A highly conformationally specific α- and β-Ala$^+$ decarboxylation pathway†

Kyo-Won Choi, Doo-Sik Ahn, Joo-Hee Lee and Sang Kyu Kim*

One-photon ionization of alanine and β-alanine induces the decarboxylation reaction which occurs with the concomitant intramolecular hydrogen transfer in a highly conformationally specific manner.

Local charges in proteins or nucleotides induced by electron transfer are responsible for a number of site-specific biological reactions which are very efficient and selective. The role of the electron–hole in chemical reactions, therefore, is an important subject indispensable for understanding of biological reactions in molecular detail. The fragmentation reactions of various peptide radical cations have been intensively studied using mass spectrometry to unravel the mechanism for the individual fragmentation channel, which is important for the understanding of the inter- and intramolecular energy transfer in biological reactions.[1–3] For instance, the decarboxylation channel from the carboxylate radical has recently been reported in the fragmentation reactions of zwitterionic peptides.[3] The associated reaction mechanism, however, needs more clarification. In this regard, a sophisticated experiment with the fine tuning of the reaction energy for the simple system would be quite useful. Here, we explore the dissociative ionization of alanine (Ala) and β-alanine (β-Ala) in the gas phase and have discovered a new dissociative ionization pathway by way of vacuum-ultraviolet (VUV) photoionization and photofragmentation spectroscopy. At the ionization threshold region, the C$_\alpha$–C bond is ruptured to liberate CO_2 with concomitant intramolecular hydrogen transfer. This decarboxylation reaction is highly conformer-specific, as demonstrated experimentally and theoretically for Ala and β-Ala monocations. Amino acids in the gas phase adopt the canonical form, NH$_2$–C$_\alpha$HR–COOH,[4–10] and bear nearly free internal rotations about the C$_\alpha$–C and C$_\alpha$–N vectors. This flexibility guarantees great polypeptide conformational diversity which gives rise to enzymatic selectivity/specificity. Understanding conformer specificity in the transformations of lone amino acids would be invaluable in understanding more complex biological systems at the atomic level.[1–3]

VUV photoionization efficiency (PIE) spectra have been obtained for Ala and β-Ala which were heated to 190–250 °C before being supersonically cooled in a free jet expansion. The VUV laser pulse ($\Delta E \sim 1.0$ cm^{-1}, $\Delta t \sim 5$ ns) in the 131–141 nm range was generated *via* a four-wave mixing process in a Kr cell

using a fixed 212.550 nm laser pulse for the two-photon Kr 5p[1/2]$_0$–4p^6 transition and a tunable laser pulse in the 430–560 nm range.[10] The VUV laser pulse was separated from UV and visible fundamentals using the edge of a collimating CaF$_2$ lens on the exit of the four-wave mixing cell. Ions generated by the VUV laser pulse were directed to the time-of-flight mass spectrometer by a pulsed electric field after the delay time of ~ 10 μs to avoid the ionization energy shift in the presence of the electric field.[10–12]

The increase of the ionization efficiency is quite smooth with increasing the excitation energy not only because of several different conformers in the jet but also because of the structural change upon ionization. Therefore, it is nontrivial to determine the ionization threshold precisely. Accordingly, the ionization threshold is estimated from the onset with relatively large uncertainties, giving the ionization energy of 8.94 ± 0.05 and 8.93 ± 0.05 eV for Ala and β-Ala, respectively, Fig. 1 and 2. These ionization energies are consistent within error with the photoelectron or photoionization-mass spectrometric values.[13,14]
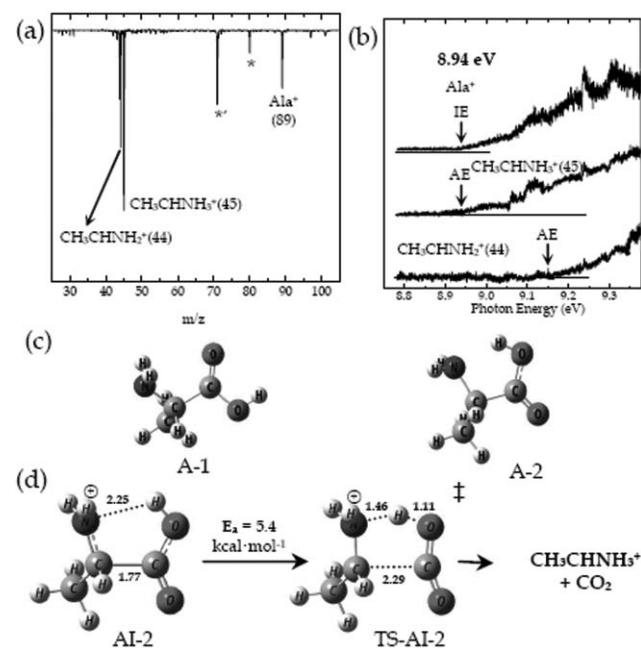

Fig. 1 (a) TOF mass spectrum of Ala (E_{ph} = 9.37 eV). The ion signal denoted by *(*′) is due to pyridazine (a fragment ion from clusters) which was introduced intentionally for monitoring of the VUV laser intensity. (b) VUV-PIE spectra of Ala and fragment ions. (c) Two most abundant conformers of Ala in the jet and (d) the calculated dissociation mechanism of Ala$^+$ showing the structure of the proposed transition state (‡). Bond lengths and activation energies predicted by the DFT (B3LYP/6-31++G**) calculation are shown.

Department of Chemistry and School of Molecular Science (BK21), Korea Advanced Institute of Science and Technology (KAIST), Daejeon, 305-701, Republic of Korea. E-mail: sangkyukim@kaist.ac.kr; Fax: +82-42-869-2810; Tel: +82-42-869-2843
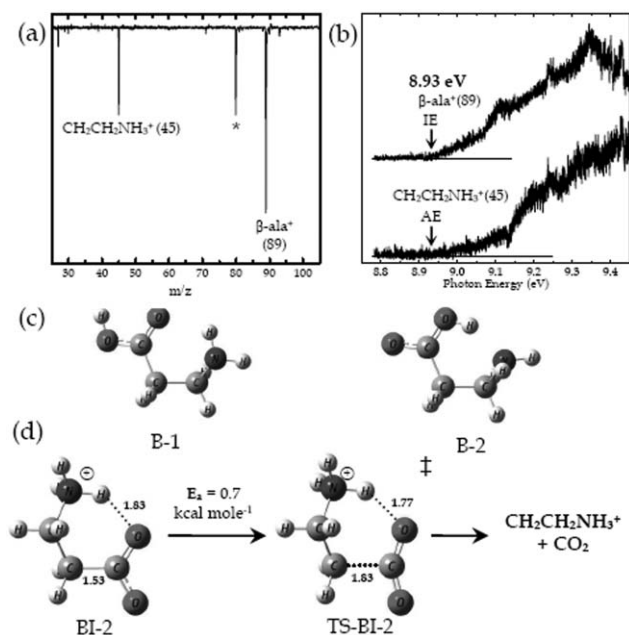
Fig. 2 (a) TOF mass spectrum of β-Ala (E_{ph} = 9.33 eV). The ion signal at m/z = 80 is due to the pyridazine ion (see the caption in Fig. 1). (b) VUV-PIE spectra of β-Ala and its fragment ion. (c) Two lowest energy conformers of β-Ala in the ground state. (d) The proposed decarboxylation mechanism of β-Ala$^+$. Structural parameters and activation energy are calculated by the DFT (B3LYP/6-31++G**) method.

A significant experimental finding is that the fragment ion signal is strongly observed even at the ionization threshold region, indicating that the amino acid undergoes molecular fragmentation simply by the ionization (Fig. 1). The m/z = 45 signal corresponds to $CH(CH_3)NH_3^+$, a formal decarboxylation product of the parent ion, while the m/z = 44 signal is assigned to $CH(CH_3)NH_2^+$ which results from the simple C–C(O) bond fission channel giving off COOH. The appearance energy for simple C–C(O) bond cleavage is ∼0.2 eV above the ionization threshold, consistent with the results of previous studies.[13,14] The appearance energy of the decarboxylation product, $NH_3CHCH_3^+$, is very close to the ionization energy of β-Ala$^+$ indicating that little or no barrier exists to the loss of CO_2 (Fig. 1). To our best knowledge, the decarboxylation reaction of Ala$^+$ in the ionization threshold region has never been observed before. It is noteworthy that the decarboxylation of glycine in the S_0 state occurs with a significant activation barrier of 73.0 kcal mol^{-1},[15] indicating that the fragment ion corresponding to m/z = 45 is not due to the ionization of thermally decomposed species of Ala. Actually, in the high backing pressure condition where the collisional decomposition of Ala is severe, the m/z = 45 signal decreases as the parent ion signal decreases, and *vice versa*.

Ala adopts the canonical form in the gas phase, NH_2–$C_\alpha H(CH_3)$–COOH, and the decarboxylation reaction of its cation requires that the C_α–C(O) bond dissociation should be accompanied by intramolecular hydrogen transfer. Earlier studies demonstrated that Ala in the supersonic jet adopts two main conformations,[16] A-1 and A-2, Fig. 1. In conformer A-2, the hydrogen in the COOH moiety is hydrogen-bonded with the amino group. The ionization of the A-2 induces a structural change where the NH_2 group becomes planar with the C_α–N bond

axis to give the conformer AI-2 (Fig. 1). Since there is a certain barrier for conformer inter-conversion,[17] the experimental fact that the fragmentation occurs even at the ionization threshold strongly indicates that the conformer A-2 (the AI-2 cation) is responsible for the cationic decarboxylation reaction. In the AI-2 cation, the hydrogen transfer from the COOH moiety to the NH_2^+ group should occur very efficiently. Intrinsic reaction coordinate (IRC) calculations using the DFT method (B3LYP/6-31++G**) confirm this reaction pathway. The calculation suggests that once a HOMO electron is removed, the hydrogen transfer from the COOH to NH_2^+ moiety occurs with simultaneous C_α–C bond elongation, Fig. 1. The transition state calculated for the decarboxylation of Ala$^+$ clearly shows that both the hydrogen-transfer and C_α–C bond elongation should be strongly coupled to the reaction coordinate. According to our calculations, the reaction barrier for the cationic decarboxylation is 5.4 kcal mol^{-1}, suggesting that the reaction proceeds *via* tunneling in the ionization threshold region. It should be noted that the decarboxylation reaction of Ala$^+$ is exothermic, giving off 7.8 kcal mol^{-1},[17–19] when heats of formation values of the parent and fragment ions are quantitatively considered. This exothermicity appears to be the driving force of the decarboxylation of the amino acid cation. The ionization-induced structural change of the Ala conformer (A-2 in Fig. 1) indicates that the vibrational modes involving NH_2 wagging and N–C_α–C bending motions will be highly activated in the one-photon ionization process, which may facilitate the intramolecular hydrogen transfer. The effect of the vibrational excitation on the hydrogen-transfer with the tunneling issue should be further investigated with the H/D isotope substitution experiment in the future.

The preference for decarboxylation from β-alanine is even more pronounced. As shown in Fig. 2, the simple C_α–C(O) bond fission pathway giving off COOH is almost absent, whereas the decarboxylation channel becomes dominant in the ionization threshold region. The (COO)H–NH_2 hydrogen-bonded conformer of β-Ala, B-2 in Fig. 2, has recently been reported to be significantly populated in the jet.[5,20] The relatively larger population of the B-2 conformer may be one of the reasons why the decarboxylation of β-Ala$^+$ is very active. The more decisive reason, however, can be found from the fact that the hydrogen-transfer in β-Ala$^+$ does not require excess energy at the ionization threshold. The calculation indicates that the (COO)H atom is already transferred to the NH_2^+ group in the β-alanine cation to adopt the structure of NH_3^+–CH_2–CH_2–COO even including a zero-point energy correction. Thereafter, the C_α–C(O) bond dissociation occurs with a small activation barrier of 0.7 kcal mol^{-1} which is much lower than that in the Ala$^+$ decarboxylation reaction. The simple C_α–C(O) bond cleavage channel of β-Ala$^+$ is therefore relatively less active at least in the ionization threshold region. The experimental facts found in the β-Ala$^+$ fragmentation confirm that the decarboxylation of amino acids does occur in the ionization threshold region, its mechanism involves the hydrogen transfer from (COO)H to the positively charged amino group, and this concomitant process is highly conformer specific.

In summary, we report that alanine and β-alanine, when ionized, undergo facile decarboxylation reactions in the ionization threshold region. This decarboxylation reaction proceeds by intramolecular hydrogen-transfer with great conformer-specificity. Experimental findings are fully supported by theoretical

calculations. The large difference of alanine and β-alanine in the chemical reactivity upon ionization has been clearly demonstrated for the first time in the gas phase. The spectroscopic selection of a specific conformer of amino acids would be much desirable for the further conformer specific molecular dynamics studies. Theoretical calculations using the *ab initio* dynamics method will be also very interesting, as had been nicely demonstrated for the ionization-driven chemical reaction of glycine.[21] The experimental and theoretical effort given to understanding small biological systems such as amino acids and DNA bases[11,12] are crucial for the detailed understanding of complex biological systems.

Notes and references

1 S. Wee, R. A. J. O'Hair and W. D. McFadyen, *Rapid Commun. Mass Spectrom.*, 2002, **16**, 884.
2 A. Karnezis, C. K. Barlow, R. A. J. O'Hair and W. D. McFadyen, *Rapid Commun. Mass Spectrom.*, 2006, **20**, 2865.
3 C. K. Barlow, D. Moran, L. Radom, W. D. McFadyen and R. A. J. O'Hair, *J. Phys. Chem. A*, 2006, **110**, 8304.
4 P. D. Godfrey and R. D. Brown, *J. Am. Chem. Soc.*, 1995, **117**, 2019.
5 S. X. Tian, *J. Phys. Chem. A*, 2006, **110**, 3961.
6 S. X. Tian and J. Yang, *Angew. Chem., Int. Ed.*, 2006, **45**, 2069.
7 D.-S. Ahn, S.-W. Park, I.-S. Jeon, M.-K. Lee, N.-H. Kim, Y.-H. Han and S. Lee, *J. Phys. Chem. B*, 2003, **107**, 14109.
8 E. G. Robertson and J. P. Simon, *Phys. Chem. Chem. Phys.*, 2001, **3**, 1.
9 E. G. Diken, N. I. Hammer and M. A. Johnson, *J. Chem. Phys.*, 2004, **120**, 9899.
10 K.-W. Choi, D.-S. Ahn, J.-H. Lee and S. K. Kim, *J. Phys. Chem. A*, 2006, **110**, 2634.
11 K.-W. Choi, J.-H. Lee and S. K. Kim, *J. Am. Chem. Soc.*, 2005, **127**, 15674.
12 K.-W. Choi, J.-H. Lee and S. K. Kim, *Chem. Commun.*, 2006, 78.
13 H.-W. Jochims, M. Schwell, J.-L. Chotin, M. Clemino, F. Dulieu, H. Baumgärtel and S. Leach, *Chem. Phys.*, 2004, **298**, 279.
14 A. F. Lago, L. H. Coutinho, R. R. T. Marinho, A. N. de Brito and G. G. B. de Souza, *Chem. Phys.*, 2004, **307**, 9.
15 J. Li and T. B. Bril, *J. Phys. Chem. A*, 2003, **107**, 5987.
16 S. Blanco, A. Lesarri, J. C. López and J. L. Alonso, *J. Am. Chem. Soc.*, 2004, **126**, 11675.
17 The barrier for the conversion from the AI-1 to AI-2 conformer has been calculated to be ~16 kcal mol^{-1}, while the reverse barrier is calculated to be ~5 kcal mol^{-1}; see ESI† for the more details.
18 J. B. Pedly, R. D. Naylor and S. P. Kirby, *Thermochemical Data of Organic Compounds*, Chapman and Hall Ltd, New York, 2nd edn, 1986.
19 S. J. Baek, K.-W. Choi, Y. S. Choi and S. K. Kim, *J. Chem. Phys.*, 2003, **118**, 11026.
20 M. E. Sanz, A. Lesarri, M. I. Peña, V. Vaquero, V. Cortijo, J. C. López and L. Alonso, *J. Am. Chem. Soc.*, 2006, **128**, 3812.
21 D. Shemesh and R. B. Gerber, *J. Chem. Phys.*, 2005, **122**, 241104.