J|A|C|S
ARTICLES

Published on Web 03/03/2009

Changes in Binding Motif of Protonated Heterodimers Containing Valine and Amines Investigated Using IRMPD Spectroscopy between 800 and 3700 cm^{-1} and Theory

Jeremy T. O'Brien,[†] James S. Prell,[†] Jeffrey D. Steill,[‡] Jos Oomens,[‡] and
Evan R. Williams*,[†]

*Department of Chemistry, University of California, Berkeley, California 94720-1460, and
FOM Institute for Plasma Physics "Rijnhuizen", Edisonbaan 14, 3439 MN Nieuwegein,
The Netherlands*

Received December 19, 2008; E-mail: williams@cchem.berkeley.edu

Abstract: Proton-bound dimers consisting of valine and basic primary and secondary amines of varying gas-phase basicity (GB) were investigated using infrared multiple photon dissociation (IRMPD) spectroscopy between 800 and 3700 cm^{-1}, collisionally activated dissociation, and theory. The low-energy dissociation of these dimers results in a sharp transition from formation of primarily protonated valine to protonated base for dimers with ethylamine and propylamine, respectively, from which a GB of ~880 kJ/mol is deduced for valine, a value that is slightly higher than previously reported. The IRMPD spectra clearly indicate that, for bases with GB values within 20 kJ/mol of that of valine, the base coordinates to the N-terminus of a nonzwitterionic form of valine. In contrast, calculations indicate that valine is zwitterionic for complexes where the base is less basic. For bases with GB values at least 20 kJ/mol greater than that of valine, the spectra indicate a transition in structure, and for diethylamine ($\Delta GB = 40$ kJ/mol), the dominant structure is one in which the base coordinates to the carbonyl oxygen of a nonzwitterionic form of valine and the carboxylic acid donates an intramolecular hydrogen bond to the N-terminus. These results are consistent with the destabilization of the N-terminally coordinated structure due to the increasing difference in proton affinities of the constituent molecules and the increasing importance of a stabililizing hydrogen bond formed in the C-terminally coordinated structure. Even when the GB of the base is 40 kJ/mol higher than that of valine, the form of the amino acid is nonzwitterionic, indicating that careful application of the kinetic method should provide reliable information about the basicity of valine and other aliphatic amino acids.

Introduction

Ionic interactions play an important role in molecular reactivity and structure. Salt bridges, in which a deprotonated acidic residue interacts with a protonated basic residue, can either stabilize or destabilize protein conformations and are often found in protein−protein interfaces.[1−6] Because water, solvent, or other molecules can stabilize ions, salt bridges and zwitterions are common in condensed phases. However, these ionic interactions can also be stable even without solvent or other molecules. The nonzwitterionic forms of isolated amino acids are more stable for all the naturally occurring amino acids in the gas phase, but the zwitterionic forms of some amino acids can be preferentially stabilized by cationization with metal ions,[7−26] attachment of an electron,[27,28] or when bound to other protonated molecules.[29−33] For example, the nonzwitterionic form of arginine is more stable than the zwitterionic form, but attachment of an electron,[27,28] larger alkali metal ions (Na, K, Rb, Cs),[10−13,23] or even another protonated arginine molecule[29,30]

[†] University of California.

[‡] FOM Institute for Plasma Physics "Rijnhuizen".

(1) Anderson, D. E.; Becktel, W. J.; Dahlquist, F. W. *Biochemistry* **1990**, *29*, 2403–2408.

(2) Errington, N.; Doig, A. J. *Biochemistry* **2005**, *44*, 7553–7558.

(3) Hendsch, Z. S.; Tidor, B. *Protein Sci.* **1994**, *3*, 211–226.

(4) Horovitz, A.; Serrano, L.; Avron, B.; Bycroft, M.; Fersht, A. R. *J. Mol. Biol.* **1990**, *216*, 1031–1044.

(5) Lee, W. S.; Park, C. H.; Byun, S. M. *J. Biochem.* **2004**, *135*, 93–99.

(6) Marqusee, S.; Sauer, R. T. *Protein Sci.* **1994**, *3*, 2217–2225.

(7) Wyttenbach, T.; Witt, M.; Bowers, M. T. *Int. J. Mass Spectrom.* **1999**, *182−183*, 243–252.

(8) Wyttenbach, T.; Witt, M.; Bowers, M. T. *J. Am. Chem. Soc.* **2000**, *122*, 3458–3464.

(9) Bertran, J.; Rodriguez-Santiago, L.; Sodupe, M. *J. Phys. Chem. B* **1999**, *103*, 2310–2317.

(10) Jockusch, R. A.; Price, W. D.; Williams, E. R. *J. Phys. Chem. A* **1999**, *103*, 9266–9274.

(11) Cerda, B. A.; Wesdemiotis, C. *Analyst* **2000**, *125*, 657–660.

(12) Bush, M. F.; O'Brien, J. T.; Prell, J. S.; Saykally, R. J.; Williams, E. R. *J. Am. Chem. Soc.* **2007**, *129*, 1612–1622.

(13) Forbes, M. W.; Bush, M. F.; Polfer, N. C.; Oomens, J.; Dunbar, R. C.; Williams, E. R.; Jockusch, R. A. *J. Phys. Chem. A* **2007**, *111*, 11759–11770.

(14) Bush, M. F.; Oomens, J.; Saykally, R. J.; Williams, E. R. *J. Phys. Chem. A* **2008**, *112*, 8578–8584.

(15) Bush, M. F.; Oomens, J.; Saykally, R. J.; Williams, E. R. *J. Am. Chem. Soc.* **2008**, *130*, 6463–6471.

(16) O'Brien, J. T.; Prell, J. S.; Steill, J. D.; Oomens, J.; Williams, E. R. *J. Phys. Chem. A* **2008**, *112*, 10823–10830.

(17) Polfer, N. C.; Oomens, J.; Dunbar, R. C. *Phys. Chem. Chem. Phys.* **2006**, *8*, 2744–2751.

(18) Constantino, E.; Rodriguez-Santiago, L.; Sodupe, M.; Tortajada, J. *J. Phys. Chem. A* **2005**, *109*, 224–230.

(19) Ai, H. Q.; Bu, Y. X.; Li, P.; Li, Z. Q.; Hu, X. Q.; Chen, Z. D. *J. Phys. Org. Chem.* **2005**, *18*, 26–34.

(20) Ai, H. Q.; Bu, Y. X.; Han, K. L. *J. Chem. Phys.* **2003**, *118*, 10973–10985.

10.1021/ja809928h CCC: $40.75 © 2009 American Chemical Society

can result in the zwitterionic form being more stable. Similarly, proline itself is not zwitterionic, but the zwitterionic form can be most stable when cationized by a metal cation[23−25] or when bound to protonated proline.[32] In contrast, glycine is not zwitterionic when monocationized[19,20,34−37] or in protonated dimers,[29−32,38,39] but the zwitterionic form can be stabilized by attachment of dications.[9,18−22]

Investigating some physical properties of amino acids or other similar molecules can be made more complicated because the form of an amino acid present in a protonated or cationized dimer can be different from that of the amino acid in isolation. For example, proton affinities or gas-phase basicities are often measured using either the kinetic method or by bracketing, which both involve formation of protonated dimers. In the kinetic method,[40−42] products formed by dissociation of protonated dimers consisting of the molecule of interest and a reference base of known basicity are measured as a function of the basicity of the reference base. In the bracketing method, these same proton-bound dimers are formed by ion-molecule reactions. In each case, if the form of the molecule in the protonated dimer is different from that in isolation, the results of these experiments may not accurately reflect the physical properties of the isolated molecule. Metal ion affinity measurements can also be affected by the structure of the amino acid in a complex. For example, lithiated proline is zwitterionic,[23,25] but results from dissociation of heterodimers consisting of lithiated proline and proline methyl ester suggest that proline is nonzwitterionic, a result attributed to destabilization of the zwitterionic form of proline in the heterodimer.[23]

Wu and McMahon recently investigated the structures of the amino acids Gly, Ala, Val, Leu, Ile, Ser, and Pro in protonated dimers with ammonia, methylamine, diethylamine, and triethylamine.[43] They reported that, for the amino acids with alkyl side chains, the zwitterionic form of the amino acid is increasingly stable with increasing basicity of the amino acid, consistent with earlier results for metal-cationized amino acids with alkyl side chains.[8,25] They also found that the stabilities of the zwitterionic forms of these amino acids decrease with increasing basicity of the base.[43]

Infrared multiple photon dissociation (IRMPD) spectroscopy has emerged as a powerful method to obtain structural information, and this method has been applied to many different protonated dimers.[31−33,38,44−48] Elegant experiments of Johnson and co-workers showed that the frequency of the asymmetric stretching band of a shared proton between two molecules depends strongly on the relative proton affinities of the two molecules.[48] IRMPD spectroscopy has been used to investigate zwitterionic structures of amino acids in the gas phase.[12−16,26,32,33,49−52] IRMPD spectra and other results for protonated glycine dimer indicate that glycine is not zwitterionic.[30−32,38,47,53] On the basis of these data, two different structures have been proposed. In one structure, the protonated N-terminus of one glycine hydrogen bonds to the N-terminus of the other glycine in its nonzwitterionic form.[31] In the other structure, the shared proton is between the protonated N-terminus of one glycine and the carbonyl oxygen of the other nonzwitterionic glycine.[32,38,47] On the basis of IRMPD spectra measured in the 1150 to 2000 cm^{-1} region, Wu and McMahon conclude that, in Gly$\cdot$NH$_4^+$, structures in which Gly is either zwitterionic or nonzwitterionic are equally populated even though ammonia is ∼32 kJ/mol less basic than Gly.[33]

Here, the structures and reactivities of protonated dimers consisting of valine and 11 basic molecules that are either primary or secondary amines with GB values bracketing that of valine are investigated using IRMPD spectroscopy, collisionally activated dissociation, and theory. In contrast to earlier results[43] and to theory at MP2/6−31++G** and B3LYP/6−31++G** levels, the IRMPD spectra clearly show that valine is not zwitterionic with any of these bases, but rather the protonated dimers undergo a gradual change in structure with increasing GB from one in which protonated base interacts with the N-terminus of nonzwitterionic valine to one in which

---

(21) Marino, T.; Toscano, M.; Russo, N.; Grand, A. *J. Phys. Chem. B* **2006**, *110*, 24666–24673.
(22) Strittmatter, E. F.; Lemoff, A. S.; Williams, E. R. *J. Phys. Chem. A* **2000**, *104*, 9793–9796.
(23) Talley, J. M.; Cerda, B. A.; Ohanessian, G.; Wesdemiotis, C. *Chem.−Eur. J.* **2002**, *8*, 1377–1388.
(24) Kapota, C.; Lemaire, J.; Maitre, P.; Ohanessian, G. *J. Am. Chem. Soc.* **2004**, *126*, 1836–1842.
(25) Lemoff, A. S.; Bush, M. F.; Williams, E. R. *J. Phys. Chem. A* **2005**, *109*, 1903–1910.
(26) Dunbar, R. C.; Polfer, N. C.; Oomens, J. *J. Am. Chem. Soc.* **2007**, *129*, 14562–14563.
(27) Xu, S. J.; Zheng, W. J.; Radisic, D.; Bowen, K. H. *J. Chem. Phys.* **2005**, *122*, 091103.
(28) Skurski, P.; Rak, J.; Simons, J.; Gutowski, M. *J. Am. Chem. Soc.* **2001**, *123*, 11073–11074.
(29) Strittmatter, E. F.; Williams, E. R. *J. Phys. Chem. A* **2000**, *104*, 6069–6076.
(30) Price, W. D.; Jockusch, R. A.; Williams, E. R. *J. Am. Chem. Soc.* **1997**, *119*, 11988–11989.
(31) Oh, H. B.; Lin, C.; Hwang, H. Y.; Zhai, H. L.; Breuker, K.; Zabrouskov, V.; Carpenter, B. K.; McLafferty, F. W. *J. Am. Chem. Soc.* **2005**, *127*, 4076–4083.
(32) Wu, R. H.; McMahon, T. B. *J. Am. Chem. Soc.* **2007**, *129*, 4864–4865.
(33) Wu, R.; McMahon, T. B. *J. Mass Spectrom.* **2008**, *43*, 1641–1648.
(34) Hoyau, S.; Ohanessian, G. *J. Am. Chem. Soc.* **1997**, *119*, 2016–2024.
(35) Shoeib, T.; Rodriquez, C. F.; Siu, K. W. M.; Hopkinson, A. C. *Phys. Chem. Chem. Phys.* **2001**, *3*, 853–861.
(36) Hoppilliard, Y.; Ohanessian, G.; Bourcier, S. *J. Phys. Chem. A* **2004**, *108*, 9687–9696.
(37) Rodriguez-Santiago, L.; Sodupe, M.; Tortajada, J. *J. Phys. Chem. A* **2001**, *105*, 5340–5347.
(38) Rajabi, K.; Fridgen, T. D. *J. Phys. Chem. A* **2008**, *112*, 23–30.
(39) Raspopov, S. A.; McMahon, T. B. *J. Mass Spectrom.* **2005**, *40*, 1536–1545.
(40) McLuckey, S. A.; Cameron, D.; Cooks, R. G. *J. Am. Chem. Soc.* **1981**, *103*, 1313–1317.
(41) McLuckey, S. A.; Cooks, R. G.; Fulford, J. E. *Int. J. Mass Spectrom. Ion Processes* **1983**, *52*, 165–174.
(42) Cooks, R. G.; Wong, P. S. H. *Acc. Chem. Res.* **1998**, *31*, 379–386.
(43) Wu, R.; McMahon, T. B. *J. Am. Chem. Soc.* **2008**, *130*, 3065–3078.
(44) Fridgen, T. D.; MacAleese, L.; Maitre, P.; McMahon, T. B.; Boissel, P.; Lemaire, J. *Phys. Chem. Chem. Phys.* **2005**, *7*, 2747–2755.
(45) Fridgen, T. D.; MacAleese, L.; McMahon, T. B.; Lemaire, J.; Maitre, P. *Phys. Chem. Chem. Phys.* **2006**, *8*, 955–966.
(46) Fridgen, T. D.; McMahon, T. B.; MacAleese, L.; Lemaire, J.; Maitre, P. *J. Phys. Chem. A* **2004**, *108*, 9008–9010.
(47) Atkins, C. G.; Rajabi, K.; Gillis, E. A. L.; Fridgen, T. D. *J. Phys. Chem. A* **2008**, *112*, 10220–10225.
(48) Roscioli, J. R.; McCunn, L. R.; Johnson, M. A. *Science* **2007**, *316*, 249–254.
(49) Armentrout, P. B.; Rodgers, M. T.; Oomens, J.; Steill, J. D. *J. Phys. Chem. A* **2008**, *112*, 2248–2257.
(50) Bush, M. F.; Forbes, M. W.; Jockusch, R. A.; Oomens, J.; Polfer, N. C.; Saykally, R. J.; Williams, E. R. *J. Phys. Chem. A* **2007**, *111*, 7753–7760.
(51) Bush, M. F.; Prell, J. S.; Saykally, R. J.; Williams, E. R. *J. Am. Chem. Soc.* **2007**, *129*, 13544–13553.
(52) Rodgers, M. T.; Armentrout, P. B.; Oomens, J.; Steill, J. D. *J. Phys. Chem. A* **2008**, *112*, 2258–2267.
(53) Price, W. D.; Schnier, P. D.; Williams, E. R. *J. Phys. Chem. B* **1997**, *101*, 664–673.

the protonated base interacts with the C-terminus of nonzwitterionic valine.

Experimental Section

Mass Spectrometry. All sustained off-resonance irradiation collisional activation dissociation (SORI-CAD) and spectroscopy experiments in the frequency range between 2800 and 3700 cm^{-1} were performed on a 2.75 T Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer at Berkeley.[12,54] Methanol, isopropylamine, *n*-butylamine, *sec*-butylamine, *tert*-butylamine, hexylamine, ethylmethylamine, diethylamine as well as hydrochloride salts of methylamine, ethylamine, propylamine, dimethylamine, and L-valine were purchased from Sigma Chemical Co. All chemicals were used without further purification. Protonated heterodimers were formed by electrospray ionization of aqueous solutions of 4 mM valine and 1−4 mM base, except for the $Val\cdot H^+\cdot$ethylmethylamine and $Val\cdot H^+\cdot$diethylamine heterodimers, which were formed from 50/50 water/methanol solutions. Ions were trapped and accumulated in a cylindrical ion cell for 3 s during which time nitrogen gas was pulsed into the chamber at a pressure of $\sim 10^{-6}$ torr to enhance trapping and thermalization. This cell is surrounded by a copper jacket that is resistively heated to 351 K. The vacuum chamber pressure returned to $\sim 10^{-8}$ torr after a pump-down delay of 3.5 s before ion isolation. Protonated dimers were isolated using a stored waveform inverse Fourier transform (SWIFT) and then irradiated with 300 pulses (30 s at 10 Hz) of IR radiation from a tunable OPO/OPA (LaserVision). Transients of 64 kB were acquired using a MIDAS data station.[55] IR laser-induced photodissociation rates as a function of frequency were obtained from the relative abundances of precursor and product ions following laser irradiation and were corrected for both laser power and blackbody infrared radiative dissociation.

For SORI-CAD experiments, ions of interest were isolated and excited using a 500-ms, 8 V_{pk-pk} waveform applied at a frequency 5000 Hz below the cyclotron frequency of the ion of interest, providing a maximum laboratory-frame translational energy of 1.0−1.5 eV, depending on the *m*/*z* of the precursor ion. Nitrogen gas was introduced into the vacuum chamber for 2 s at a pressure of $\sim 10^{-6}$ torr to increase the number of collisions. The pressure returned to $\sim 10^{-8}$ torr after a 3 s delay before ion detection. Under these conditions, the percentage of the precursor dissociated ranged from ~90% for methylamine to ~99% for diethylamine.

IRMPD spectra between 800 and 1900 cm^{-1} were obtained using a 4.7 T FT-ICR mass spectrometer at the FOM Institute for Plasma Physics.[56,57] Briefly, the protonated dimers were formed by electrospray ionization from 50/50 water/methanol solutions of 1 mM valine and 4 mM methylamine, or 2 mM valine and 4 mM diethylamine, infused at a rate of 10 μL/min. Precursor ions were mass selected using SWIFT and subsequently irradiated for 2.5 to 3 s using tunable irradiation from the free-electron laser. For these experiments, IRMPD spectra were obtained from the sum of the photodissociation product ion intensities divided by the total ion intensity measured as a function of photon energy and linearly power-corrected.[57]

Computational Methods. Candidate low-energy structures for $Val\cdot H^+\cdot B$, where B = methylamine, ethylamine, hexylamine, and diethylamine, were obtained by Monte Carlo conformational searching using the MMFF94 force field in Macromodel 8.1 (Schrödinger, Inc.). At least 5000 conformations were generated for protonated dimers with methylamine, ethylamine, and hexylamine, and 10 000 conformers were generated for diethylamine. Starting structures for $Val\cdot H^+\cdot B$, B = propylamine, isopropylamine, *n*-butylamine, *sec*-butylamine, or *tert*-butylamine, were generated by substituting the corresponding alkyl groups into the low-energy candidate structures obtained for B = ethylamine. Because the relatively short alkyl chains of these primary amines do not interact significantly with the valine molecule, conformational searching for each base was not performed. Initial structures for $Val\cdot H^+\cdot B$, where B = dimethylamine and ethylmethylamine, were similarly generated by alkyl group substitution into the candidate structures identified for diethylamine.

Geometries of candidate low-energy structures were subsequently optimized with Jaguar v. 6.5 (Schrödinger, Inc.) at the B3LYP/6−31G* level of theory. The resulting low-energy structures were then optimized further at B3LYP/6−31++G** using Q-Chem 3.0 (Q-Chem, Inc.),[58] and harmonic vibrational frequencies and intensities were calculated analytically. Each final structure was found to have all positive frequency vibrational modes, indicating that these structures are local minima. Energies were obtained using B3LYP/6−31++G**, with zero-point energy corrections calculated using unscaled vibrational frequencies. For comparisons with the experimental data, calculated vibrational frequencies between 2800 and 3700 cm^{-1} were scaled by 0.956. This scaling factor has been used previously in an IR study in this region to compare experimental IR action spectra of lithiated valine water cluster to spectra calculated at the same level of theory as used in this study.[59] Calculated spectra in this frequency region were convolved using 20 and 30 cm^{-1} full-width at half-maximum (fwhm) Lorentzian peak shapes for the free OH and hydrogen-bonded features, respectively, which provide a good fit to the observed peak shapes. Vibrational frequencies between 800 and 1900 cm^{-1} were scaled by 0.975, which has been used previously in other IR studies in this region to compare experimental IR action spectra and spectra calculated at similar levels of theory as used in this study.[14−17,50,60] In this region, the calculated spectra were convolved with a 50 cm^{-1} fwhm Lorentzian peak shape to approximate the width of the observed bands.

Additional calculations were performed for methylamine and diethylamine. Geometry optimization and frequency calculations using the B3LYP/6−311++G** level of theory were performed on the 6−31++G** optimized structures. In addition, MP2/6−31++G** single-point energies were calculated for the B3LYP/6−31++G** optimized structures. The zero-point energy and temperature corrections from the B3LYP/6−31++G** calculations were added to the MP2 single-point energies to obtain relative Gibbs free energies at the temperature of the experiments.

Results and Discussion

Dissociation Pathways. Dissociation of $Val\cdot H^+\cdot B$ with SORI-CAD at low energy results in formation of either $Val\cdot H^+$ or $B\cdot H^+$ and the corresponding neutral molecule. For $Val\cdot H^+\cdot MeA$, predominantly $Val\cdot H^+$ is observed, consistent with the 15 kJ/mol higher basicity of Val. For bases with GB values greater than that of hexylamine, only protonated base is observed. The ratio of protonated valine to protonated base product intensities changes for $Val\cdot H^+\cdot B$ between B = methylamine and hexylamine (Figure 1a). The ratio of $[Val\cdot H^+]$ to $[EtA\cdot H^+]$ is 75:25, indicating that valine is more basic than ethylamine. On the basis of the results of these SORI-CAD experiments, the gas-phase basicity and proton affinity of valine are ~880 and ~914 kJ/mol, respectively. In contrast, results

---

(54) Schnier, P. D.; Jurchen, J. C.; Williams, E. R. *J. Phys. Chem. B* **1999**, *103*, 737–745.

(55) Senko, M. W.; Canterbury, J. D.; Guan, S. H.; Marshall, A. G. *Rapid Commun. Mass Spectrom.* **1996**, *10*, 1839–1844.

(56) Valle, J. J.; Eyler, J. R.; Oomens, J.; Moore, D. T.; van der Meer, A. F. G.; von Helden, G.; Meijer, G.; Hendrickson, C. L.; Marshall, A. G.; Blakney, G. T. *Rev. Sci. Instrum.* **2005**, *76*, 023103.

(57) Polfer, N. C.; Oomens, J.; Moore, D. T.; von Helden, G.; Meijer, G.; Dunbar, R. C. *J. Am. Chem. Soc.* **2006**, *128*, 517–525.

(58) Shao, Y.; et al. *Phys. Chem. Chem. Phys.* **2006**, *8*, 3172–3191.

(59) Kamariotis, A.; Boyarkin, O. V.; Mercier, S. R.; Beck, R. D.; Bush, M. F.; Williams, E. R.; Rizzo, T. R. *J. Am. Chem. Soc.* **2006**, *128*, 905–916.

(60) Prell, J. S.; Demireva, M.; Oomens, J.; Williams, E. R. *J. Am. Chem. Soc.* **2008**, *131*, 1232–1242.

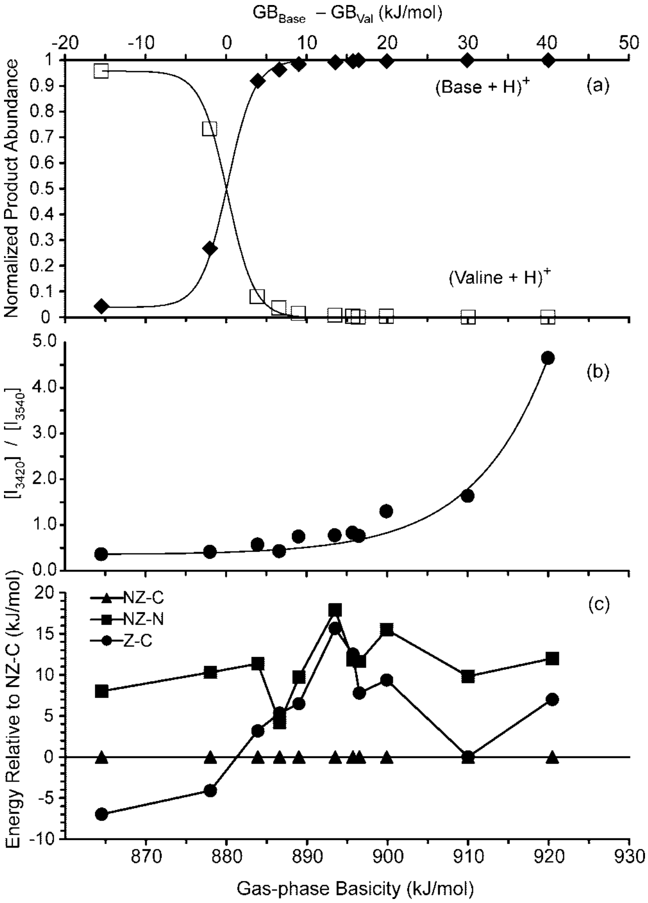

Figure 1. (a) SORI-CAD product ion abundances for dissociation of Val•H$^+$•B. (b) Ratio of the band intensity at 3420 cm^{-1}, [I_{3420}], to the intensity at 3540 cm^{-1}, [I_{3540}], from the IRMPD spectra of the proton-bound valine-base dimers. (c) Gibbs free energies of the NZ−N and Z−C structures relative to that of NZ−C at 351 K calculated at the B3LYP/6−31++G** level of theory as a function of the gas-phase basicity of the base. The GB$_{Base}$ − GB$_{Val}$ values are based on a GB$_{Val}$ = 880 kJ/mol.

Table 1. Relative Energies at 0/351 K, B3LYP/6−31++G** (MP2/6−31++G** Single-Point Energies with B3LYP ZPE and Temperature Corrections)

molecule	gas-phase basicity kJ/mol[a]	NZ−C kJ/mol	NZ−N kJ/mol	Z−C kJ/mol
valine	880			
methylamine	864.5	4/7 (4/7)	11/15 (2/6)	0/0 (0/0)
ethylamine	878	0/4	10/14	~0/0
propylamine	883.9	0/0	12/11	5/3
butylamine	886.6	0/0	7/4	2/5
isopropylamine	889	1/0	6/10	0/6
hexylamine	893.5	0/0	10/18	7/16
sec-butylamine	895.7	0/0	7/12	~0/13
dimethylamine	896.5	0/0	11/12	7/8
tert-butylamine	899.9	0/0	10/16	3/9
methylethylamine	910	0/0	9/10	5/~0
diethylamine	919.9	0/0 (7/5)	14/12 (5/5)	9/7 (0/0)

[a] Basicity values except for valine from ref 63. Valine value from this work.

from both bracketing[61] and prior kinetic method[62] experiments indicate that ethylamine is more basic than valine. On the basis of these and other results, proton affinity values ranging from 900 to 912 kJ/mol have been reported.[63,64] Changing the maximum laboratory-frame translational energy in these SORI-CAD experiments from 1.5 to 4.4 eV resulted in a decrease in the [Val•H$^+$]/[EtA•H$^+$] ratio from 75:25 to 45:55, respectively. These results are consistent with those of Li and Harrison, who measured the effects of collision energy in kinetic method experiments and found that the ratio of protonated ethylamine to protonated valine increased with increasing energy, indicating that formation of EtA•H$^+$ is entropically favored.[62] The higher basicity value of valine determined in our experiments is consistent with a much lower internal energy deposition in these SORI-CAD experiments and the relative entropy for dissociation by the two channels differing markedly. There is an intramolecular hydrogen bond from the hydroxyl group of the C-terminus to the nitrogen of the N-terminus of valine that hinders rotation about the N−C$_\alpha$ bond (vide infra). This hydrogen bond is significantly stronger for Val•H$^+$ than for neutral valine. Formation of EtA•H$^+$ and neutral valine are likely entropically favored because neutral valine can more freely rotate around the N−C$_\alpha$ bond than can Val•H$^+$.

The same products formed by SORI-CAD are observed by photodissociation, although the ratio of products can differ. For Val•H$^+$•EtA, the ratio of protonated valine to protonated ethylamine is ~90:10, indicating that basicity of valine is slightly higher than 880 kJ/mol and that the energy deposition and resulting effective temperatures in these photodissociation experiments are even lower than those in the SORI-CAD experiments. The branching ratio of the photodissociation products does not depend on photon energy.

IRMPD Spectra. IRMPD action spectra of protonated valine-base heterodimers were measured for each of 11 different bases (Table 1) at a cell temperature of 351 K in the frequency range from 2800 to 3700 cm^{-1} (Figure 2). These protonated dimers dissociate more readily at elevated cell temperatures as a result of their interaction with the surrounding blackbody radiation field that increases their internal energy. The temperature was chosen so that, after 30 s of exposure to just blackbody radiation, about 5−12% of the precursor ions dissociate. Under these conditions, absorption of even a single IR photon from the laser is expected to increase the rate of dissociation, although sequential absorption of multiple IR photons also occurs. Laser-induced photodissociation rates were between 0 and 150% of the respective blackbody infrared radiative dissociation rates.

The spectral range from 2800 to 3700 cm^{-1} can be roughly divided into three regions corresponding to free OH stretches (3500−3600 cm^{-1}), NH stretches (3200−3500 cm^{-1}), and a region with both CH stretches and hydrogen-bonded OH and NH stretches (2800−3200 cm^{-1}). CH stretches can appear as weak bands in IR action spectra because of the weak transition dipole moment for this mode.[12,31,59]

For complexes containing a base with a primary amine, there are three bands in the NH stretch region at roughly 3275, 3335, and 3420 cm^{-1}, although the band at 3275 cm^{-1} is not resolved from the band at 3335 cm^{-1} for methylamine and ethylamine. The IR action spectra of complexes containing bases with secondary amines (dimethylamine, methylethylamine, and diethylamine) have bands at ~3275 and ~3420 cm^{-1}, but no band at ~3335 cm^{-1}. These spectra are consistent with the IR action

---

(61) Gorman, G. S.; Speir, J. P.; Turner, C. A.; Amster, I. J. *J. Am. Chem. Soc.* **1992**, *114*, 3986–3988.

(62) Li, X. P.; Harrison, A. G. *Org. Mass Spectrom.* **1993**, *28*, 366–371.

(63) Hunter, E. P. L.; Lias, S. G. *J. Phys. Chem. Ref. Data* **1998**, *27*, 413–656.

(64) Bleiholder, C.; Suhai, S.; Paizs, B. *J. Am. Soc. Mass Spectrom.* **2006**, *17*, 1275–1281.

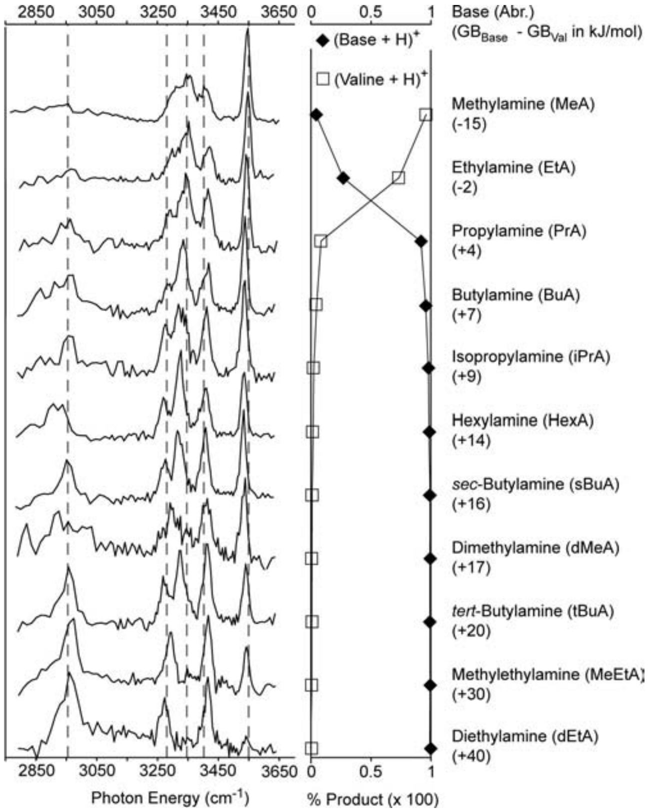

Figure 2. Dissociation of Val•H$^+$•B: IRMPD spectra from 2750 to 3700 cm^{-1} (left) and SORI-CAD product ratios (right). The identities of B and $GB_{Base} - GB_{Val}$ (kJ/mol) are indicated to the right of the experimental data.

spectra of other cationized amino acids that have NH stretch bands between 3250 and 3450 cm^{-1}.[12,65]

All the IR action spectra have a distinct band at ~3540 cm^{-1} consistent with the free OH stretch of a carboxylic acid group (Figure 2), although this is only a minor feature for diethylamine. A plot of the intensity of the band at ~3420 cm^{-1} relative to the intensity of the band at ~3540 cm^{-1} as a function of the GB of the amine is shown in Figure 1b. The relative intensities of these bands change only slightly for bases that have GB values less than 20 kJ/mol greater than valine, indicating that the population of structures that have free OH stretches does not change significantly for these bases. In contrast, this ratio increases significantly for bases that are 20 kJ/mol or more basic than valine. This indicates that the relative population of structures with free OH stretches decreases significantly for complexes with these more basic bases. These results are consistent with a transition in structure from one in which the base is coordinated to the N-terminus to one in which it is coordinated to the C-terminus. When the base coordinates to the C-terminus, either the OH group of nonzwitterionic valine forms a hydrogen bond and the OH stretch vibration occurs at a much lower frequency, or valine is zwitterionic and does not have an OH stretching mode.

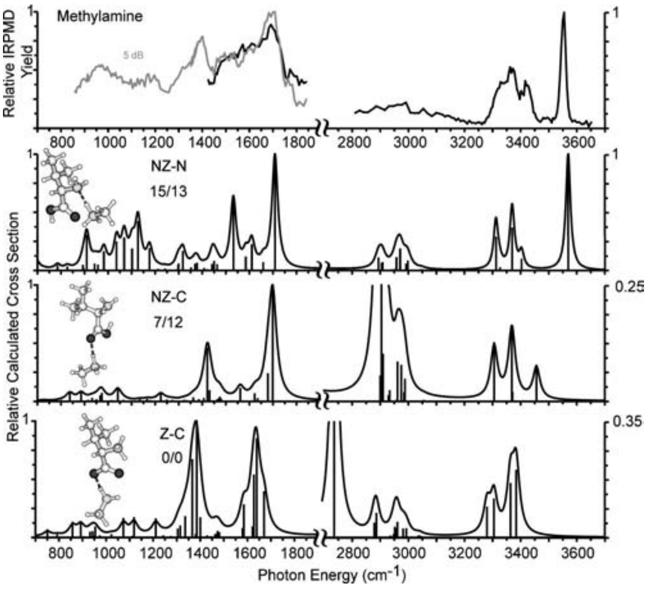

Figure 3. IRMPD spectra of Val•H$^+$•MeA from 800 to 1900 cm^{-1} and 2800 to 3700 cm^{-1} obtained at 298 and 351 K, respectively, and calculated absorption spectra for three low-energy structures, NZ−N, NZ−C, and Z−C, at the B3LYP/6−31++G** level of theory. The relative Gibbs free energies at 351 K at the (B3LYP/6−31++G**)/(MP2//B3LYP/6−31++G**) levels of theory are reported.

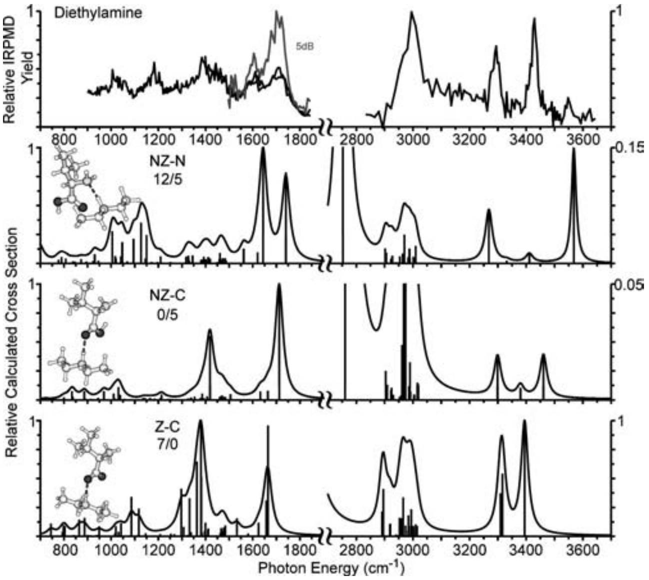

Figure 4. IRMPD spectra of Val•H$^+$•Et$_2$A from 800 to 1900 cm^{-1} and 2800 to 3700 cm^{-1} obtained at 298 and 351 K, respectively, and calculated absorption spectra for three low-energy structures, NZ−N, NZ−C, and Z−C, at the B3LYP/6−31G++** level of theory. The relative Gibbs free energies at 351 K at the (B3LYP/6−31++G**)/(MP2//B3LYP/6−31++G**) levels of theory are reported.

Spectra of Val•H$^+$•MeA and Val•H$^+$•Et$_2$A were also measured at room temperature using tunable radiation at lower energies, ~850−1850 cm^{-1}, generated by a free electron laser (Figures 3 and 4). This spectral range covers carbonyl stretching modes, as well as NH and OH bending modes, which can provide structural information complementary to that obtained from the higher photon energy range. The bands in the IRMPD spectra of Val•H$^+$•MeA and Val•H$^+$•Et$_2$A are relatively broad and weak between ~850 and 1850 cm^{-1} (Figures 3 and 4). Similarly broad features are observed in the IR action spectrum of Gly•NH$_4^+$ measured in this same spectral region.[33] The bands at ~1700 cm^{-1} are likely an overlap of a carbonyl stretching mode and NH bending modes. The carbonyl stretch of a carboxylic acid group has been observed to occur between ~1740 and 1790 cm^{-1} for protonated amino acids[13,16,50] and between 1700 and 1800 cm^{-1} for metal-cationized amino

---

(65) Stearns, J. A.; Mercier, S.; Seaiby, C.; Guidi, M.; Boyarkin, O. V.; Rizzo, T. R. *J. Am. Chem. Soc.* **2007**, *129*, 11814–11820.

acids[13−17,24,49,50,52,57] and Gly·NH_4^+.[33] NH bending modes are reported to occur between 1500 and 1700 cm^{-1}.[13−17,33,50,57] The bands near 1400 cm^{-1} are consistent with the OH bend of a carboxylic acid that is weakly hydrogen bonded to the N-terminus as observed for some cationized amino acids.[13−17,49,50,52] The weak bands at ~975 and 1175 cm^{-1} are consistent with CH bending modes coupled with OH and NH bends.

The spectra of Val·H^+·MeA and Val·H^+·Et_2A are similar between 850 and 1850 cm^{-1}. The structural difference clearly indicated by the spectra in the higher frequency range (2800−3700 cm^{-1}) is not apparent from the lower frequency data, although the relatively poor signal-to-noise of these data may obscure any differences.

Calculated Low-Energy Structures. For each of the protonated heterodimers, the lowest-energy structures fall into three structural families, all of which consist of formally neutral valine and a protonated base. These structural families are illustrated for methylamine and diethylamine in Figures 3 and 4, respectively. In structure NZ−N, the protonated base coordinates to the N-terminus of nonzwitterionic valine, where it forms an ionic hydrogen bond to the nitrogen. A structure in which protonated valine donates an ionic hydrogen bond to the neutral base is calculated to be stable only for methylamine. Surprisingly, calculations predict that this structure is 28 kJ/mol higher in energy (351 K) than the NZ−N structure in which methylamine is protonated, despite the 12 kJ/mol higher basicity of valine compared to that of methylamine (i.e., the proton is predominantly associated with the less basic molecule in this dimer). Similarly, in recently reported calculations for Gly·NH_4^+, ammonia is protonated in the three most stable reported structures despite the much higher (+33 kJ/mol) basicity of glycine.[33]

In the other nonzwitterionic structure, NZ−C, the protonated base donates a single hydrogen bond to the carbonyl oxygen of valine, and there is an intramolecular hydrogen bond between the acidic hydrogen and the nitrogen of valine. In the zwitterionic structure, Z−C, the protonated base coordinates to the carboxylate group of zwitterionic valine, donating a hydrogen bond to each oxygen atom. For all structures, one position of the side chain is consistently calculated to be lowest in energy. In this preferred position, both methyl groups of the side chain are gauche relative to the N-terminal amine group (Figures 3 and 4). Other orientations of the valine side chain are generally 3−8 kJ/mol higher in energy. These results are consistent with the low-energy structures reported by Wu and McMahon for protonated valine alkyl-ammonium complexes.[43]

The B3LYP/6−31++G** relative Gibbs free energies at 0/351 K are given in Table 1. The relative energies of these three structures are similar for each amine base, spanning a range of less than 20 kJ/mol for each protonated heterodimer. A plot of the 351 K relative energies of these three structures as a function of the gas-phase basicity of the base is shown in Figure 1c. At this level of theory, the NZ−C structures are calculated to be lowest in energy at 351 K for all the protonated heterodimers except for those with methylamine and ethylamine, for which the Z−C structures are most stable. The 0 K relative energies are similar, typically within 5 kJ/mol of the 351 K values. There is no other apparent trend in the relative energies of these three structures with the GB of the base at either 0 or 351 K. These results are surprising because zwitterionic structures of valine are calculated to be stable for the two least basic bases at this level of theory. Calculations at both B3LYP/6−311+G* and MP2(full)/6−311++G** by Wu and McMahon also indicate that the Z−C structure is most stable for Val·H^+·MeA.[43]

To investigate the effect of basis set size and MP2 correlation on the observed trends, calculations were done on the protonated heterodimers consisting of the least basic (methylamine) and most basic (diethylamine) at the B3LYP/6−311++G** level of theory and, using the B3LYP/6−31++G** optimized geometries, MP2/6−31++G** single-point energies were also obtained (Table 1). The B3LYP/6−311++G** relative energies are within 2 kJ/mol of the 6−31++G** energies, indicating the larger basis set has little effect on the relative energies. The MP2 results for methylamine indicate the Z−C structure is again most stable and the relative energies of the NZ−C and NZ−N structures are almost equal. For diethylamine, the Z−C structure is favored with MP2 and the NZ−C structure with B3LYP. Calculated spectra for these structures are shown for methylamine and diethylamine in Figures 3 and 4, respectively. The calculated spectra for the other primary amine heterodimers are very similar to the corresponding spectra of Val·H^+·MeA structures, although the vibrational frequencies vary slightly for each base within a roughly ±15 cm^{-1} range.

IRMPD and Calculated Spectra of Val·H^+·MeA. Calculated spectra for each of the candidate low-energy structures of Val·H^+·MeA are shown in Figure 3, along with the IRMPD spectra of these respective ions in both the 800−1850 and 2800−3700 cm^{-1} regions. For Val·H^+·MeA, the strong band at ~3540 cm^{-1} clearly indicates the presence of a free carboxylic acid OH stretch. Only structures such as NZ−N with the amine coordinated to the N-terminus of nonzwitterionic valine have a free carboxylic acid OH stretch (3570 cm^{-1}). An NZ−N structure in which valine is protonated instead of methylamine also has a free carboxylic acid OH stretch, but this structure cannot be distinguished from the NZ−N structure with protonated methylamine based on their very similar calculated spectra. The OH band in NZ−C is substantially red-shifted owing to hydrogen bonding to the amine nitrogen and is calculated to occur around 2900 cm^{-1}. Despite very large calculated intensities, hydrogen-bonded features are often observed as relatively weak broad bands in IRMPD spectra.[66,67] Many factors can contribute to this discrepancy, including peak broadening, calculation uncertainties, and anharmonicity. Structure Z−C does not have a carboxylic acid OH band, and this structure can be eliminated on the basis of the absence of this band.

The symmetric and asymmetric stretches of the free methylamine NH bonds for NZ−N are at 3315 and 3375 cm^{-1}, consistent with the broad band between 3260 and 3400 cm^{-1} in the experimental spectrum. The band at 3410 cm^{-1} in the IRMPD spectrum is consistent with the asymmetric stretch of the free NH bonds of the N-terminus, which is calculated to occur at 3400 cm^{-1}.

Bands for NZ−C at 3300, 3370, and 3450 cm^{-1} correspond to the symmetric NH stretch of methylamine, an overlap of the symmetric NH stretch of the N-terminus and the asymmetric NH stretch of methylamine, and the asymmetric stretch of the free NH bonds of the N-terminus, respectively. The absence of both this latter band and a carboxylic acid free OH stretch above 3500 cm^{-1} indicates that this structure is not significantly populated.

---

(66) Bush, M. F.; Saykally, R. J.; Williams, E. R. *ChemPhysChem* **2007**, *8*, 2245–2253.

(67) O'Brien, J. T.; Williams, E. R. *J. Phys. Chem. A* **2008**, *112*, 5893–5901.

In the lower frequency region of the spectrum, the band at ~1700 cm^{-1} is consistent with the asymmetric carbonyl stretch of a carboxylic acid group. The frequency of this stretch for a carboxylate group is lower and occurs at ~1625 cm^{-1} for Z−C. This result, along with the carboxylic acid OH stretch, clearly indicates that valine is not zwitterionic in this complex. NZ−C has a band at 1400 cm^{-1} corresponding to the OH bend of the carboxylic acid group. The presence of a distinct band at 1400 cm^{-1} in the IRMPD spectrum suggests that a small population of NZ−C may be present.

These results clearly indicate that valine is not zwitterionic in the protonated dimer with methylamine. The IRMPD spectrum in the hydrogen stretch region is most consistent with the NZ−N structure, but the lower frequency data indicate that a minor population of NZ−C could be present as well. In contrast with these experimental results, calculations using both B3LYP and MP2 methods including MP2(full)/6−311++G** values reported by Wu and McMahon indicate that Z−C is the most stable.[43]

IRMPD and Calculated Spectra of Val·H$^+$·Et$_2$A. Calculated spectra for each of the candidate low-energy structures Val·H$^+$·Et$_2$A are shown in Figure 4, along with the IRMPD spectra in both the 800−1850 and 2700−3700 cm^{-1} regions. The presence of a small band at ~3540 cm^{-1} indicates that a small population of structures that have a free carboxylic acid OH stretch, such as NZ−N, must be present. The calculated NH stretching bands at 3410 and 3260 cm^{-1}, respectively, and the carboxylic acid stretch at 3570 cm^{-1} are consistent with those in the measured spectrum, but the low intensity of the carboxylic acid stretch indicates that the majority of the ion population must have structures where the base is coordinated to the C-terminus of valine.

As was the case for Val·H$^+$·MeA, the substantial photodissociation observed at 1700 cm^{-1} is consistent with the carbonyl stretch of a carboxylic acid (1710 cm^{-1} for NZ−C) and not of a carboxylate (1660 cm^{-1} for Z−C). The separation between the two NH stretching bands (3415 and 3275 cm^{-1}) in the experimental spectrum is ~140 cm^{-1}, and the separation in the corresponding bands in the calculated spectra of the NZ−C and Z−C structures is ~150 and 80 cm^{-1}, respectively. Thus, structure NZ−C appears to be the best fit to the experimental spectrum. There is significant photodissociation intensity at ~3000 cm^{-1} consistent with the calculated intramolecularly hydrogen-bonded carboxylic acid OH stretch in NZ−C. The intensity is consistent with previous studies in which hydrogen-bonded features have been observed as very broad, weak features despite the high intensity of the calculated bands.[66,67] Band intensities are calculated using a double harmonic approximation that results in attendant uncertainties in comparing with experimental spectra. CH stretches also occur in this region, but these bands are typically very weak. The lack of significant photodissociation intensity in this region of the IRMPD spectrum of Val·H$^+$·MeA provides additional support for the assignment of an NZ−N structure for Val·H$^+$·MeA. For Val·H$^+$·Et$_2$A, the weak band in the free OH stretch region indicates that a small population of the NZ−N structure is present, but the strong bonded OH feature indicates that the dominant structure is NZ−C.

In electrospray ionization, large protein ions can be kinetically trapped and can retain a "memory" of their solution-phase structures.[68−70] In aqueous solution, valine is zwitterionic whereas valine is nonzwitterionic in these protonated dimers, and thus kinetic trapping of higher-energy structures is unlikely in these experiments.

Conclusions

The structure and reactivities of proton-bound dimers consisting of valine and basic primary and secondary amines of varying gas-phase basicity were investigated using IRMPD spectroscopy between 800 and 3700 cm^{-1}, collisionally activated dissociation, and theory. Low-energy dissociation of these dimers results in a sharp transition from formation of protonated valine for bases with GB values below 880 kJ/mol to protonated base for bases with GB values above 880 kJ/mol, but the ratio of these products for bases with similar GB to valine depends on ion internal energy. The results from these experiments at low internal energy indicate that the GB of valine is approximately 880 kJ/mol, which is slightly higher than previously reported values.[63]

The IRMPD spectra clearly indicate a transition in structure with increasing basicity of the base, but this transition occurs only for bases that are ~20 kJ/mol more basic than valine. Quantum chemical calculations indicate that the lowest-energy structure of protonated heterodimers of valine and methylamine is a salt-bridge structure in which protonated methylamine interacts with the carboxylate group of the zwitterionic form of valine. This result is consistent with previous computational results, including MP2(full)/6−311++G** results for Val·H$^+$·MeA and calculations for Gly·NH$_4$$^+$.[33,43] However, the IRMPD spectra in the higher frequency region clearly rule out this form of the complex. Spectroscopic data are most consistent with a structure in which methylamine coordinates to neutral valine via a strong ionic hydrogen bond at the N-terminus. For the most basic bases, a gradual structural transition occurs. For protonated heterodimers of valine and diethylamine, the most basic amine in this study, the predominant structure is one in which protonated diethylamine hydrogen bonds to the carbonyl oxygen of neutral valine and an internal hydrogen bond between the carboxylic acid hydrogen and the N-terminus of valine is formed. However, even for this cluster, a small population of structures in which the base is N-terminally bound is present.

The binding energies of proton-bound dimers are greatest when the proton affinities of the two molecules are similar.[71] In the NZ−N structure, the proton is shared between the most basic sites in each molecule, and this is the predominant structure when the proton affinity of the basic molecule is within 20 kJ/mol of that of valine. The change from an N-terminally bound to a C-terminally bound structure with bases that have even higher proton affinities can be attributed to a destabilization of the NZ−N structure because of the larger difference in proton affinities of the two constituent molecules. This difference is even greater for the NZ−C structure, where the proton is not shared with the most basic site of valine. However, an intramolecular hydrogen bond between the N- and C-termini of valine stabilizes this structure. The stability gained from this neutral hydrogen bond becomes more important as the difference in the proton affinities of the two molecules increases and the stability of the ionic hydrogen bond decreases.

---

(68) Gross, D. S.; Zhao, Y.; Williams, E. R. *J. Am. Soc. Mass Spectrom.* **1997**, *8*, 519–524.

(69) Myung, S.; Badman, E. R.; Lee, Y. J.; Clemmer, D. E. *J. Phys. Chem. A* **2002**, *106*, 9976–9982.

(70) Robinson, E. W.; Williams, E. R. *J. Am. Soc. Mass Spectrom.* **2005**, *16*, 1427–1437.

(71) Meot-Ner, M. *Chem. Rev.* **2005**, *105*, 213–284.

Calculations show that the most favorable protonation site in these dimers is on the base, even for bases with GB values that are lower than valine. However, the calculations incorrectly predict that the zwitterionic form of valine is most stable for protonated dimers with methylamine, the least basic base in this study. The calculations also do not correctly account for the change in structure of these protonated dimers with increasing GB of the base that is clearly observed from the changes in the IRMPD spectra in the higher frequency region. These results suggest that structural conclusions based on calculations of similar protonated complexes may not be reliable at this level of theory. These results also clearly demonstrate the complementary structural information obtained in the high and low frequency regions of the IRMPD spectra.

Because valine is nonzwitterionic in isolation and also in these protonated dimers with a base, even when the GB of the base is greater than valine by 40 kJ/mol, the kinetic method, with the caveats noted previously by others,[42,72−75] should provide reliable information about the basicity and proton affinity of this and other aliphatic amino acids. For more basic amino acids, such as arginine, which has a greater propensity to form zwitterionic structures in the presence of a charge, the structure of the amino acid in the protonated dimers may be significantly different from that in isolation. IRMPD spectroscopy should provide detailed information about the structures of these complexes and could provide useful information about how major structural differences of the form of a molecule in a protonated dimer vs in isolation may influence results from kinetic method experiments.

Acknowledgment. We thank Dr. Matthew F. Bush for helpful discussions and Professor Alan G. Marshall and Dr. Gregory T. Blakney for the loan of and support for the modular data acquisition system (MIDAS). IRMPD spectra were measured at the FOM Institute for Plasma Physics "Rijnhuizen", which is financially supported by the Nederlandse Organisatie voor Wetenschappelijk Onderzoek (NWO), and we thank Dr. B. Redlich and the FELIX staff for excellent support. Financial support was generously provided by the National Science Foundation (Grants CHE-0718790 and OISE-730072).

Supporting Information Available: Full citation for ref 58. This material is available free of charge via the Internet at http://pubs.acs.org.

JA809928H

---

(72) Armentrout, P. B. *J. Mass Spectrom.* **1999**, *34*, 74–78.

(73) Armentrout, P. B. *J. Am. Soc. Mass Spectrom.* **2000**, *11*, 371–379.

(74) Cooks, R. G.; Koskinen, J. T.; Thomas, P. D. *J. Mass Spectrom.* **1999**, *34*, 85–92.

(75) Drahos, L.; Vekey, K. *J. Mass Spectrom.* **1999**, *34*, 79–84.