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ORIGINAL ARTICLE

Synchrotron vacuum ultraviolet (VUV) photo-induced fragmentation of cyclic dipeptides radical cations

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Abstract Cyclic dipeptides, due to their chemical properties and various bioactivities, are very attractive for medicinal chemistry. Fragmentations of three simple cyclic dipeptides including cyclo(Gly–Gly), cyclo(Ala–Ala) and cyclo(Gly–Val) in the gas-phase are determined with synchrotron vacuum ultraviolet (VUV) photoionization mass spectrometry (VUV PIMS) and theoretical calculations. Cyclo(Gly-Gly) and cyclo(Ala-Ala) show the similar fragmentation pathways. The primary decomposition reactions of cyclo(Gly-Gly) and cyclo(Ala-Ala) radical cations are found to be HNCO loss and CO elimination. The appearance energies (AEs) of fragment ions $[CH_2NHCOCH_2]^{+\bullet}$ and $[CH_3CHNHCOCHCH_3]^{+\bullet}$ are measured to be 10.21 and 9.66 ± 0.05 eV, respectively, which are formed from cyclo(Gly-Gly) and cyclo(Ala-Ala) radical cations with HNCO elimination. Due to the stabilization of the radical cation of cyclo(Gly-Val) with isopropyl side group, the dominant fragment ion *m/z* 114 assigned as $[C_4H_6N_2O_2]^{+\bullet}$ is produced by γ-H migration and *i* cleavage to lose propylene. The ionization energies (IEs) of three cyclic dipeptides decrease in the order cyclo(Gly-Gly) (9.33 ± 0.05 eV) > cyclo(Ala-Ala) (9.21 ± 0.05 eV) > cyclo(Gly-Val) (9.09 ± 0.05 eV) from measurements of photoionization efficiency spectra. It implies that IEs of cyclic dipeptides are affected by substituent groups and symmetrical characterization of molecular structures. These observations of the chemical properties of cyclic dipeptides radical ion ($M^{+\bullet}$) may be important for understanding gas-phase molecular reactivity of 2,5-diketopiperazines and guiding diketopiperazine-based drug design.

Keywords Cyclic dipeptide · VUV photoionization · Mass spectrometry · Fragmentation

Introduction

Cyclic peptides, a very important family of bioactive compounds, are easily available from natural sources (plants, animals or microorganisms) or by means of synthetic methods. They have various bioactivities including antitumour (Kanoh et al. 1999; Kanzaki et al. 2000; Nicholson et al. 2006), antiviral (Sinha et al. 2004), antifungal (Asano 2003; Houston et al. 2004), antibacterial (Fdhila et al. 2003; Kanokmedhakul et al. 2002; Sugie et al. 2001), and antihyperglycaemic (Hwang et al. 2003; Kwon et al. 2000; Song et al. 2003). Cyclic peptides, due to their conformational rigidity, have enhanced receptor-binding affinities, specificity and stability relative to their linear counterparts. These features make cyclic peptides attractive for drug discovery and biomedical research. Cyclic dipeptides are the smallest among them. They have the general structure of diketopiperazines (DKPs) as shown in Fig. 1. The important molecular properties of DKPs include substituent group stereochemistry (defined and controlled in up to four combinations), donor and acceptor groups for hydrogen bonding (favouring interactions with biological targets), resistance to proteolysis and conformational rigidity. Therefore, DKPs as pharmacophore are

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usually studied for the development of new drug (Fischer 2003a, b; Horton et al. 2000; Wang et al. 2002).

The well-established method of finding and identifying DKPs is to isolate a compound by chromatography and then to characterize it by spectroscopic methods such as nuclear magnetic resonance (NMR), X-ray crystallography, infrared spectroscopy (IR) and mass spectrometry (MS) (Borthwick et al. 2006; Fischer 2003b; Leung and Grant 1997; Martins and Carvalho 2007). Due to its high sensitivity and selectivity, MS has been the most useful tool for analysis of cyclic dipeptides. A variety of ionization methods for analysis of cyclic peptides have been used including electron ionization (EI), photoionization (PI) (Ling and Lifshitz 1998), chemical ionization (CI) (Wabnitz et al. 1996), fast atom bombardment (FAB) (van der Merwe et al. 2008) and electrospray ionization (ESI) (Furtado et al. 2007; Guo et al. 2009a, b). The fragmentation pathways of protonated and deprotonated cyclic dipeptides have been studied by ESI-MS/MS (Furtado et al. 2007; Guo et al. 2009a, b; Wabnitz et al. 1996). It is found that the protonated cyclic dipeptides tend to eliminate CO, $HCONH_2$, amino acid residue or saturated substituent molecule (R + H) to form various ions (Guo et al. 2009b). For the proline derivatives of DKPs, loss of CO directly from the protonated molecule was found to be a fragmentation process, which is common to all the compounds (Furtado et al. 2007). However, the deprotonated cyclic dipeptides tend to eliminate an unsaturated substituent molecule (R − H), a substituent radical, successively eliminates a CO and a saturated substituent molecule (R + H) (Guo et al. 2009a). Bowie's group has studied the mass spectra of some deprotonated cyclic dipeptides in the negative CI mode (Wabnitz et al. 1996). It was found that there were two major fragmentation pathways of $[M - H]^-$ ions for symmetrical and unsymmetrical cyclic dipeptides. One pathway was loss of the characteristic side-chain and the other one was an unusual loss of RCHO (R is the substituent). In addition, the cyclo(Pro-Gly) radical cation was investigated by Lifshitz using EIMS/MS and PIMS. They found loss of HNCO and CO from cyclo(Pro-Gly) radical cation to be the primary decomposition reactions (Ling and Lifshitz 1998).

In this paper, we report photon-induced fragmentation of cyclic dipeptides with infrared laser desorption/synchrotron vacuum ultraviolet (VUV) photoionization mass spectrometry (IRLD/VUVPIMS). Recently, IRLD/VUVP-IMS has been successfully applied for the studies of photoionization and photodissociation of biomolecules and drugs, including amino acids (Guo et al. 2010; Pan et al. 2009b; Zhang et al. 2009), steroids (Pan et al. 2009a) and drugs (Deng et al. 2010; Pan et al. 2008a). In this study, photoionization mass spectra of three cyclic dipeptides were measured at different photon energies. Moreover, fragmentations of three cyclic dipeptides were investigated according to theoretical calculations.

Experimental section

The experiments were performed with IR LD/VUV-PIMS apparatus, which has been reported in detail previously (Pan et al. 2008a, b, 2010). Commercial available cyclic dipeptides were deposited on to a stainless steel substrate without any matrix and desorbed by the fundamental output (1,064 nm) of a Nd:YAG laser (Surelite I-20,Continuum, USA). The desorbed neutral molecules were then ionized by tunable VUV light and analyzed by a home-made reflectron time-of-flight (RTOF) mass spectrometer. Cyclo(Gly-Gly) and cyclo(Ala-Ala) were obtained from TCI (Japan), while cyclo(Gly-Val) was obtained from Sigma-Aldrich. Their structures are shown in Fig. 1. All chemicals were used without any purification.

To confirm the structural assignment of fragment ions for proposing the fragmentation pathways, accurate mass measurements were performed with EI TOF-MS (Micromass, Manchester, UK) with 70 eV electron impact and trap current of 10 mA. The instrument was calibrated at a mass resolution of 8000 (FWHM) using heptacosafluorotributylamine as internal reference and the single point lock-mass was set at *m/z* 218.9856. Sample analysis, exact mass measurements and elemental composition determination were performed automatically using the OpenLynx software (Micromass).

Computational method

All the theoretical calculations were carried out using Gaussian03 program (Frisch et al. 2004). The main decomposition pathways of three DKPs cations were

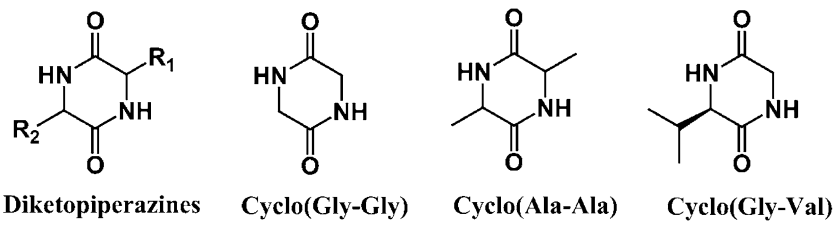


Fig. 1 The structures of diketopiperazines, cyclo(Gly-Gly), cyclo(Ala-Ala) and cyclo(Gly-Val)

calculated using density functional theory employing the Becke-3-Lee-Yang-Parr (B3LYP) hybrid function and the 6-31++g(d,p) basis set for geometry optimization and frequency calculation for these molecules (Becke 1993). Single point energy calculation was performed with a combination of B3LYP/6-31++G(2df,p) and MP2/6-31+G(2df,p) methods, which has been verified to have higher accuracy (Li et al. 2009; Wolken et al. 2007). Therefore, accurate energies were calculated using the following Eq. 1, which ZPE is the zero point energy from the B3LYP/6-31++G(d,p) frequency calculation

$$E(\mathrm{B3}-MP2) = (E[\mathrm{B3LYP/6{-}31{+}{+}G(2df,p)}] + E[\mathrm{MP2/6{-}31{+}{+}G(2df,p)}]) \times 0.5 + \mathrm{ZPE} \quad (1)$$

Further, the half-and-half functional BH and HLYP method with 6-31 ++G(d,p) basis set was also used to calculate the reaction pathways. The stationary points were identified with frequency calculations at the same level to verify minima and transition state structures with zero and one imaginary frequencies, respectively. Zero-point energy (ZPE) corrections were also evaluated from the frequency calculations. In the dissociation pathways of three cyclic dipeptide cations, the energies of corresponding neutral molecules were defined to be zero. Appearance energy (AE) of ionic fragment is defined as $E_{AE} = E_{max} - E_0$, in which E_{max} refers to the highest energy barrier involved in the formation pathway of corresponding ionic fragment and E_0 is the absolute energy of neutral precursor.

Results and discussion

Cyclo(Gly-Gly)

VUV photoionization mass spectra of cyclo(Gly-Gly) at different photon energies are displayed in Fig. 2. The synchrotron VUV photoionization is a soft ionization process which can avoid the interference of fragment ions. Only cyclo(Gly-Gly) molecular ion ($M^{+\bullet}$) is obtained at photon energy of 10.00 eV (Fig. 2a). Many literatures were reported on the mass spectrometric analysis of DKPs (Chen et al. 2009, 2004; Furtado et al. 2007; Guo et al. 2009a, b; Ryan et al. 2009). But the exclusive cyclic dipeptide radical ion ($M^{+\bullet}$) has scarcely been reported, which is important for understanding gas-phase molecular reactivity and chemical properties of DKPs. The peptide radical cations exhibit a more diverse loss of small species (radicals and neutrals). These reactions show complexity, low activation barriers, and poor selectivity due to the high reactivity of the radical and the small energy differences among reaction channels.

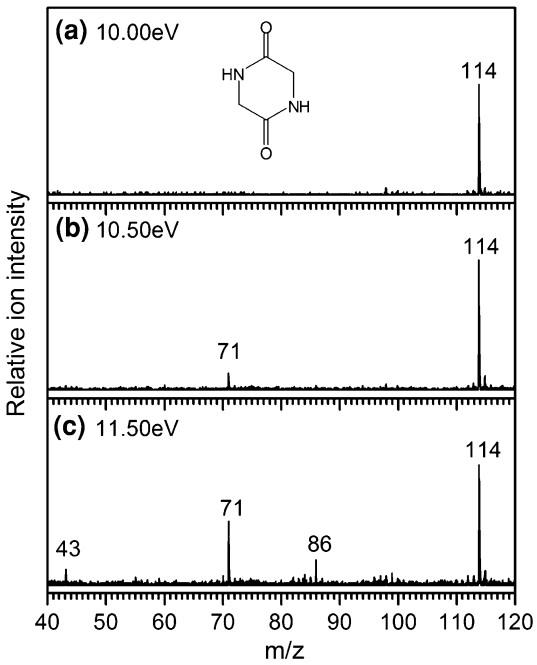


Fig. 2 Photoionization mass spectra of cyclo(Gly-Gly) at 10.00, 10.50 and 11.50 eV photon energies

Fragment ions *m/z* 71, 86 and 43 are yielded gradually as the photon energy increases, which are assigned as $[M–HNCO]^{+\bullet}$, $[M–CO]^{+\bullet}$ and $[M–CO–HNCO]^{+\bullet}$. The photoionization efficiency (PIE) spectra of cyclo(Gly-Gly) radical ion ($M^{+\bullet}$) and fragment $[M–HNCO]^{+\bullet}$ (*m/z* 71) were measured from photon energies 8.85–11.00 eV. As shown in Fig. 3a, IE of cyclo(Gly-Gly) is measured to be 9.33 ± 0.05 eV and AE of the fragment ion *m/z* 71 is 10.21 ± 0.05 eV, respectively, which are listed in Table 1. Considering the accuracy of calculation, we chose the energies coming from B3-MP2 method using the Eq. 1 in this present study (Wolken et al. 2007). The calculated AEs of other ions are 10.04 eV for *m/z* 86 and 10.36 eV for *m/z* 43. However, the fragment of *m/z* 71 appeared first with increasing photon energy. The large discrepancy between the calculated values and experimental measurement may be due to temperature effects and numerous possible conformations of the fragments. The numerous possible conformations of each fragment would make it more difficult to find a reaction pathway with lower activation energy. The IE of the precursor and AE of the fragment ion *m/z* 71 are calculated to be 9.39 and 10.50 eV, respectively, which are in relatively good agreement with experimental values. Generally IEs and AEs are related to 0 K bond dissociation energies of neutrals and cations, such values are of fundamental importance to chemistry (Ng 2000). To the best of our knowledge, no previous study was reported on IE of cyclo(Gly-Gly).

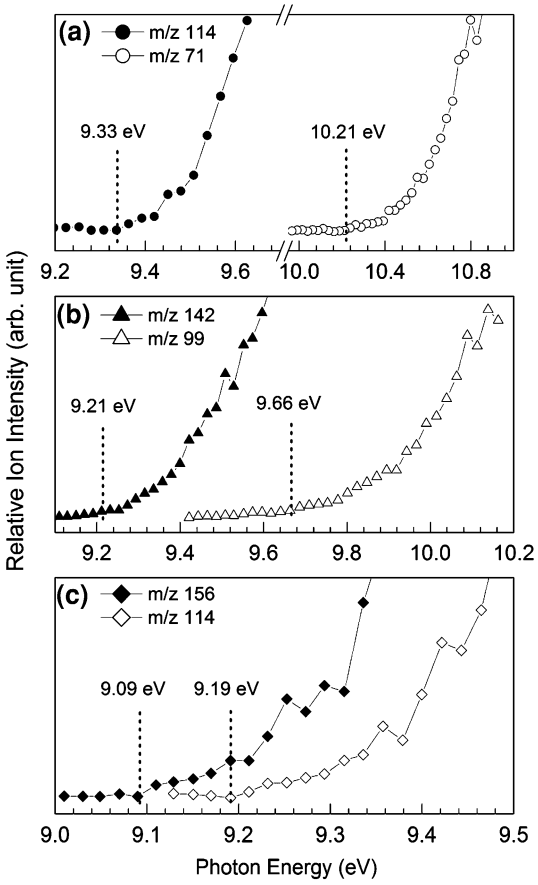


Fig. 3 Photoionization efficiency spectra of cyclo(Gly-Gly) (**a**), cyclo(Ala-Ala) (**b**) and cyclo(Gly-Val) (**c**) molecular ion and their main fragment ions

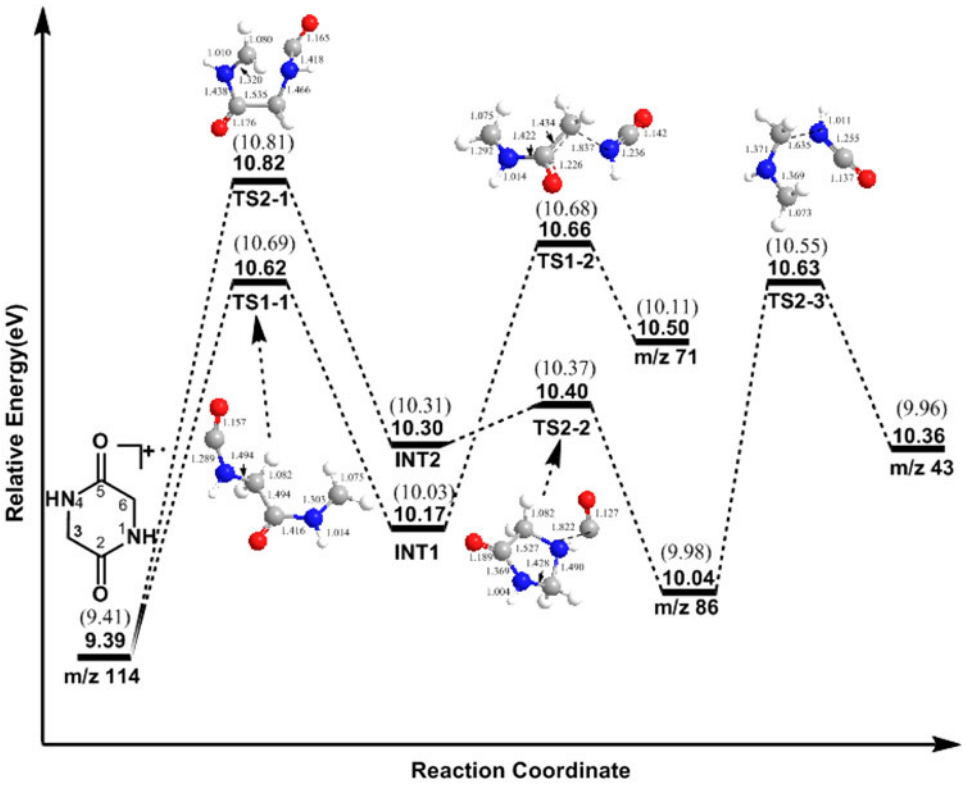
Accurate mass measurements were performed with EI TOF-MS, provided in the supplementary as Fig. S1. Combining the main fragments of IRLD/VUVPIMS and theoretical calculations, the fragmentation pathways of cyclo(Gly-Gly) are proposed and shown in Scheme 1. All calculated 3D structures and Cartesian coordinates are shown in Fig. S2 and Table S1. For the formation of fragment *m/z* 71, the bond between C2 and C3 in cyclo(Gly-Gly) radical ion ($M^{+\bullet}$) cleaves to form intermediate INT1 via transition state TS1-1 overcoming an energy barrier of 1.23 eV. INT1 eliminates HNCO neutral group via N1-C6 bond cleavage, and the corresponding dissociation energy is 0.49 eV. For the formation of fragment *m/z* 86, the CO group is transferred to N1 to form intermediate INT2 via transition state TS2-1 overcoming an energy barrier of 1.43 eV. INT2 eliminates CO neutral group via N–C bond cleavage, and the corresponding dissociation energy is 0.10 eV. Further loss of the HNCO neutral group from fragment ion *m/z* 86 yields fragment ion *m/z* 43 with a dissociation energy of 0.59 eV, which is assigned as $[CH_2NHCH_2]^{+\bullet}$.

Cyclo(Ala-Ala)

Figure 4 displays mass spectra of cyclo(Ala-Ala) at the photon energies of 9.18, 10.00, 11.50 and 12.50 eV. Only molecular ion of cyclo(Ala-Ala) is observed at photon energy of 9.18 eV. The fragment ion *m/z* 99, which is the analogous fragment ion *m/z* 71 for cyclo(Gly-Gly), is assigned as the $[M–HNCO]^{+\bullet}$. The main fragment ions at

Table 1 Experimental and calculated ionization energies (IEs) of three cyclic dipeptides and appearance energies (AEs) of the major fragment ions

Cyclic dipeptide	*m/z*	Ion assignment	IE/AE (eV)		
			(B3-MP2)	BH and HLYP	Experimental
Cyclo(Gly-Gly)	114	$[C_4H_6N_2O_2]^{+\bullet}$	9.39	9.41	9.33 ± 0.05
	86	$[C_3H_6N_2O]^{+\bullet}$	10.04	9.98	
	71	$[CH_2NHCOCH_2]^{+\bullet}$	10.50	10.11	10.21 ± 0.05
	43	$[CH_2NHCH_2]^{+\bullet}$	10.36	9.96	
Cyclo(Ala-Ala)	142	$[C_6H_{10}N_2O_2]^{+\bullet}$	9.11	9.15	9.21 ± 0.05
	114	$[C_5H_{10}N_2O]^{+\bullet}$	9.70	9.18	
	99	$[CH_3CHNHCOCHCH_3]^{+\bullet}$	9.74	9.82	9.66 ± 0.05
	71	$[CH_3CHNHCHCH_3]^{+\bullet}$	9.43	9.18	
	44	$[CH_3CHNH_2]^{+\bullet}$	11.90	11.36	
Cyclo(Gly-Val)	156	$[C_7H_{11}N_2O_2]^{+\bullet}$	9.00	9.08	9.09 ± 0.05
	128	$[C_6H_{11}N_2O]^{+\bullet}$	9.61	9.15	
	114	$[C_4H_6N_2O_2]^{+\bullet}$	9.11	8.78	9.19 ± 0.05
	85	$[CONHCH2CO]^{+\bullet}$	9.83	9.42	
	70	$[NHCHCH(CH_3)_2]^{+\bullet}$	11.20	10.91	



Scheme 1 The potential energy surface for dissociation pathways of cyclo(Gly-Gly) cation. The energies without brackets were calculated with B3-MP2 method using the Eq. 1. The energies in brackets were calculated at the BHandHLYP/6-31 ++g(d,p) level. The energy of the neutral molecule is defined as zero. All energies are electronic energies with ZPE correction (E_0)

12.50 eV are m/z 114, 99, 71 and 44. The PIE spectra of cyclo(Ala-Ala) radical ion ($M^{+\bullet}$) and fragment ion $[M–HNCO]^{+\bullet}$ were measured, as shown in Fig. 3b. IE of cyclo(Ala-Ala) is determined to be 9.21 ± 0.05 eV and AE of the fragment *m/z* 99 is 9.66 ± 0.05 eV. The calculated AEs for other ions are 9.70 eV for *m/z* 114, 9.43 eV for *m/z* 71 and 11.90 eV for *m/z* 44.The fragment assignment and the structures were listed in Table 1 and Scheme 2. The accurate mass measurements were also performed with EI TOF-MS (see Fig. S3). IE of cyclo(Gly-Gly) (9.33 ± 0.05 eV) is higher than that of cyclo(Ala-Ala) (9.21 ± 0.05 eV) (Fig. 3). Cyclo(Gly-Gly) and cyclo(Ala-Ala) are homo-cyclic dipetides with the symmetric structures. The methyl group of C3 and C6 could make the cyclo(Ala-Ala) radical ion more steady. The lower IE of cyclo(Ala-Ala) may be due to the effect of degree of branching of substituent groups at C3 and C6.

Combining the main fragments of IRLD/VUVPIMS and theoretical calculations, the fragmentation pathways of cyclo(Ala-Ala) are proposed and shown in Scheme 2. All calculational 3D structures and Cartesian coordinates are shown in Fig. S4 and Table S2. In the formation pathway of fragment *m/z* 99, the bond between C2 and C3 in cyclo(Ala-Ala) radical ion ($M^{+\bullet}$) cleaves to form intermediate INT3 via transition state TS3-1 overcoming an energy barrier of 1.33 eV. INT3 eliminates HNCO neutral group via N1–C6 bond cleavage with dissociation energy of 0.82 eV. However, for the formation of fragment ion

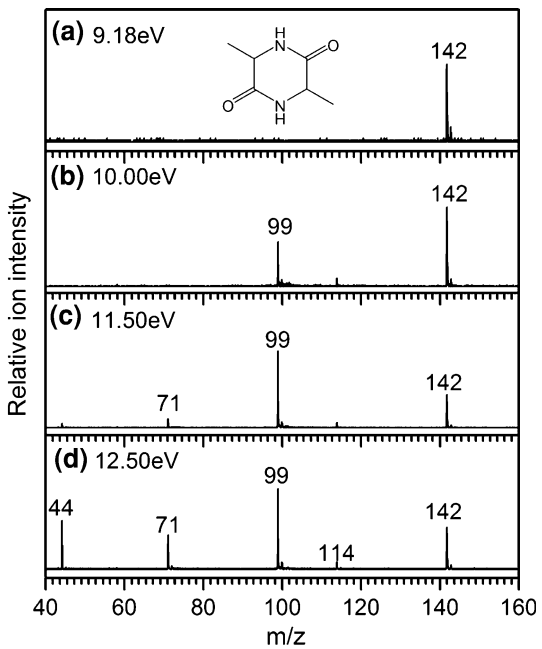
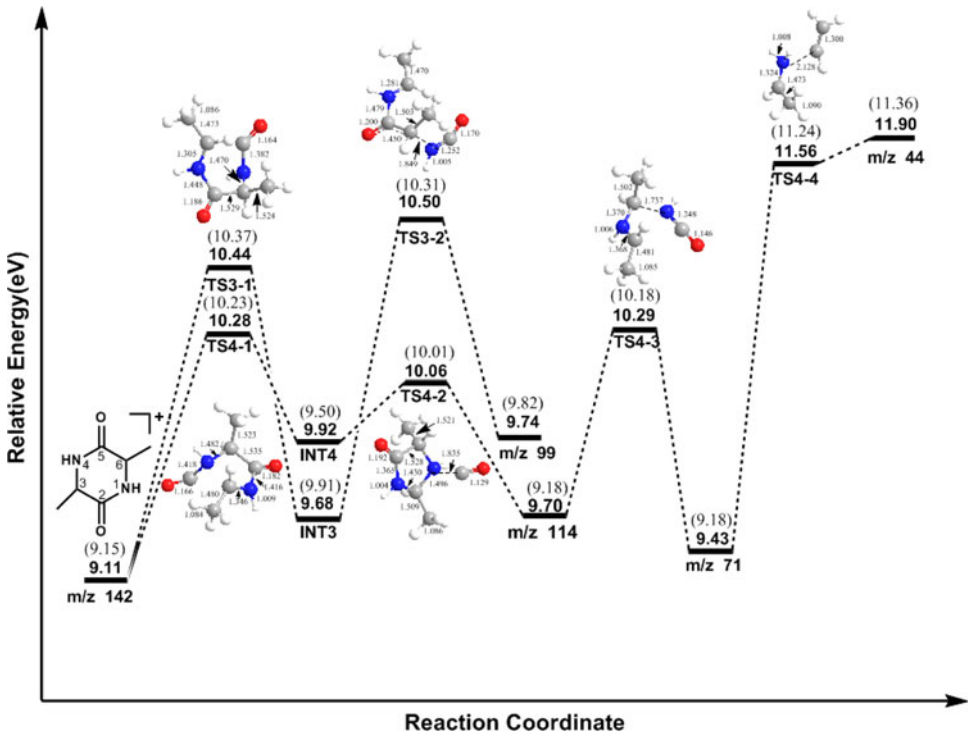


Fig. 4 Photoionization mass spectra of cyclo(Ala-Ala) at 9.18, 10.00, 11.50 and 12.50 eV photon energies

m/z 114, the CO group is transferred to N1 to form intermediate INT4 via transition state TS4-1 overcoming an energy barrier of 1.27 eV. INT4 eliminates CO neutral group via N–C bond cleavage, and the corresponding dissociation energy is 0.14 eV. Further loss of the HNCO



Scheme 2 The potential energy surface for dissociation pathways of cyclo(Ala-Ala) cation. The energies without brackets were calculated using the Eq. 1, and the energies in brackets were calculated at the BHandHLYP/6-31 ++g(d,p) level. The energy of the neutral molecule is defined as zero. All energies are electronic energies with ZPE correction (E_0)

neutral group from fragment ion at *m/z* 114 yields the fragment ion *m/z* 71 with the dissociation energy of 0.59 eV. The fragment ion *m/z* 71 loses the $CH_2CH^{\bullet}$ radical group to yield the fragment ion *m/z* 44 via transition state TS4-4 overcoming an energy barrier of 2.13 eV.

The homo-cyclic dipeptides show the similar fragmentation pathways. The base fragment ions at *m/z* 71 and 99 are produced from parent ion $[M^{+\bullet}]$ by eliminating HNCO neutral group. The fragment ions at *m/z* 86 and 114 are produced from ion $[M^{+\bullet}]$ by eliminating CO group. But HNCO elimination is found to be about three times more intense than CO elimination at the 11.50 eV (Figs. 2, 4). Previous study indicates that protonation occurs at the nitrogen of DKP ring in the collision-induced dissociation (CID) process. For the protonated ions, the base peak is produced by a loss of 45 Da to form ions $[M + H–HCONH_2]^+$ (Guo et al. 2009b). But it was observed that the product ions for a series of DKPs, isolated from *Aspergillus fumigates*, included fragment ions $[M + H–CO–HCONH_2]^+$ and $[M + H–H_2O–HCONH_2]^+$ (Furtado et al. 2007). As we know, the protonation of DKPs in ESI multistage mass spectrometry is effect on the protonated molecular structures in the CID process and their subsequent fragmentation reactions. But in the VUV PIMS, no proton was migrated to cyclic dipeptides. Their subsequent fragmentation reactions are driven by radical or charge at the different photon energies. Furthermore, in a typical CID experiment the average internal energy deposited in the precursor ion is higher than the AE of ionic fragment. As a result, the detailed dissociation processes are poorly understood. Considering the stabilization of $[M–HNCO]^{+\bullet}$ radical ion due to methyl groups at C3 and C6, it is well understood that the AE of fragment ion *m/z* 99 from cyclo(Ala-Ala) is lower than fragment ion *m/z* 71 from cyclo(Ala-Ala). These results could help us to understand well the DKPs dissociation processes.

Cyclo(Gly-Val)

Cyclo(Gly-Val) has been reported to inhibit colon and cervical carcinoma cell lines, which may be a potential molecule in the treatment of cancer (van der Merwe et al. 2008). It has an unsymmetrical structure. As shown in Fig. 5, the exclusive radical ion ($M^{+\bullet}$) [*m/z* 156] is hard to be observed due to that the molecular ion directly eliminates propylene to yield fragment ion *m/z* 114. The PIE spectrum indicates that IE of the parent ion is 9.09 ± 0.05 eV and the AE of fragment ion *m/z* 114 is 9.19 ± 0.05 eV (Fig. 3c). Therefore, the molecular ion *m/z* 156 and the fragment ion *m/z* 114 are both produced at photon energy of 9.28 eV. When the photon energy increasing to 13.50 eV, more fragments are yielded. The cyclo(Gly-Val) radical ion could be stabilized by the isopropyl group at C3 of diketopiperazine ring. The γ-H of methyl easily transfers to adjacent carbonyl group. Subsequently, the C–C bond at adjacent carbonyl group generates *i* cleavage to lose propylene and yield fragment ion *m/z* 114. Therefore, IE of cyclo(Gly-Val) is the lowest one

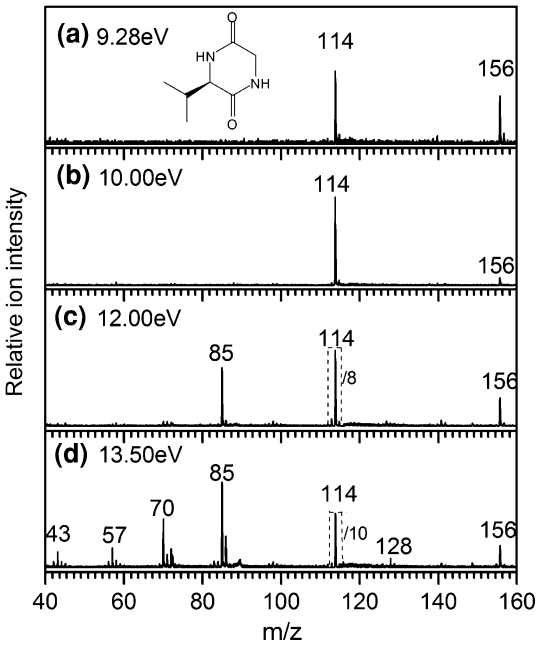
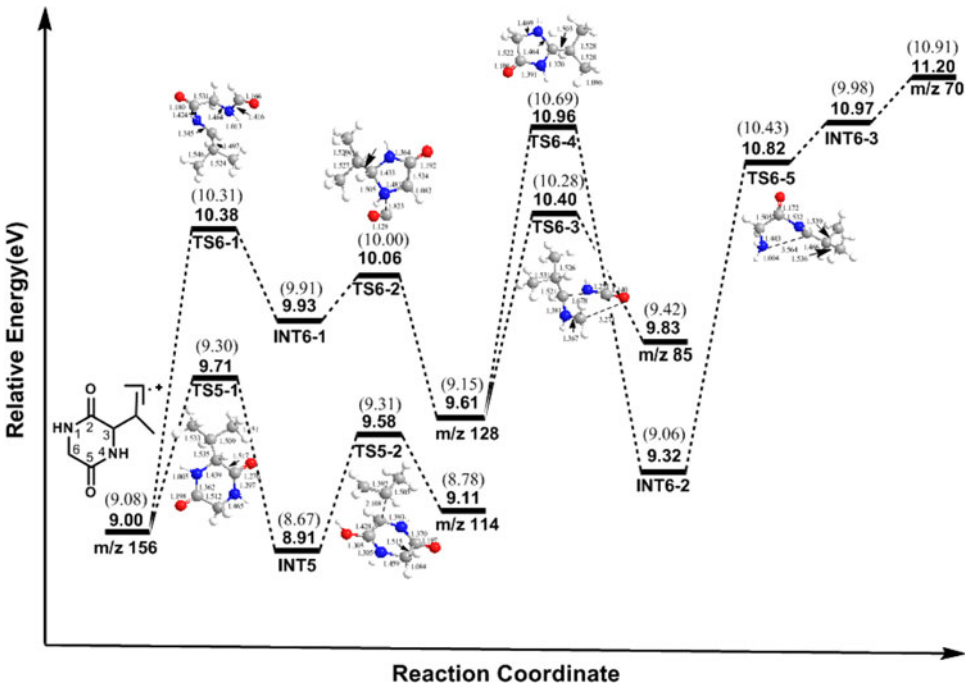


Fig. 5 Photoionization mass spectra of cyclo(Gly-Vla) at 9.28, 10.00, 12.00 and 13.50 eV photon energies

among the three cyclic dipeptides. The dominant peak is produced by a loss of 42 Da ($CH_3CH{=}CH_2$) to form fragment ion *m/z* 114, but no fragment ion *m/z* 113 is observed via HNCO elimination from the molecular ion.

Combining theoretical calculations, the fragmentation pathways of cyclo(Gly-Val) are proposed and shown in Scheme 3 and AEs of the fragment ions are listed in Table 1. The accurate mass measurement is provided in Fig. S5. Additionally, all calculated 3D structures and Cartesian coordinates are shown in Fig. S6 and Table S3. Isopropyl group, the only substituent in diketopiperazine ring for cyclo(Gly-Val) molecule, easily cleaves from parent molecule to yield 2,5-diketopiperazine ion (*m/z* 114) and propylene by γ-H migration and *i* cleavage of intermediate INT5. However, at the higher photon energy (above 12.00 eV), the cyclo(Gly-Val) radical cation may yield a fragment ion *m/z* 128 by a five-membered ring intermediate INT6-1 with loss of CO. Further, intramolecular proton transfer is forming intermediate INT6-2 and fission of N1–C2 and N4–C5 bonds leads to loss of NH_2CH_2CO radical and produces the fragmentation ion at *m/z* 70. In addition, the fragment ion *m/z* 128 could directly lose HNCO group to yield fragment ion *m/z* 85 with 1.35 eV dissociation energy by photo-induced dissociation. However, the fragment ion *m/z* 114 loses CH_2=NH neutral molecule to yield fragment radical ion *m/z* 85 as well. While fragment ion *m/z* 85 can further dissociate to yield fragment ions *m/z* 43 and 57 with lose CH_2CO radical and CO neutral molecule, respectively. The proposed fragmentation pathways is loss of CH_2=NH from the ion *m/z* 114 to form ion *m/z* 85 and then successive loss of 28 Da (CO) or 42 Da (CH_2CO) to form ions *m/z* 57 or 43. The ions mentioned above are observed at higher photon energies, as shown in Fig. 5. Although in neutral fragment reionization mass spectrometry (N_fRMS) of glycine-contained peptides, the loss of CH_2=NH neutral molecule was also observed in the course of cyclo(Gly-Gly) ion *m/z* 114 forming ions *m/z* 85 (Cordero and Wesdemiotis 1994; Polce et al. 2000). All these results indicate that hetero-

Scheme 3 The potential energy surface for dissociation pathways of cyclo(Gly-Val) cation. The energies without brackets were calculated using the Eq. 1, and the energies in brackets were calculated at the BHandHLYP/6-31 ++g(d,p) level. The energy of the neutral molecule is defined as zero. All energies are electronic energies with ZPE correction (E_0)



cyclic dipeptide and homo-cyclic dipeptides in this study show different fragmentation pathways.

CO elimination from cyclo(Gly-Val) was found to be the same as in cyclo(Gly-Gly) and cyclo(Ala-Ala) although the intensity of *m/z* 128 is very low. This may be due to the fragment ion *m/z* 128 instantly losing the glycine residue (NH_2CH_2CO group) to yield fragment ion *m/z* 70 or the HNCO group to yield fragment ion *m/z* 85. In the protonated cyclic dipeptides, the elimination of an amino acid residue from fragment ion was also observed (Guo et al. 2009b). It is indicated that different cyclic dipeptides show the similar reaction pathways even under different condition. HNCO elimination is the common characteristic induced by photoionization. The HNCO group elimination from the cyclo(Pro-Gly) radical cation was studied by time-resolved photoionization mass spectrometry (Ling and Lifshitz 1998). Therefore, these salient features of cyclic dipeptides enrich the information of gas-phase reactions of cyclic peptide database and have some directive function for DKP-based new drug design.

Conclusion

VUV PIMS spectra of three cyclic dipeptides were investigated and the fragmentation pathways were elucidated. The primary peak is produced by a loss of 43 Da to form ions $[M–HNCO]^{+\bullet}$ from cyclo(Gly-Gly) and cyclo(Ala-Ala) radical cations. The CO group elimination from cyclo(Gly-Gly) and cyclo(Ala-Ala) is further important pathway. But in the case of cyclo(Gly-Val) molecular ion, due to the feature of isopropyl group and unsymmetric molecular structure, the major primary decomposition reaction is loss of propylene to yield fragment ion *m/z* 114. The IEs of three cyclic dipeptides and AEs of some major fragment ions are obtained from measurements of the PIE spectra. The present study is of great significance for understanding the gas-phase reaction and chemical characteristics of DKPs.

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