Zwitterions

DOI: 10.1002/ange.200600597

Progressive Stabilization of Zwitterionic Structures in $[H(Ser)_{2-8}]^+$ Studied by Infrared Photodissociation Spectroscopy**

Xianglei Kong, I-An Tsai, Sahadevan Sabu, Chau-Chung Han, Yuan T. Lee, Huan-Cheng Chang, Shih-Yu Tu, A. H. Kung, and Chih-Che Wu*

Although amino acids are known to exist as zwitterions in aqueous solution and crystals, they should exist in their neutral form in the gas phase because zwitterionic charge separation is not energetically favored in the absence of a solvent.[1–3] However, it is also known that zwitterionic amino acids can be stabilized by noncovalent interactions with proximal molecules or ions.[4,5] For example, monomeric glycine is non-zwitterionic in the gas phase,[2,6] but clustering with five water molecules transforms glycine into its zwitterion.[7] Recently, the clusters of serine have attracted much attention from both experimentalists and theorists.[8–14] The protonated serine octamer, $[H(Ser)_8]^+$, first appeared as a "magic number" cluster in electrospray ionization (ESI) mass spectra and showed a pronounced preference for homochirality.[8,9] Several structures have been proposed for this cluster ion based on tandem mass spectrometry, ion mobility, and H/D exchange experiments.[8–12] Although most of the proposed structures contain all zwitterionic serine constituents, an H/D-exchange study indicated that isomers with both neutral and zwitterionic structures can form in the ESI source.[12b]

Infrared spectroscopy in combination with mass spectrometry and ab initio calculations has been proven to be an effective approach To deduce the structural information of gas-phase cluster ions.[15] McLafferty and co-workers demonstrated that it is possible to obtain infrared photodissociation spectra of protonated amino acid complexes with a pulsed infrared laser coupled to a Fourier-transform ion-cyclotron resonance (FTICR) mass spectrometer;[13] the spectra of $[H(Ser)_n]^+$ can be similarly obtained. Herein, we present infrared spectra of these cluster ions ($n = 2$–8) and address the questions of whether the serine dimer has a neutral structure and how the zwitterionic structure evolves as n increases. Results of ab initio calculations based on density functional theory (DFT) for the isomeric structures of $[H(Ser)_2]^+$ are also reported. By closely examining the infrared spectra of $[H(Ser)_{2-8}]^+$ in the frequency range 3400–3750 cm^{-1} for the free carboxy and hydroxymethyl OH stretches, a size effect on the structural transition from neutral to zwitterionic forms of serine subunits has been identified.

Figure 1a shows a typical FTICR mass spectrum of homochiral serine clusters produced by ESI. The spectrum is dominated by two peaks that correspond to $[H(Ser)_2]^+$ and $[H(Ser)_8]^+$.[16] The abundance of cluster ions of intermediate size ($n = 3$–7) is clearly too low to acquire their infrared photodissociation spectra. In a first attempt to overcome this difficulty, $[H(Ser)_8]^+$ was isolated in the ICR cell by radio-frequency (RF) sweeps and then fragmented by collision with pulsed argon gas (2×10^{-7} Torr) to produce smaller cluster ions of $n = 3$–6 (Figure 1b). The seemingly less stable heptamer was later produced by in-source collision-induced dissociation (CID) by increasing the ion-transmission energy in the region of the ESI source (Figure 1c). Mass selection was performed in the ICR cell to isolate the desired fragment ions for infrared spectroscopic measurements with an optical parametric oscillator (OPO) laser.

A typical infrared spectrum of $[H(Ser)_2]^+$ at room temperature is shown in Figure 2b. The spectrum was obtained by recording the dissociation fractions of all fragment ions versus total ions against laser frequency. The general feature of the spectrum (normalized with laser power) is in good agreement with the result of Oh et al., who used a similar setup but with a low-repetition-rate pulsed

---

[*] Dr. X. Kong, S. Sabu, Dr. C.-C. Han, Dr. Y. T. Lee, Dr. H.-C. Chang, S.-Y. Tu, Dr. A. H. Kung
Institute of Atomic and Molecular Sciences
Academia Sinica
Taipei 106 (Taiwan)
Fax: (+886) 2362-0200
E-mail: hcchang@po.iams.sinica.edu.tw

I.-A. Tsai, Dr. Y. T. Lee
Department of Chemistry
National Taiwan University
Taipei 106 (Taiwan)

Dr. C.-C. Han, Dr. Y. T. Lee, Dr. H.-C. Chang
Genomics Research Center
Academia Sinica
Nankang 115 (Taiwan)

Dr. A. H. Kung
Department of Photonics
National Chiao Tung University
Hsinchu 300 (Taiwan)

Dr. C.-C. Wu
Department of Applied Chemistry
National Chi Nan University
Puli, Nantou 545 (Taiwan)

[**] This study was supported by the Academia Sinica and the National Science Council (Grant No. NSC 92-3112-B-001-012-Y under the National Research Program for Genomic Medicine) of Taiwan, Republic of China. We thank Professors O. P. Charkin, H. B. Oh, and F. W. McLafferty for insightful discussions.

Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

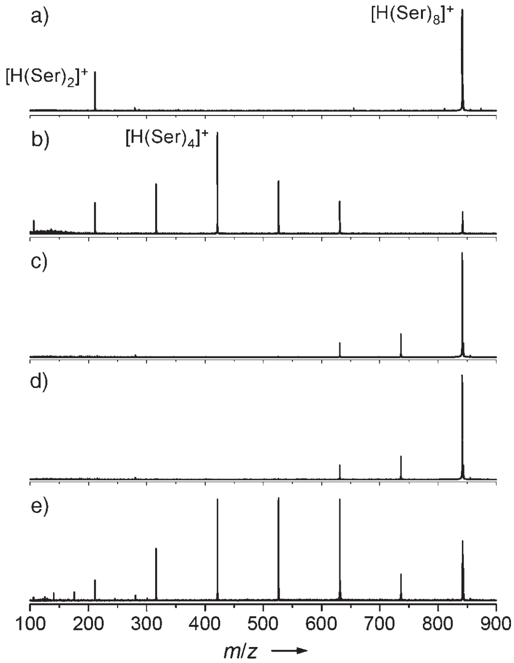

Figure 1. FTICR mass spectra of: a) protonated serine clusters produced directly from ESI; b) protonated serine cluster fragments after in-cell CID of isolated octamers; c) protonated serine clusters with in-source CID; d,e) protonated serine cluster fragments after infrared photodissociation of isolated octamers at 3672 cm^{-1} for 0.5 and 2.0 s, respectively.

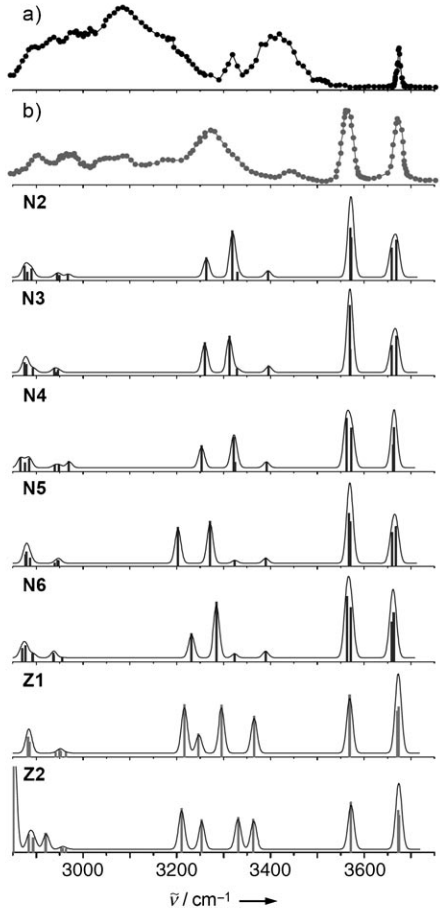

Figure 2. Comparison of experimental infrared spectra of a) [H(Ser)$_8$]$^+$ and b) [H(Ser)$_2$]$^+$ in the frequency range 2850–3750 cm^{-1}. The calculated [H(Ser)$_2$]$^+$ spectra shown underneath were obtained by the B3LYP/6-311++G(d,p) level of calculation and scaled with a single factor, ×0.954. The structures of isomers **N2**–**N6**, **Z1**, and **Z2** are depicted in Figure 3.

OPO laser.[13] The stronger absorption peaks at 3565 and 3672 cm^{-1} are ascribed to the stretching modes of free carboxy OH bonds on the C terminus and free hydroxymethyl OH bonds of the serine side chains, respectively. To assist the spectral interpretation, calculations performed at the B3LYP/6-31+G* level identified 86 stable isomers for this dimeric ion. Of these isomers, only seven are zwitterionic. The main reason that the number of stable neutral structures is far more than that of zwitterionic structures is because the COOH group can act as a proton acceptor, proton donor, or simultaneously as both, thus the probability of forming hydrogen bonds is high. The differences in total energy of these isomers are less than 20 kcal mol^{-1} (see the Supporting Information). Table 1 gives the energetics of the six lowest-energy neutral structures and the two lowest-energy zwitterionic structures, as depicted in Figure 3.[17] As can be seen, the neutral structures (**N1**–**N6**) are all significantly more stable than the zwitterionic structures (**Z1** and **Z2**) according to the MP2/6-311++G(d,p) and CCSD/6-31+G(d,p) levels of calculations. Although the structure of the serine dimer cannot be determined uniquely as a result of the complexity of the hydrogen-bonding network, a comparison of the experimental spectrum with the calculated spectra indicates that the [H(Ser)$_2$]$^+$ isomer with a neutral structure is most likely to be responsible for our observation (Figure 2b).

Table 1: Relative energies (in kcal mol^{-1}) of eight low-energy isomers obtained at three different levels of calculations.

Method[a]	N1[b]	N2	N3	N4	N5	N6	Z1	Z2
B3LYP/6-311++G(d,p)	0.00	0.66	1.22	1.19	1.16	1.50	1.91	3.18
	(0.00)[c]	(0.73)	(1.05)	(1.19)	(1.23)	(1.47)	(1.90)	(3.24)
	(0.00)[d]	(1.79)	(1.77)	(1.77)	(1.91)	(1.92)	(1.81)	(3.81)
MP2/6-311++G(d,p)	0.00	−4.07	−2.79	−2.93	−2.46	−1.95	2.06	3.27
	(0.00)[c]	(−4.00)	(−2.95)	(−2.93)	(−2.39)	(−1.98)	(2.06)	(3.33)
CCSD/6-31+G(d,p)	0.00	−3.87	−2.66	−2.47	−2.47	−1.72	2.63	3.96
	(0.00)[c]	(−3.80)	(−2.83)	(−2.47)	(−2.41)	(−1.76)	(2.63)	(4.02)

[a] All energy calculations were conducted based on the structures optimized by B3LYP/6-311++G(d,p). The calculated total electronic energies of **N1** with the B3LYP, MP2, and CCSD methods are −798.5996675, −796.538688, and −796.278142 hartree, respectively. [b] Structures of the isomers are depicted in Figure 3. [c] Values in this row include zero-point energy (ZPE) corrections based on the frequencies calculated by B3LYP/6-311++G(d,p). [d] Values in this row represent Gibbs free energies at 298 K.

For example, a comparison of the intensities of the absorption bands ascribable to the free OH stretching vibrations of the carboxy group and the hydroxymethyl group yields an experimentally observed ratio of 1.3 ± 0.1, which agrees favorably with that calculated for **N2**–**N6** (≈1.4), but is in

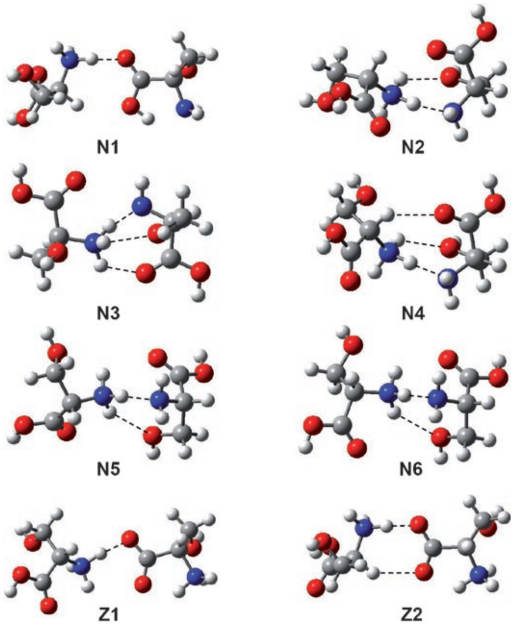

Figure 3. Lowest-energy isomers of $[H(Ser)_2]^+$ optimized by B3LYP/6-311++G(d,p), with six neutral (**N1**–**N6**) and two zwitterionic (**Z1**–**Z2**) structures. The red, blue, gray, and white spheres denote O, N, C, and H atoms, respectively. Note that the structures of **N2**–**N6** are all dominated by linear neutral and ionic $N{-}H^+\cdots N$ hydrogen bonds, instead of $N{-}H^+\cdots O$, because the amino group has a much higher proton affinity than both the carboxy group on the C terminus and the hydroxy group on the serine side chain.

apparent disparity with that calculated for **Z1** and **Z2** (≈ 0.7). We assign the bands at approximately 2900 cm^{-1} to CH stretching vibrations and the bands at 3270 cm^{-1} predominantly to the stretching modes of NH_3^+ groups involved in intermolecular or intramolecular $N{-}H\cdots O$ hydrogen bonding.

Figure 2a shows the photodissociation spectrum of $[H(Ser)_8]^+$. Mass spectra of the corresponding photofragments are given in Figure 1d,e, in which evidence is given of the octamer degrading successively into smaller clusters upon exposure to resonating infrared photons. The sharp feature observed at 3674 cm^{-1}, again, corresponds to the free aliphatic OH stretching mode of serine. Its frequency matches that of the free OH stretch of CH_3OH in $[H(CH_3OH)_{4,5}]^+$ to within 3 cm^{-1}.[18a] Relative to that of $[H(Ser)_2]^+$, the bandwidth of this OH stretching absorption band is significantly smaller (8 versus 18 cm^{-1}), thus indicating that the aliphatic OH bonds in this octamer have more uniform environments. Distinct from the dimer case, however, no sign of the free carboxy OH stretching absorption was found in the spectrum within the limit of our experimental sensitivity. Additionally, a new and relatively sharp band (bandwidth ≈ 33 cm^{-1}) appears in the spectrum of $[H(Ser)_8]^+$ at 3324 cm^{-1}. The band can be ascribed to the free NH stretches of NH_3^+ groups in zwitterionic serines, as its frequency is close to the corresponding vibration in $[NH_4(H_2O)_3]^+$, 3375 cm^{-1}.[19] The observation of this distinct free NH stretching absorption band for the NH_3^+ ion cores, together with the disappearance of the free OH stretches of the COOH groups, corroborates the suggestion that the dominant structure of the $[H(Ser)_8]^+$ cluster probed in our experiment is zwitterionic.[20]

It is instructive to compare our infrared spectroscopic observations with previous measurements of ion mobility. For $[H(Ser)_8]^+$, Counterman and Clemmer[10] and Julian et al.[9b] independently determined the collisional cross section of this octamer to be 191 and 187 Å^2, respectively. By comparing these measured values with calculated cross sections for various neutral and zwitterionic structures, they concluded that only compact, cagelike structures stabilized by electrostatic interactions between the carboxyate groups and the ammonium groups of zwitterionic serines are consistent with their experimental findings. The present observation is in good agreement with their conclusion but additionally indicates that not all hydroxymethyl groups of the serine subunits are involved in hydrogen bonding, as manifested by the sharp band at 3674 cm^{-1} in Figure 2a.

Between the two extreme cases studied, $n=2$ and $n=8$, we found evidence for the progressive stabilization of the zwitterionic structure with increasing cluster size. Figure 4 shows the size-dependent spectra of $[H(Ser)_{2-8}]^+$ in the free OH stretching region.[21] The band intensity ratio of the carboxy OH stretches versus the hydroxymethyl OH stretches decreases progressively from 1.3 to 0.2 as n increases from 2 to 6. The most notable changes occur between $n=5$ and $n=6$. Similar to that of $[H(Ser)_8]^+$, the spectrum of $[H(Ser)_6]^+$ shows only a very weak band that corresponds to free carboxy OH stretches at approximately 3565 cm^{-1}, which reflects that the structure of $[H(Ser)_6]^+$ is also compact and predominantly zwitterionic. In contrast, the intensity ratio of these two absorption bands in the spectra of $[H(Ser)_{3-5}]^+$ and $[H(Ser)_7]^+$ varies substantially from 0.4 to 1.2, which strongly suggests that the structures of these clusters are mixtures of neutral and zwitterionic forms.

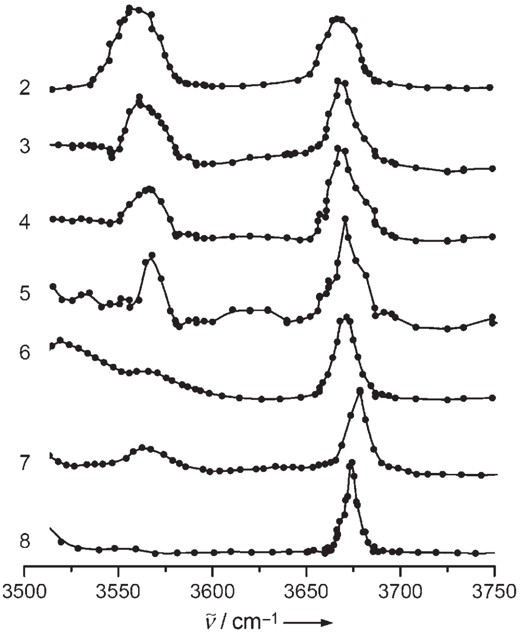

Figure 4. Infrared photodissociation spectra of sized-selected $[H(Ser)_n]^+$, $n=2$–8, in the free OH stretching region. Note the progressive decrease in intensity of the free carboxy OH stretching absorption band with increasing cluster size at ≈ 3565 cm^{-1}.

To conclude, we have provided infrared spectroscopic evidence for progressive stabilization of the zwitterionic structures in homochiral $[H(Ser)_{2-8}]^+$ clusters. Our results are in line with those reported by Beauchamp and co-workers,[9b] who pointed out this intriguing effect based on DFT calculations. However, to provide further insight into this size-dependent structural transition, which is also expected to be found in other amino acid clusters, more calculations and experiments are required.

Experimental Section

Mass spectra of protonated serine clusters were acquired by using an FTICR mass spectrometer (APEX IV, Bruker-Daltonics) equipped with a 7.0 tesla actively shielded superconducting magnet and an external ESI source. Protonated homochiral serine clusters were produced by spraying a sample solution (1 mM L-serine in 30% methanol solution containing 1% formic acid) through an electrically grounded, gas-assisted nebulization system. The ESI-generated ions, after passing through the differentially pumped regions, were accumulated in a hexapole ion trap for ≈1 s and finally pulsed into the ICR cell. Inside the cell, the ions of interest were isolated by RF clean-up sweeps and thermalized with pulsed argon gas at a pressure of $\approx 2\times10^{-7}$ Torr.

Infrared photodissociation spectra of $[H(Ser)_{2-8}]^+$ were obtained by excitation of the OH, NH, and CH stretching vibrations in the frequency range of 2800–3800 cm^{-1} by using a home-built pulsed OPO laser.[22] The singly resonant OPO was constructed with a periodically poled lithium niobate (PPLN) crystal in a grazing-incidence grating optical cavity. The PPLN crystal was pumped by an acousto-optic Q-switched Nd:YAG laser (210S, Lightwave Electronics) at a repetition rate of 4 kHz. As the storage time of ions in the ICR cell is in the order of seconds, a high-repetition-rate light source provided a better duty cycle in signal acquisition than a low-repetition-rate laser of 10–30 Hz.[23] By resonating the signal wavelength, the idler output wavelength of the OPO laser was tunable from 2.5 to 3.5 μm with a bandwidth of ≈1 cm^{-1}. The output wavelength was measured with an optical spectrum analyzer (86142 A, Hewlett Packard) and calibrated against atmospheric water absorption with an uncertainty of ±1 cm^{-1}. The typical laser output power was 100 mW.

Ab initio calculations were carried out extensively for $[H(Ser)_2]^+$. Because of the complexity of the system, even for monomeric serine itself,[24,25] a complete search for all possible potential minima was impractical. Nonetheless, a wide range of hydrogen-bonding network structures, including those of neutral and zwitterionic forms, were designed based on our previous studies of protonated ammonia,[19] methanol,[18] and formamide[26] clusters and optimized with the semi-empirical method, AM1. Ten salt-bridge conformations and 100 ion-molecule conformations were then selected and optimized by the DFT calculation at the B3LYP/6-31+G* level. A split valence double-ζ basis set and extra polarization and diffuse functions (6-311++G**) were further used for geometry optimization and frequency calculation for eight lowest-energy isomers predicted by B3LYP/6-31+G*. The final energies were refined by single-point calculations with MP2/6-311++G(d,p) and CCSD/6-31+G(d,p) methods. All calculations were performed by using the Gaussian 03 program.[27]

Received: February 15, 2006

Keywords: cluster compounds · density functional calculations · IR spectroscopy · mass spectrometry · zwitterions

[1] a) J. H. Jensen, M. S. Gordon, *J. Am. Chem. Soc.* **1991**, *113*, 7917–7924; b) J. H. Jensen, M. S. Gordon, *J. Am. Chem. Soc.* **1995**, *117*, 8159–8170.

[2] Y. Ding, K. Krogh-Jespersen, *Chem. Phys. Lett.* **1992**, *199*, 261–266.

[3] C. J. Chapo, J. B. Paul, R. A. Provencal, K. Roth, R. J. Saykally, *J. Am. Chem. Soc.* **1998**, *120*, 12956–12957.

[4] T. Wyttenbach, M. Witt, M. T. Bowers, *J. Am. Chem. Soc.* **2000**, *122*, 3458–3464.

[5] R. R. Julian, J. L. Beauchamp, W. A. Goddard III, *J. Phys. Chem. A* **2002**, *106*, 32–34.

[6] a) M. J. Locke, R. L. Hunter, R. T. McIver, *J. Am. Chem. Soc.* **1979**, *101*, 272–273; b) M. J. Locke, R. T. McIver, *J. Am. Chem. Soc.* **1983**, *105*, 4226–4232.

[7] S. J. Xu, M. Nilles, K. H. Bowen, *J. Chem. Phys.* **2003**, *119*, 10696–10701.

[8] a) R. G. Cooks, D. Zhang, K. J Koch, F. C. Gozzo, M. N. Eberlin, *Anal. Chem.* **2001**, *73*, 3646–3655; b) Z. Takats, S. C. Nanita, R. G. Cooks, G. Schlosser, K. Vekey, *Anal. Chem.* **2003**, *75*, 1514–1523; c) Z. Takats, S. C. Nanita, R. G. Cooks, *Angew. Chem.* **2003**, *115*, 3645–3647; *Angew. Chem. Int. Ed.* **2003**, *42*, 3521–3523; d) S. C. Nanita, R. G. Cooks, *J. Phys. Chem. B* **2005**, *109*, 4748–4753; e) S. C. Nanita, R. G. Cooks, *Angew. Chem.* **2006**, *118*, 568–583; *Angew. Chem. Int. Ed.* **2006**, *45*, 554–569.

[9] a) R. Hodyss, R. R. Julian, J. L. Beauchamp, *Chirality* **2001**, *13*, 703–706; b) R. R. Julian, R. Hodyss, B. Kinnear, M. F. Jarrold, J. L. Beauchamp, *J. Phys. Chem. B* **2002**, *106*, 1219–1228.

[10] A. E. Counterman, D. E. Clemmer, *J. Phys. Chem. B* **2001**, *105*, 8092–8096.

[11] C. A. Schalley, P. Weis, *Int. J. Mass Spectrom.* **2002**, *221*, 9–19.

[12] a) P. Ustyuzhanin, J. Ustywhanin, C. Lifshitz, *Int. J. Mass Spectrom.* **2003**, *223*, 491–498; b) U. Mazurek, O. Geller, C. Lifshitz, M. A. McFarland, A. G. Marshall, B. G. Reuben, *J. Phys. Chem. A* **2005**, *109*, 2107–2112.

[13] H. B. Oh, C. Lin, H. Y. Hwang, H. Zhai, K. Breuker, V. Zabrouskov, B. Y. Carpenter, F. W. McLafferty, *J. Am. Chem. Soc.* **2005**, *127*, 4076–4083.

[14] F. Pollreisz, A. Gomory, G. Schlosser, K. Vekey, I. Solt, A. G. Csaszar, *Chem. Eur. J.* **2005**, *11*, 5908–5916.

[15] H.-C. Chang, C.-C. Wu, J.-L. Kuo, *Int. Rev. Phys. Chem.* **2005**, *24*, 553–578.

[16] The identities of these cluster ions were confirmed based on isotope separations in a high-resolution mass spectrum (see the Supporting Information).

[17] The most-stable structures suggested in ref. [14] have energies of 1–4 kcal mol^{-1} higher than **N1**–**N6** and **Z1** and **Z2**. It is therefore very unlikely that they are the global-minimum structures of $[H(Ser)_2]^+$.

[18] a) H.-C. Chang, J. C. Jiang, S. H. Lin, Y. T. Lee, H.-C. Chang, *J. Phys. Chem. A* **1999**, *103*, 2941–2944; b) C.-C. Wu, J. C. Jiang, D. W. Boo, S. H. Lin, Y. T. Lee, H.-C. Chang, *J. Chem. Phys.* **2000**, *112*, 176–188.

[19] Y.-S. Wang, H.-C. Chang, J. C. Jiang, S. H. Lin, Y. T. Lee, H.-C. Chang, *J. Am. Chem. Soc.* **1998**, *120*, 8777–8788.

[20] It is possible that the disappearance of the transition for the free carboxy OH stretch can be caused by a red shift of the band because of intramolecular or intermolecular hydrogen bonding. However, experiments of H/D exchange reactions between CH_3OD and $[H(Ser)_8]^+$ with and without ion isolation in our ICR mass spectrometer showed only one type of isomer (see the Supporting Information). A comparison of our experimental conditions and results with those of Mazurek et al.[12b] does not lend support to the presence of neutral structures.

[21] As previously found for other protonated molecular clusters,[15,18,19,26] the absorption bands of hydrogen-bonded OH

and NH stretches are broad and congested and thus provide little structural information.

[22] C. S. Yu, A. H. Kung, *J. Opt. Soc. Am. B* **1999**, *16*, 2233–2238.

[23] X. Qian, T. Zhang, C. Y. Ng, A. H. Kung, M. Ahmed, *Rev. Sci. Instrum.* **2003**, *74*, 2784–2790.

[24] S. Gronert, R. A. J. O'Hair, *J. Am. Chem. Soc.* **1995**, *117*, 2071–2081.

[25] R. Miao, C. Jin, G. Yang, J. Hong, C. Zhao, L. Zhu, *J. Phys. Chem. A* **2005**, *109*, 2340–2349.

[26] a) C.-C. Wu, J. C. Jiang, I. Hahndorf, C. Chaudhuri, Y. T. Lee, H.-C. Chang, *J. Phys. Chem. A* **2000**, *104*, 9556–9565; b) C. Chaudhuri, J. C. Jiang, C.-C. Wu, X. Wang, H.-C. Chang, *J. Phys. Chem. A* **2001**, *105*, 8906–8915.

[27] M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, J. A. Montgomery, Jr., T. Vreven, K. N. Kudin, J. C. Burant, J. M. Millam, S. S. Iyengar, J. Tomasi, V. Barone, B. Mennucci, M. Cossi, G. Scalmani, N. Rega, G. A. Petersson, H. Nakatsuji, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, M. Klene, X. Li, J. E. Knox, H. P. Hratchian, J. B. Cross, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, P. Y. Ayala, K. Morokuma, G. A. Voth, P. Salvador, J. J. Dannenberg, V. G. Zakrzewski, S. Dapprich, A. D. Daniels, M. C. Strain, O. Farkas, D. K. Malick, A. D. Rabuck, K. Raghavachari, J. B. Foresman, J. V. Ortiz, Q. Cui, A. G. Baboul, S. Clifford, J. Cioslowski, B. B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. L. Martin, D. J. Fox, T. Keith, M. A. Al-Laham, C. Y. Peng, A. Nanayakkara, M. Challacombe, P. M. W. Gill, B. Johnson, W. Chen, M. W. Wong, C. Gonzalez, J. A. Pople, Gaussian 03, Revision B.01, Gaussian, Inc., **2004**.