrostatic bonding. Simply, the strong ionic hydrogen bond binding structure **C** is not as strong as the ion-dipole interaction when the protonated amino acid interacts with the strong dipole of the neutral amino acid which is about 4.5[47] and 5.0[48] for glycine and alanine, respectively. Computational studies of mixed protonated dimers have shown that ion-dipole complexes are preferred over "normal" ionic hydrogen-bonded proton-bound dimers.[49] The mixed proton-bound dimer is an especially interesting example since the proton affinity of glycine is lower than that of alanine by 15 kJ mol^{-1}. The ion-dipole interaction between protonated glycine and alanine is stronger than that between protonated alanine and glycine such that **A1** is only 3.5 kJ mol^{-1} less stable than **A** for the mixed proton-bound dimer.

In studies which use the kinetic method to determine relative proton affinities of amino acids (i.e., ref 26), the conclusion that the lowest-energy structure is that of structure **A** should not affect these measurements for reasons discussed now. The mixed alanine/glycine proton-bound dimer, for example, will form N-protonated alanine and neutral glycine as its lowest-energy dissociation pathway. One might be tempted to think that structure **A** will dissociate to an O-protonated glycine and neutral alanine, but these products would be very much higher

than N-protonated glycine and neutral alanine. When this proton-bound dimer begins to dissociate to protonated glycine and alanine, it would most likely isomerize to the **C** type ions prior to dissociation as summarized in Scheme 1. This isomerization prior to dissociation has been predicted previously for dimethyl ether proton-bound dimers.[50] As long as the energy barrier for interconversion between **A** and **C** is low compared to the dissociation energies, the kinetics for dissociation producing N-protonated glycine and N-protonated alanine will be governed by this dissociation enthalpy, and the measurements can be used to determine the relative proton affinities. While a complete computation of the potential energy surface of dissociating amino acid proton-bound dimers is beyond the scope of this paper, it is the subject of a future study.

3.6. Comparison of the Aliphatic Amino Acid Proton-Bound Dimer Spectra. In Figure 7 the IRMPD spectra for glycine, alanine, and valine homogeneous proton-bound dimers as well as the mixed alanine/glycine proton-bound dimer are compared. These proton-bound dimers clearly have very similar spectra in this region which might make it difficult to distinguish between the different proton-bound dimers. The similarity in spectra probably reflects the similarity in structure about the central proton. The only real distinguishing feature is that, in the 1100 cm^{-1} region, the most intense feature for glycine is one band whereas for all the other proton-bound dimers there is a splitting of this feature due to the CH_3 rock/CN stretch which occurs in this region as well. This difficulty in distinguishing between amino acid proton-bound dimers is in fact predicted by the calculations as seen in Figure 8a. However, distinguishing between the proton-bound dimers composed of amino acids with different aliphatic side chains may be possible at higher frequencies as shown in Figure 8b due to the differing asymmetric stretching frequencies in 2400−2600 cm^{-1} range, which is most likely due to the difference in proton affinities between the ends of the amino acids which are bound by the proton. Also, in the 2900−3100 cm^{-1} region, it may be possible to distinguish between the proton-bound dimers due to the differing positions for the hydrogen-bonded N−H and O−H stretching absorption.

4. Conclusion

The IRMPD spectra of amino acid proton-bound dimers with aliphatic side chains in the 700−2100 cm^{-1} range have been recorded. The dominant absorptions in this range are C═O stretching and NH_2 bending as well as COH bending. According to computed IR spectra and the difference in computed free energies the most stable structures are determined for each of the glycine, alanine, and valine proton-bound dimers as well as the mixed alanine/glycine proton-bound dimer, and these proton-bound dimers are concluded to be very similar in structure.

For the glycine proton-bound dimer, structure **A** (Figure 1) is assigned to be the most dominant structure according to our IRMPD spectrum in the 700−2100 cm^{-1} range and by reanalyzing the spectrum of Oh et al.[38] in the 3050−3800 cm^{-1} range. Although in their paper[38] the structure for the proton-bound dimer was assigned to structure **C**, the calculated IR spectra for structure **A** shows a much better agreement with their spectrum as well as the one presented in this study. Furthermore, the computed thermochemistry favors structure **A** in agreement with previous equilibrium studies.[27]

Comparing the experimental IRMPD spectrum and the calculated IR spectra for both the alanine and valine proton-bound dimers shows that structure **A** is also the most probable structure in agreement with the calculated energies. For the alanine/glycine proton-bound dimer, the IRMPD spectrum is only recorded in the 950−1600 cm^{-1} region. However, the predicted lowest-energy structures are very similar to the homogeneous proton-bound dimers (i.e., glycine proton-bound dimer and alanine proton-bound dimer), and the portion of the spectrum recorded is also comparable to the experimental spectra for the homogeneous proton-bound dimers such that a full spectrum in this region would not likely be very helpful. However, on the basis of the calculated thermochemistry, 3.5 kJ mol^{-1} difference in free energy, two very similar isomers would be expected to exist in a sample. The only region for differentiating the two structures is the asymmetric stretching vibration for the central proton which occurs at 2595 and 2466 cm^{-1} for **A** and **A1**, respectively, as well as hydrogen-bonded O−H stretching at around 3000 cm^{-1}. It is also determined that distinguishing the proton-bound dimers composed of different aliphatic amino acids should be possible in the 2000−3200 cm^{-1} range.

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Note Added in Proof. Following initial submission, a similar spectrum for the glycine proton-bound dimer was published, and the same structural assignment was made.[51]

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