Zwitterionic Structures

DOI: 10.1002/anie.200605174

Stabilization of the Zwitterionic Structure of Proline by an Alkylammonium Ion in the Gas Phase**

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It is well known that amino acids exist in the zwitterionic form in the solid state and in aqueous solution; however, in the gas phase, their structures are in the canonical form. In biological systems, the electric field created by zwitterionic structures is the driving force that determines the structure, function, and activity of amino acids, peptides, and proteins. Many studies of zwitterionic structure have been reported,[1] but direct experimental evidence for their existence in the gas phase is extremely limited.[2] The stabilization of zwitterionic amino acids by metal ions has been investigated relatively extensively.[2a,3] Although charged organic groups are ubiquitous in biological media, their role in stabilizing zwitterionic amino acids remains largely unstudied. In the present work, direct experimental evidence that zwitterionic proline can be effectively stabilized by protonated methylamine in the gas phase is demonstrated from both accurate binding-energy measurements and the IR fingerprint spectrum of this ionic cluster. These results imply that organic ions and other charged groups may provide a very effective means of stabilization of zwitterions and provide further insight into salt-bridge interactions and the formation of zwitterionic structures. Such species may thus serve as models to facilitate understanding of the interactions among the various moieties in more-complex biological molecules, such as proteins.

For the simple amino acids, the zwitterionic form is not a local minimum on the potential-energy surface (PES),[4] and, according to theoretical calculations, the most stable transition structure resembling a zwitterion of glycine lies about 20 kcal mol^{-1} higher in energy than the most stable nonzwitterionic form.[5] Bowen and co-workers suggested that at least five water molecules and an electron could transform glycine into a solvated zwitterion.[1d] Proline, a secondary amine, plays a very important role in determining the secondary and tertiary structures and the biochemical functions of peptides and proteins.[6] Relative to glycine, proline forms a zwitterionic structure somewhat more easily; the zwitterionic form lies about 15 kcal mol^{-1} higher in energy than its canonical form. However, it is still not a local minimum on the PES.

The interactions between amino acids and other molecules or ions play a determinant role in the stabilization of zwitterionic structures,[1–3] and salt bridge interactions involving zwitterionic structures are very important noncovalent interactions. High-pressure mass spectrometry (HPMS) is a very powerful technique for investigation of the interactions between ions and molecules.[7]

Exact binding enthalpy and entropy changes can be directly determined by measuring equilibrium constants as a function of temperature. For the association reaction of protonated proline with methylamine [Eq. (1)], the equilib-

$$\mathrm{ProH^+ + CH_3NH_2 \rightleftharpoons Pro(CH_3NH_2)H^+} \quad (1)$$

rium constant can be obtained from the relative equilibrium ionic abundances and the known partial pressure of methylamine. According to the van't Hoff relation, the enthalpy and entropy changes ΔH and ΔS can be obtained as -26.6 kcal mol^{-1} and -30.3 cal mol^{-1} K^{-1}, respectively.

Ab initio calculations were carried out with the Gaussian 03 program to explore the nature of this ion–molecule interaction and the structure of the resulting cluster.[8] The structures were optimized at the B3LYP/6-311+G(d,p) level of theory and the four most stable structures are shown in Figure 1. In each case, a proline ring-puckering isomer exists,

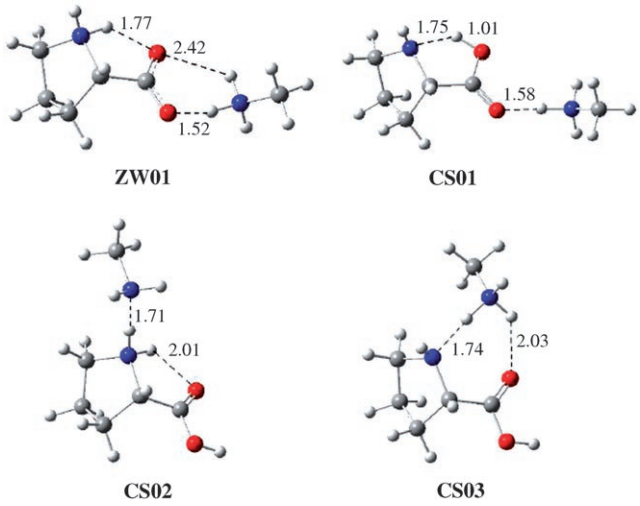

Figure 1. The structures of the four most stable isomers of Pro-$(CH_3NH_2)H^+$ calculated at the B3LYP/6-311+G(d,p) level.

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[**] The generous financial support of this work by the Natural Sciences and Engineering Research Council of Canada (NSERC) is gratefully acknowledged. We are very grateful for the award of beam time at the CLIO FEL facility and for the valuable assistance of the CLIO team, P. Maitre, J. Lemaire, D. Scuderi, J. M. Bakker, T. Besson, and J. M. Ortega. The financial support of the European Commission through the NEST/ADENTURE program (EPITOPES) (project number 15637) is also gratefully acknowledged.

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

but the species shown are each the most stable by less than or equal to 0.5 kcal mol^{-1}. Single-point energies were computed at the MP2(full)/6-311++G(2d,2p)//B3LYP/6-311+G(d,p) level to obtain more accurate interaction enthalpies. These calculated values are summarized in Table 1 together with the experimental values.

Table 1: The experimental and calculated enthalpy and entropy changes of the association reaction for various isomers.

		ΔH_{298} [kcal^{-1} mol^{-1}]	ΔS [cal^{-1} mol^{-1} K^{-1}]
Exptl		-26.6 ± 0.5[a]	-30.3 ± 3.0[a]
Calcd	**ZW01**	-25.7[b] (-27.5[c])	-33.1[b]
	CS01	-22.2[b] (-22.9[c])	-31.5[b]
	CS02	-19.9[b] (-21.7[c])	-30.8[b]
	CS03	-18.9[b] (-21.3[c])	-33.1[b]
	CS04	-17.3[b] (-20.1[c])	-32.2[b]

[a] These uncertainties are greater than those obtained from the van't Hoff plot because of the assumed additional maximum uncertainties in temperature and pressure measurements. [b] The enthalpy and entropy changes were obtained at the B3LYP/6-311+G(d,p) level of theory. [c] Single-point calculations were performed at the MP2(full)/6-311++G(2d,2p)//B3LYP/6-311+G(d,p) level including zero-point energies and thermal energy corrections at 298 K from the calculation results at B3LYP/6-311+G(d,p).

Significantly, the most stable isomer (**ZW01**) involves a zwitterionic proline. In the formation of this isomer from the interaction of protonated proline with methylamine, barrierless proton transfer occurs from the protonated proline to methylamine, which subsequently gives rise to the formation of two hydrogen bonds, involving two hydrogen atoms of the amine function and the two carboxylate oxygen atoms of the zwitterionic proline. These two hydrogen bonds are markedly different. The stronger of the two hydrogen bonds is very short and has a bond length of only 1.52 Å. Evidently, the other intermolecular hydrogen bond is much weaker and longer, as a result of formation of an additional intramolecular hydrogen bond to the carbonyl oxygen atom. The calculated binding energy for this isomer is 27.5 kcal mol^{-1}, which is about 4.6 kcal mol^{-1} lower in energy at 298 K than the second most stable isomer (**CS01**). This latter structure is the nonzwitterionic form. In **CS01** and **CS03**, endothermic proton transfer has also occurred from the protonated proline to methylamine, in contrast to **CS02** and **CS04** (Figure S1 in the Supporting Information), in which a protonated proline structure is retained. Both **CS02** and **CS03** have very similar binding energies of 21.7 and 21.3 kcal mol^{-1}, respectively.

Although the theoretical calculations reveal that several isomers of the cluster investigated herein are possible, it is of interest to ascertain whether all such species are energetically accessible under the experimental conditions employed. To address this question, an exhaustive search of the PES for several key cluster ions was undertaken to explore their interconversion. The calculation of the PES was also carried out at the same B3LYP/6-311+G(d,p) level. The resulting PES based on relative energy differences for interconversion of the various Pro(CH_3NH_2)H$^+$ cluster species at 0 K is shown

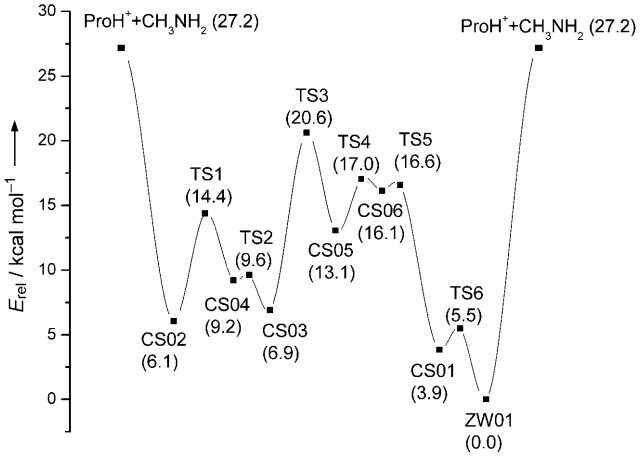

Figure 2. Potential-energy surface for isomerization of protonated proline/methylamine clusters calculated at the B3LYP/6-311+G(d,p) level. The relative energies (E_{rel}) at 0 K are given in parentheses.

in Figure 2. The corresponding PES at 298 K including zero-point-energy and thermal-energy corrections, as well as the structures of every stationary point are shown in Figure S1 (in the Supporting Information). The proton affinity of proline is about 5 kcal mol^{-1} higher than that of CH_3NH_2, so that the more favorable dissociation channel of Pro(CH_3NH_2)H$^+$ should be ProH$^+$ and CH_3NH_2. The highest barrier on the PES is 20.6 kcal mol^{-1} (19.2 kcal mol^{-1} at 298 K) higher in energy than the most stable isomer. However, this transition state is still significantly lower in energy than that of the separated reactants. Therefore, under the experimental conditions, the complete PES can be populated to give a statistical distribution of isomeric species. For a Boltzmann distribution of possible isomers and the calculated values of ΔH and ΔS, the zwitterionic isomer is the dominant species (99.8%) under thermal equilibrium conditions throughout the experimental temperature range examined. The experimental enthalpy change (-26.6 kcal mol^{-1}) is in very good agreement with the calculated binding energy for **ZW01** and is notably higher than that of any other isomer. This result thus constitutes experimental evidence that protonated methylamine may indeed effectively stabilize the zwitterionic structure of proline.

Direct vibrational spectroscopic evidence for the existence of the zwitterionic proline in this cluster was also obtained by using the infrared multiple photon dissociation (IRMPD) technique. IRMPD spectroscopy is a very useful tool for elucidating clearly the structures of both ions and ionic clusters in the gas phase in combination with theoretical calculations.[9] Experiments were carried out with a free-electron laser (FEL) at CLIO coupled to an electrospray ionization–ion trap mass spectrometer (Bruker Esquire3000+). The FEL facility at CLIO has been described in detail previously.[10] The experimental IRMPD spectrum of Pro(CH_3NH_2)H$^+$ (Figure 3a), is expressed as the natural logarithm of the fragmentation efficiency as a function of the photon energy (in cm^{-1}).[10] To identify the structure, the calculated spectra of the two most stable isomers, **ZW01** and **CS01**, are also included. These calculated

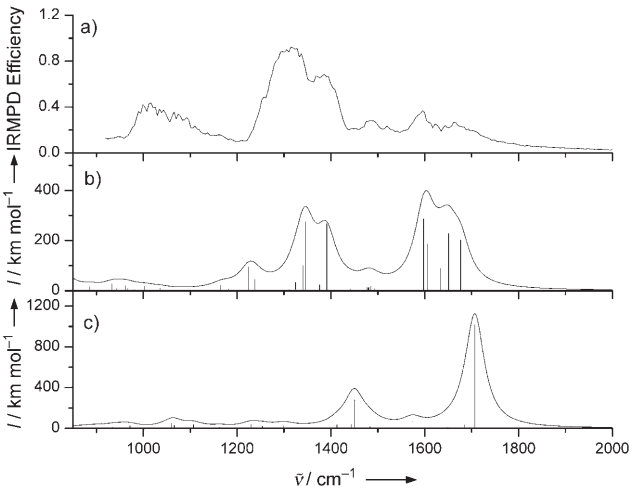

Figure 3. a) IRMPD spectrum of Pro(CH_3NH_2)H^+ and calculated spectra of the two most stable isomers: b) **ZW01** and c) **CS01**.

spectra were obtained at the B3LYP/6-311+G(d,p) level with a scaling factor of 0.985. The calculated band frequencies and intensities were convoluted by assuming a Lorentzian profile with a full width at half-maximum of 50 cm^{-1} to take into account the rotational bandwidth and the dynamics of the multiphoton excitation.[9e] The experimental and calculated band intensities are often inconsistent, which may be the result of a number of possible factors. Firstly, the multiple-photon excitation process may not precisely mimic the calculated single-photon absorption spectrum. Secondly, the calculated IR intensities may themselves be subject to some error, particularly, for modes involving hydrogen bonds. Finally, the IRMPD spectrum is a function not only of the absorption spectrum, but also of the fragmentation efficiency of the ion.

As can be seen from Figure 3, most of the bands of the experimental IRMPD spectrum correspond well to those calculated for **ZW01**. The poorest agreement is found for the experimental band near 1000 cm^{-1}, which differs by some 50 cm^{-1} from the weak calculated band because of a combination of the ring deformation vibrations of proline and the C–N stretch of $CH_3NH_3^+$. The calculated band at about 1230 cm^{-1} for CH and NH wagging motions quite possibly corresponds to the poorly resolved shoulder at about 1250 cm^{-1} in the strongest band in the experimental spectrum. The strong band at 1320 cm^{-1} may be assigned to the twisting motions of the CH_2 and NH_2 of the proline ring. The band at 1390 cm^{-1} is the symmetric stretching vibration of the carboxylate group, which is in very good agreement with the calculated value of 1391 cm^{-1}. This band is characteristic of zwitterionic amino acids and can be considered to be diagnostic of a zwitterionic structure.[2a,11] The weak band at 1484 cm^{-1} is also consistent with the calculated -CH_2 scissors modes of proline and $CH_3NH_3^+$. The band at 1595 cm^{-1} may be assigned as the combination of the umbrella mode of the -NH_3 group of $CH_3NH_3^+$ and the scissors mode of the -NH_2 group of proline. Finally, the band at 1660 cm^{-1} corresponds to a combination of the asymmetric stretch of the carboxylate group and the scissors mode of the -NH_3 group in $CH_3NH_3^+$. The intensities of the two bands at 1595 and 1660 cm^{-1} in the experimental spectrum are notably weaker than those in the calculated spectrum. This may be due, at least in part, to weaker laser intensity in this range.

The calculated spectrum of **CS01** exhibits two strong bands; the stronger, at 1707 cm^{-1}, corresponds to the stretching vibration of the carbonyl group, and the weaker, near 1450 cm^{-1}, is due to the OH bending vibration. The experimental spectrum shows no significant peak above 1700 cm^{-1}, even though the calculated intensity of the peak at 1707 cm^{-1} (1019 km mol^{-1}) is predicted to be much stronger than all peaks in the **ZW01** spectrum; the greatest intensity in this range for **ZW01** is calculated to be 287 km mol^{-1} for the peak at 1597 cm^{-1}. The calculated spectra of **CS02** and **CS03** have also been compared. Like that of **CS01**, they are markedly different from the experimental IRMPD spectrum. These results demonstrate that **ZW01** is almost certainly the dominant species and there is no significant contribution of the other isomers to the population of the cluster under these experimental conditions.

Thus, the **ZW01** cluster, containing a zwitterionic proline moiety, has been demonstrated to exist in the gas phase by a combination of HPMS and IRMPD experiments and ab initio calculations. This result indicates that the zwitterionic structure of proline may be effectively stabilized by protonated methylamine. Zwitterionic proline may also be stabilized by Na^+, as has also been confirmed by IRMPD and theoretical calculations.[2a] The calculated and experimental results for glycine[2a] indicated that the zwitterionic isomer is not the most stable one in the cluster of Na^+, and the relative energy of the zwitterionic structure is still 1–3 kcal mol^{-1} higher than that of the most stable nonzwitterionic structure.[2a,3b,13] However, for the cluster of glycine and NH_4^+, a zwitterionic isomer is equivalent in energy to the most stable nonzwitterionic isomer according to calculations at the MP2(full)/6-311++G(2d,2p)//B3LYP/6-311+G(d,p) level.[12] Therefore, NH_4^+ may be more effective than Na^+ in stabilizing the zwitterionic structure of glycine, as has been confirmed by IRMPD spectra, which show that several isomeric glycine species coexist in the gas phase.[14] Moreover, it has been shown that zwitterionic proline also exists in the protonated proline dimer.[15]

As ammonium ions are prevalent in biological media as protonated amino acids, peptides, proteins, and nucleic acid bases, all of which exhibit zwitterionic structures, the present results for the adduct of protonated proline and methylamine serve as an illuminating model to aid in the understanding of the stabilization of zwitterionic structures. Even in an isolated protein molecule, a charged group may thus stabilize a zwitterionic structure, with very important consequences relating to the functions of the protein.

Received: December 21, 2006
Published online: March 30, 2007

Keywords: ab initio calculations · gas-phase chemistry · proline · vibrational spectroscopy · zwitterions

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