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The mechanism of C-terminal fragmentations in alkali metal ion complexes of peptides

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In honor of the fundamental accomplishments of Professor Jack Beauchamp on the occasion of his 60th birthday.

Abstract

A combination of mass spectrometry and ab initio calculations (MP2/6-31+G(d)//HF/6-31+G(d)) has been used to study the mechanism of C-terminal residue cleavage in gas phase peptide/alkali metal ion complexes. Although previous workers had suggested a mechanism relying on a concerted cleavage of an oxazolidin-5-one intermediate, the present calculations indicate that this pathway has a high barrier and is not competitive. Instead, it appears that the mechanism involves a rearrangement to an anhydride intermediate that fragments to give the observed products. The computational data indicates that this mechanism has a much lower activation energy than a concerted pathway and should be viable. Moreover, compelling evidence for the mechanism is found in experiments involving the lithium complexes of dipeptides. In the proposed mechanism, the two amino acids of a dipeptide are in equivalent positions in the anhydride intermediate (i.e., sequence information is lost) and therefore, fragmentation of either sequence of a dipeptide should give the same result. This was confirmed for eight pairs of dipeptides by collision-induced dissociation (CID) of their lithium complexes in a quadrupole ion trap mass spectrometer. Although the CID spectra are not identical, the yields of the products that would pass through the anhydride intermediate are nearly equivalent, independent of the original sequence. Finally, additional computational work shows that the mechanism does not rely on the presence of a metal and is also viable as a charge-remote fragmentation pathway. (Int J Mass Spectrom 222 (2003) 117–134)

Keywords: Anhydride; Fragmentation; Pathway; Peptide; Lithium

1. Introduction

In recent years, there has been considerable interest in the use of mass spectrometry as a tool for determining the sequences of peptides [1–12]. Most work has focused on cationized peptides, and protonated as well as metallated peptides have been widely studied. Several groups have observed that complexes of peptides with alkali metal ions (Li^+, Na^+, and K^+) undergo a particularly useful fragmentation during collision-induced dissociation (CID) [13–28]. In most cases, the complexes lose the C-terminal residue in a highly efficient process leading to a complex of the alkali metal ion and the shortened peptide. Lin and Glish [26] have shown that the process can be repeated in MS^n experiments and as a result, the sequence of a

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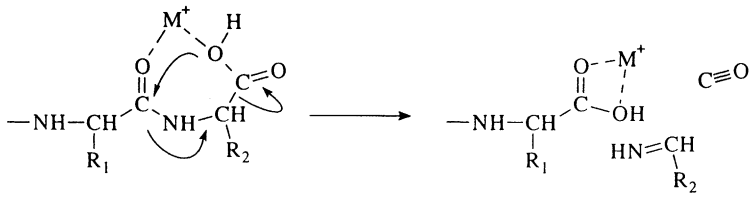
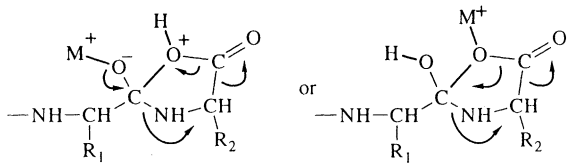


Scheme 1.

peptide can be definitively determined by the repeated loss of C-terminal residues. Although the process is not very common in simple protonated peptides [29,30], it has been observed in systems with localized positive charges such as charge appended species (e.g., phosphonium salts [31,32]) or arginine-containing species (see accompanying paper [33]).

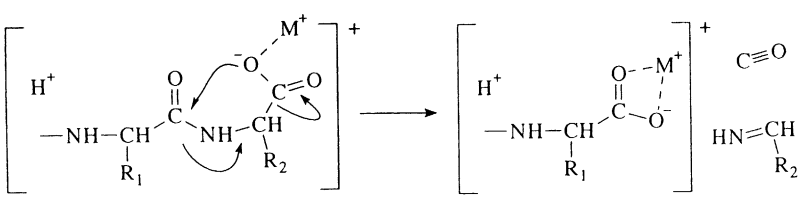
There is no consensus on the mechanism of the process in the metallated systems. One view is that the alkali metal cation coordinates to the carbonyl of the amide bond and activates it toward nucleophilic attack by the C-terminal carboxyl group (Scheme 1) [20–22].

A feature of this mechanism is that of all the functional groups in peptides, the amides have nearly the highest alkali metal cation affinities [34–36]. The intermediate (or transition state) resulting from nucleophilic attack on the amide carbonyl is believed to break down by the loss of the C-terminal residue as CO and an imine. Alternatively, the peptide could adopt a zwitterion-type structure with the alkali metal ion coordinated to a C-terminal carboxylate and the positive charge carried remotely by a protonated nitrogen or oxygen (N-terminal amine or other basic group) on the peptide chain [15,16,19]. Structures of this type have been implicated recently in related fragmentation processes [25]. The metallated carboxylate then acts as a nucleophile and attacks the amide bond to initiate a similar fragmentation of the C-terminal residue (Scheme 2). In this case, deprotonation of the C-terminal carboxyl group enhances its nucleophilicity and activates the process. The fact that C-terminal esters and amides do not undergo this process provides some support for this mechanism [15,21].



Scheme 3.

Although there is no agreement on the starting point for the mechanism, there has been a consensus that the key player is a five-membered ring species (oxazolidin-5-one derivative) that exists as either a transition state or an intermediate on the potential energy surface (Scheme 3). This species then directly leads to the observed products. It has been assumed that the breakdown of the oxazolidin-5-one derivative by one of the six-electron, pericyclic processes outlined in Scheme 3 would be relatively facile (zwitterionic and conventional structures are shown in Scheme 3).



Scheme 2.

As a part of a larger project focussed on the interaction of peptides with metals, we became interested in the mechanism of the C-terminal cleavage process. In the course of modeling the reaction by ab initio calculations, it became clear that the direct cleavage of an oxazolidin-5-one intermediate (Scheme 3) involves a high activation barrier and is an unlikely pathway in the fragmentation process. This paper describes our search for an alternative C-terminal cleavage mechanism. Specifically, we have used computational methods to explore the fragmentation process in a simple model system, lithiated glycylglycine, and discovered a relatively low-energy pathway involving an anhydride intermediate. We have also completed an experimental study of the CID of a series of lithiated dipeptides and the results provide strong support for the anhydride intermediate. Independently, Farrugia and O'Hair [33] have suggested an analogous intermediate in a related system (see accompanying paper). Finally, we have used computational methods to assess the role that the metal plays in the process by studying the analogous fragmentation of neutral glycylglycine and found that the metal cation only has a modest effect on the potential energy surface.

2. Experimental

2.1. Mass spectrometry

All measurements were made with a Finnigan LCQ ion trap mass spectrometer operating with a background helium pressure of 1.75×10^{-3} Torr. Peptides were obtained from Sigma Chemical Co. (St. Louis, MO) and used without further purification. Typical operating conditions involve an ESI needle voltage of about 4 kV, a solution flow rate of 5–10 uL/min, and a heated capillary temperature of about 150 °C. The samples (peptide + LiBr) were prepared as methanol/water solutions ($\sim 10^{-3}$ M). The precursor ions generated by ESI were isolated using the LCQ advanced scan software (release 1.1) and activated for 30 ms with an activation voltage of ~0.5–0.7 V. As in all quadrupole ion traps, CID is a multi-collision process. Control experiments showed that varying the activation voltage in this range had only a minor effect on the product distributions. Extensive signal averaging was done in the case of weak signals and the reported ratios were the result of integrating the areas under the appropriate peaks.

2.2. Calculations

Calculations were completed with the GAUSSIAN 94 [37] or GAUSSIAN 98 [38] quantum mechanical packages on an SGI Octane, an IBM 39H, an HP 735, or a Pentium III computer. Optimizations were completed without constraints and harmonic frequency calculations were done at the HF/6-31+G(d) level. All relative energies are corrected for zero point vibrational energies (HF) scaled by 0.9135 [39]. Multiple rotamers were considered at low levels of theory (PM3) [40] and the most stable conformation was used in subsequent calculations. Although this process may not identify the preferred conformation in every case, it is a reasonable compromise given the size of the systems and the fact that we are only seeking a semi-quantitative picture of the reaction processes. For the lithiated systems, energies were refined with single-point calculations at the MP2/6-31+G(d) level. For the neutral systems, a more rigorous approach was adopted and energies were refined by optimizations at the MP2/6-31+G(d) level with single-point calculations at the MP2/6-31+G(d,p) level.

3. Results and discussion

3.1. Mechanism of the C-terminal cleavage process in metallated peptides

Based on the previous work in the literature, we initially focussed on the pathways presented in Schemes 1–3. To provide a simple model system, we have used the lithium complex of glycylglycine. A sample structure for ground-state lithiated glycylglycine (**I**) is shown in Fig. 1. This structure was obtained from optimizations at the HF/6-31+G(d) level

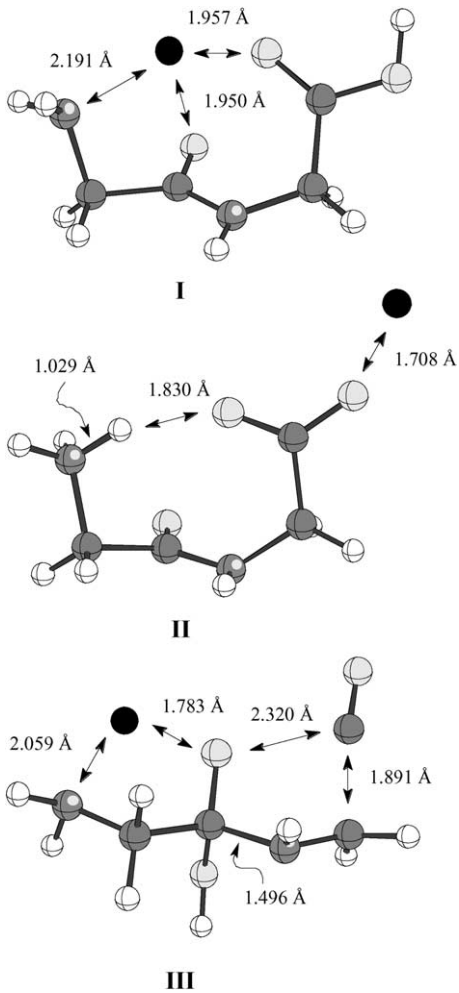


Fig. 1. Structures of the lithium complexes of glycylglycine and transition state for direct fragmentation. Geometries at HF/6-31+G(d) level (carbon: dark gray; hydrogen: white; oxygen: light gray; nitrogen: patterned; lithium: black).

and is one of multiple conformations for the complex. In this case, the lithium cation interacts with the amide carbonyl as well as the carbonyl of the C-terminal carboxylic acid and the N-terminal amine. An alternative binding scheme for a metallated peptide would be a zwitterion (salt-bridge) structure with the alkali metal cation coordinated to a C-terminal carboxylate and the positive charge provided by a protonated functional group in the peptide (e.g., N-terminal amine or side-chain group). In Fig. 1, an example of a salt-bridge structure, **II**, is also given for lithiated glycylglycine. Here the anionic carboxylate coordinates to the lithium cation and hydrogen-bonds to the ammonium cation. These types of structures are stabilized by a favorable ion-triplet electrostatic interaction (+ − +) and have been identified for metal complexes of proline [34] and arginine [41,42]. Of course the high basicity of these amino acids makes them well suited for forming salt-bridge structures. In contrast, we and others have presented evidence that lithiated and sodiated dipeptides generally prefer conventional rather than salt-bridge structures [43,44]. The computational data supports this conclusion (Table 1). At the MP2/6-31+G(d)//HF/6-31+G(d) level, the conventional structure, **I**, is about 18 kcal/mol more stable than the salt-bridge structure, **II**. These are only representative conformations (i.e., they are the result of only a partial survey of the total conformational space of each binding scheme) so it is possible that more stable conformations exist; however, the large energy difference between structures **I** and **II** makes it unlikely that any salt-bridge conformation would be more stable than **I**. Nonetheless, this does not rule out a mechanism that passes through an intermediate involving a zwitterion. Structures such as **II** would be energetically accessible under CID conditions and Beauchamp has used a zwitterion intermediate to explain the aspartic acid mediated cleavages of sodiated peptides [25].

The transition state for the concerted ring opening in lithiated glycylglycine is surprisingly unstable relative to the starting complex suggesting a very large barrier for the process. Several conformations and coordination schemes were tried at various levels of theory, but the most stable transition state that we located, **III**, is 65 kcal/mol less stable than the initial lithium/glycylglycine complex, **I** (Table 1). In transition state **III**, the original amide carbonyl is protonated and the lithium is associated with an oxygen from the carboxylic acid component of the molecule. Consequently, it is most closely related to the cyclization of a zwitterionic complex, $H_2NCH_2C(OH)^+NHCH_2$-$CO_2^-Li^+$. It appears to be a late transition state with nearly complete cleavage of the C–O bond (2.320 Å)

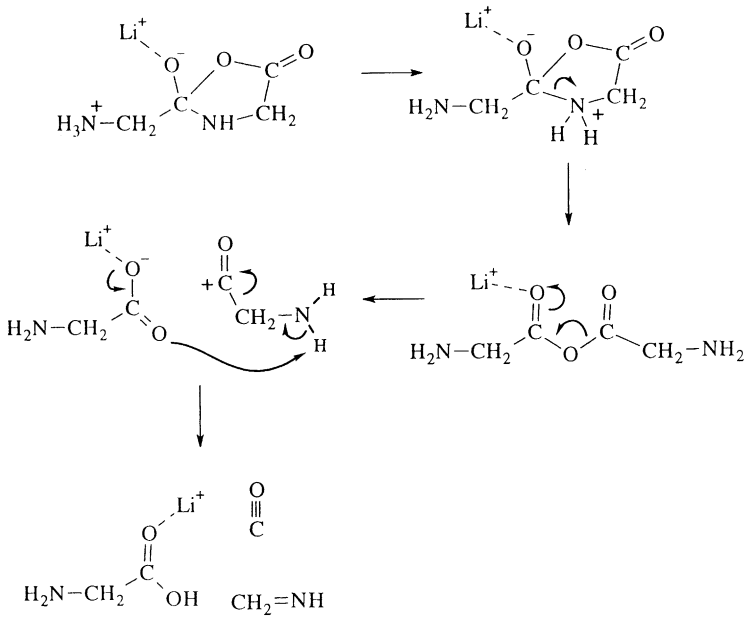
Table 1
Energies of intermediates and transition states in C-terminal cleavage process of lithiated glycylglycine[a]

Species	HF/6-31+G(d)	MP2//HF/6-31+G(d)[b]	ZPE[c]	Relative energies	
				HF	MP2
I	−497.01167	−498.38620	0.15125	0	0
II	−496.97423	−498.35880	0.15245	24.2	17.9
III	−496.89484	−498.27956	0.14785	71.4	65.0
IV	−496.92555	−498.32070	0.15199	54.5	41.5
V	−496.94084	−498.32765	0.15257	45.2	37.5
VI	−496.93157	−498.32046	0.15248	51.0	42.0
VII	−496.94891	−498.33734	0.15256	40.1	31.4
VIII	−496.93740	−498.31902	0.14882	45.2	40.8
IX	−496.96631	−498.33655	0.14896	27.1	29.8
X	−496.92951	−498.31543	0.14710	49.2	42.0
XI	−496.93334	−498.30520	0.14556	45.9	47.6

[a] Absolute energies in hartree. Relative energies in kcal/mol.
[b] MP2/6-31+G(d)//HF/6-31+G(d) level of theory.
[c] Zero point vibrational energy (unscaled). Values scaled by 0.9135 [39] are used in the calculation of relative energies.

and significant cleavage of the C–C bond (1.891 Å). However, there is a high degree of asynchronicity in the transition state and C–N cleavage (1.496 Å) is lagging behind. Fragmentation transition states with the acidic proton and the lithium cation bound to alternative sites also were located (i.e., various zwitterion and conventional bonding arrangements), but these structures are all less stable (3–16 kcal/mol) than **III**. A number of coordination schemes were considered for the cyclic transition state including: (i) proton on N-terminus amine with lithium on amide carbonyl (81.1 kcal/mol relative to **I**); (ii) neutral carboxylic acid group with lithium on amide carbonyl (68.4 kcal/mol relative to **I**); and (iii) proton on amide nitrogen with lithium on amide carbonyl (collapses to transition state **VIII** on the anhydride path). *Having surveyed several possible transition states, we have concluded that the direct cleavage pathways shown in Scheme 3 are not viable under CID conditions*. First, the barriers are higher than the expected lithium (or sodium) binding energy of the dipeptide. For example, glycylglycine has a lithium cation affinity of only about 55 kcal/mol [43]. Second, with a barrier of over 60 kcal/mol, one would expect other processes to be very competitive which is inconsistent with the high efficiency normally observed in these C-terminal cleavages. Although it is difficult to judge the absolute accuracy of the ab initio calculations, it is unlikely that this level of theory would produce errors that are large enough to shift the barrier into a reasonable range. Overall, there appears to be a severe problem with orbital overlap in this process such that a fully conjugated transition state is not possible. In particular, it does not appear that the carbonyl and imine π-bonds can form simultaneously. As a result, a highly asynchronous reaction occurs with nearly complete cleavage of the C–O bond before the C–N and C–C bonds break.

Assuming that the transition states suggested in Scheme 3 are inaccessible, another pathway must be available. This led us to an essentially de novo computational search for alternative pathways. Numerous possible routes to the products were explored at low levels of theory and promising ones were pursued at the ab initio level. In this process, we identified a pathway involving an anhydride intermediate that appeared to be a viable alternative. It is outlined in Scheme 4 for lithiated glycylglycine. We start from the oxazolidin-5-one intermediate in one of its zwitterionic forms [45]. Instead of directly fragmenting to CO and the imine, a proton is transferred to the ring nitrogen leading to C–N cleavage to give a metallated



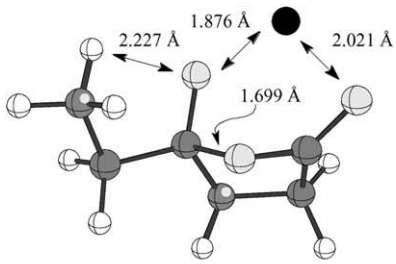
Scheme 4.

anhydride. The anhydride then can fragment directly to give a complex of an acyl cation and a metal carboxylate. Decomposition of the acyl cation (loss of CO) gives an iminium ion that can transfer a proton to the carboxylate yielding the lithiated amino acid product (i.e., C-terminal cleavage).

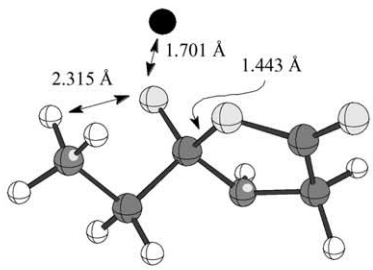
An attractive feature of the mechanism outlined in Scheme 4 is that it is simply the completion of a nucleophilic acyl substitution reaction, a very well established process in organic chemistry. Acyl substitutions are relatively facile reactions in solution although the conversion of an amide to an anhydride is generally endothermic. Nonetheless, it is likely that none of the steps will exhibit excessive barriers.

In an effort to model the mechanism computationally, we were forced to limit the survey of the potential energy surface to a series of likely transition states and intermediates on the proposed reaction path. Given that the system is large and has exceptional conformational freedom, it is simply impractical to fully investigate the conformational space of all the species on the pathway. Nonetheless, this is a reasonable approach because we are only trying to determine if the path is viable and can compete with the direct cleavage mechanism (i.e., transition state **III**). Moreover, errors associated with not locating lowest energy conformations will lead to barriers that are too large relative to the starting complex and therefore, this approach leads to an upper bound on the true barrier (within the accuracy of this level of theory).

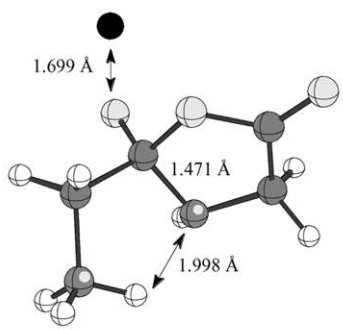
As noted before, the reaction begins by the formation of a cyclic intermediate. After investigating a variety of possible cyclic structures, **V** (Fig. 2) was found on the potential energy surface. This structure results from cyclization of the zwitterionic form of glycylglycine with the carboxylate attacking the amide carbonyl. The old carbonyl is in the form of a neutral lithium alkoxide and the charge is carried on an N-terminal ammonium group (in a larger peptide, it is likely that another functional group would accept the proton). At the MP2/6-31+G(d)//HF/6-31+G(d) level, **V** is 37.5 kcal/mol (Table 1) less stable than the initial complex, **I**, or about 20 kcal/mol less stable than the zwitterionic lithium complex, **II**. A transition state linking **II** to **V** was located (**IV**) and it is 41.5 kcal/mol above the lowest energy lithium complex, **I**. This is



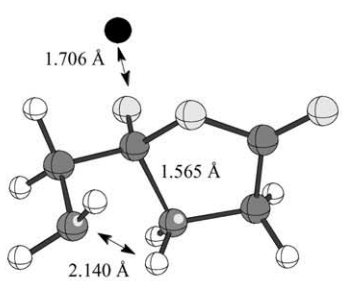
IV



V



VI



VII

Fig. 2. Structures of intermediates and transition states in the conversion of lithiated glycyglycine to the anhydride complex. Geometries at HF/6-31+G(d) level (carbon: dark gray; hydrogen: white; oxygen: light gray; nitrogen: patterned; lithium: black).

a significant barrier, but it should be accessible under CID conditions. A component of this transition state is the transfer of the lithium from the carboxylate carbonyl to the developing alkoxide (i.e., old amide carbonyl). Attempts to find non-zwitterionic, cyclic intermediates failed because the trial structures collapsed without a barrier to the starting complex, **I**. Cyclic complex **V** is only marginally stable and the barrier to ring opening to return to **II** is just 4 kcal/mol. It was stated earlier that there has been a controversy over whether the cyclic intermediate is formed from a conventional or zwitterionic metal ion complex (Scheme 1 vs. Scheme 2). This may not be a critical question in terms of the reaction pathway. The calculations clearly indicate that a conventional structure is preferred for the metal ion complex, but we have identified a cyclization pathway starting from the zwitterion complex. It is also possible that a pathway from **I** to **V** exists that involves cyclization in concert with proton transfer, but in any case, the results show that identification of an intermediate (**V**) related to a zwitterionic precursor (i.e., **II**) does not imply that the global minimum is a zwitterion. Because proton transfers can occur readily within these types of complexes, the question of zwitterionic vs. conventional intermediates may not be easily resolved nor provide important insights into the mechanism.

The next step in the cleavage process involves transferring a proton from the terminal ammonium group to the ring nitrogen. This is exothermic by about 6 kcal/mol and leads to **VII** via transition state **VI**. This transition state mainly involves rotation around a C–C bond to place the N-terminal ammonium group in a position to transfer a proton to the ring nitrogen, and a part of the barrier is associated with breaking the hydrogen-bond between the ammonium group and the lithium alkoxide. Throughout this process, the lithium cation remains coordinated to the alkoxide group. Having protonated the ring nitrogen, the system is poised for C–N cleavage to give the anhydride intermediate, **IX** (Fig. 3). The process is slightly exothermic and passes through transition state **VIII**. The barrier is about 9 kcal/mol (40.8 kcal/mol relative to **I**). An alternative structure for the anhydride

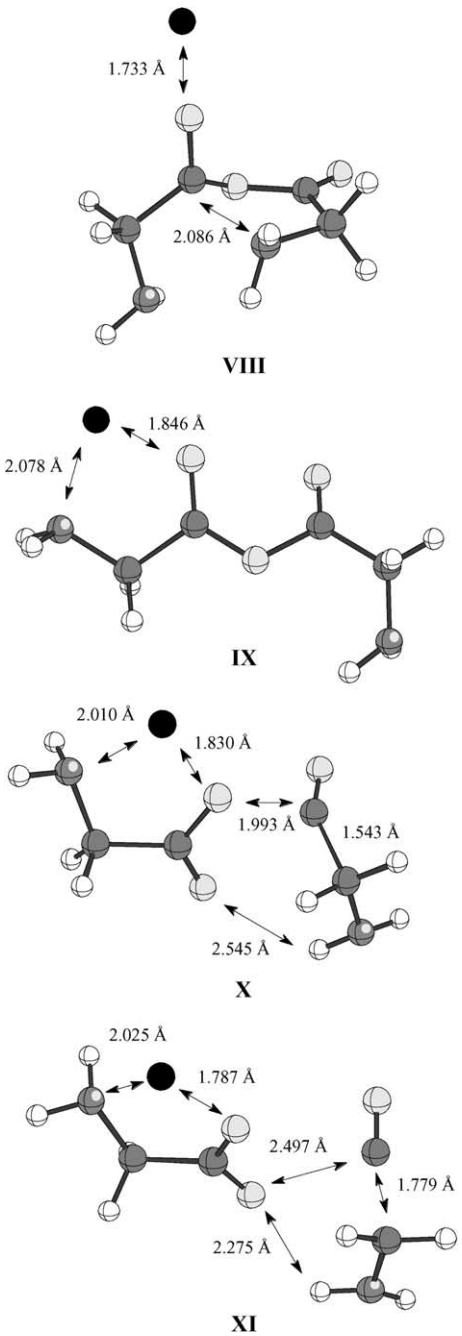


Fig. 3. Structures of intermediates and transition states in the fragmentation of lithium/anhydride complex. Geometries at HF/6-31+G(d) level (carbon: dark gray; hydrogen: white; oxygen: light gray; nitrogen: patterned; lithium: black).

with the lithium coordinated to both carbonyls was located, but it is slightly less stable than **IX**. From the anhydride, cleavage of the C–O bond occurs through transition state **X** which is 12.2 kcal/mol above the anhydride. This initially leads to a weakly bound complex (not shown) of the metal salt of the amino acid and $H_2NCH_2CO^+$ which loses CO through transition state **XI**. Proton transfer after the expulsion of CO eventually leads to the imine, CO and a lithiated amino acid. This proton transfer process appears to have little or no barrier other than its endothermicity and a transition state was not located.

Overall, the highest energy transition state, **XI**, on the pathway in Scheme 4 lies 47.6 kcal/mol above the starting complex, **I**; therefore, the entire fragmentation process occurs via transition states with barriers of about 45 kcal/mol or less relative to the starting lithium complex (Fig. 4). These are reasonable barriers for a CID process and therefore, this mechanism should be viable. Moreover, all the barriers on the anhydride pathway are at least 17 kcal/mol below the direct cleavage transition state, **III**. Consequently, the ab initio calculations provide strong support for the mechanism outlined in Scheme 4. By separating the fragmentation into two steps, rearrangement to

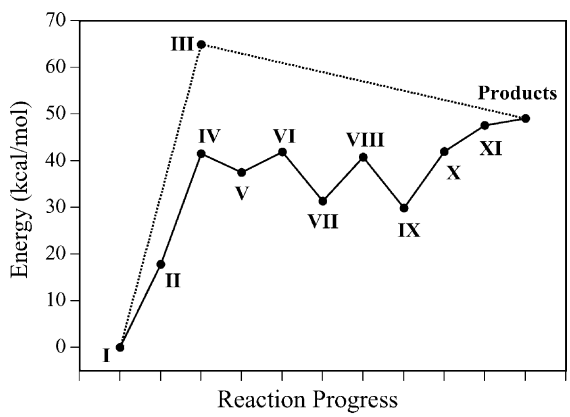


Fig. 4. Representation of the potential energy surfaces for the two pathways for fragmenting lithiated glycylglycine. Only the stationary points that were located as a part of this study are included. In several cases, intermediates or transition states that link species together have been neglected in this drawing. See text for details.

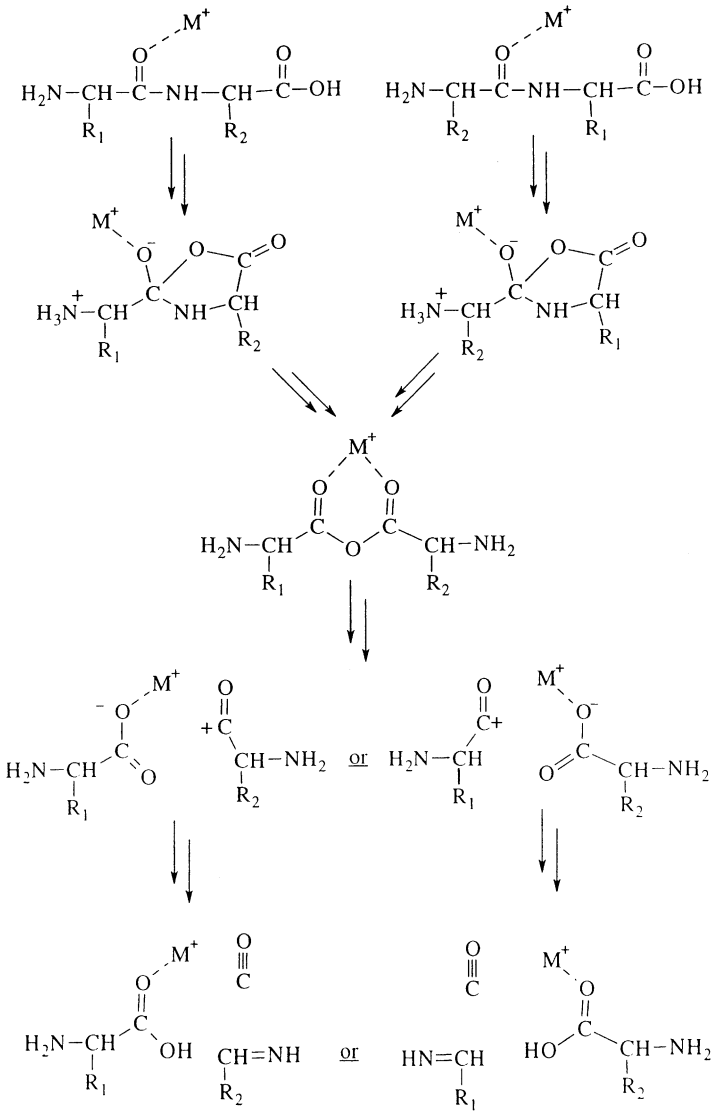
the anhydride followed by CO expulsion, the system is able to greatly reduce the activation barrier.

Although the barriers on this pathway are well below those on the direct cleavage pathway (i.e., **III**), they are close to the dissociation energy of the complex to simply give glycylglycine and a lithium cation (∼55 kcal/mol). If one assumes that the lithium dissociation channel is entropically much more favorable than the pathway outlined before, then it might seem that loss of lithium would dominate during CID. We observe good recovery yields during our CID studies, so it appears that lithium loss is not the dominant channel. This can be explained by taking two factors into account. First, metal dissociation from large, flexible, multidentate ligands is not a facile process and significant kinetic shifts are observed in threshold dissociation experiments. For example, Armentrout and co-workers [46] have noted kinetic shifts ranging from 10 kcal/mol for the dissociation of lithium cations from dimethoxyethane to 70 kcal/mol for the dissociation of lithium cations from 12-crown-4. They point out that dissociation from a flexible, multidenatate ligand may involve a pathway which is narrow in phase space compared to dissociation from a rigid, monodentate ligand. Second, the tight transition states on the C-terminal cleavage pathway are all 13 kcal/mol or more below the expected dissociation limit. This is a significant advantage and could outweigh the entropic advantages of lithium cation loss. The highest energy transition states on the cleavage pathway are involved in the dissociation of the anhydride and should be relatively loose as it fragments via a pair of bond cleavages to give three products, each with rotational degrees of freedom. These factors appear to be sufficient to favor the C-terminal cleavage pathway in the lithium complexes. However, it should be noted that when we attempted similar experiments with sodium complexes of dipeptides, we found that metal loss was preferred to some extent over the C-terminal cleavage pathway, presumably due to weaker binding of the sodium cation (∼42 kcal/mol for glycylglycine) [43]. However, with larger peptides sodium binding energies are higher and metal dissociation should be suppressed.

The mechanism in Scheme 4 makes a powerful prediction about the way in which metallated dipeptides should fragment because in the anhydride intermediate, both of the amino acids are in equivalent positions. In other words, the sequence information is lost. This is illustrated in Scheme 5 (the anhydride is shown with symmetric coordination to the lithium cation for clarity). Consequently, the mechanism requires that a metallated dipeptide would give metal complexes of both the amino acids as products. Moreover, the ratio of those products should be the same (independent of the starting sequence) because the anhydride represents a common intermediate for both sequences. These requirements provide a rigorous experimental test for the proposed mechanism.

3.2. Experimental support for the mechanism

Data supporting the mechanism can be found in early studies by Grese and Gross [16] on the metastable decay of lithiated dipeptides. In this work, they found that the fragmentation led to the production of the lithium complexes of both amino acids along with other products (Scheme 6). One amino acid complex is from the C-terminal cleavage process, $[b_1 + OH + Li]^+$ ions, and the other is a y-type ion, $[y_1+H+Li]^+$. Fortunately, they happened to include a number of pairs of dipeptides with reversed sequences in their study. Focusing only on the relative yields of the $[b_1+OH+Li]^+$ and $[y_1+H+Li]^+$ ions, the similarity in the product ratios from these pairs is striking. For example, lithiated Ala–Gly and Gly–Ala both mainly give lithiated Gly (85 and 88%, respectively) during metastable decay (Scheme 6). The same is true (i.e., similar product distributions from both dipeptides) for five other pairs of dipeptides. A significant difference in the metastable decay product distributions was observed with only one of the paired systems in their study, the Ala, Leu pair (41 and 68% $AlaLi^+$). Grese and Gross [16] also investigated the lithiated dipeptide complexes under high energy CID conditions. Although the $[y_1+H+Li]^+$ ions were generally more abundant under these conditions, the patterns still suggested an underlying lack of sequence specificity. This



Scheme 5.

$$[A_1\text{–}A_2\ Li^+] \xrightarrow[\text{decay}]{\text{metastable}} \begin{matrix} [A_1\ Li^+] \quad \text{and} \quad [A_2\ Li^+] \\ [b_1 + OH + Li]^+ \qquad [y_1 + H + Li]^+ \end{matrix}$$

$$\left\{ \begin{matrix} A_1 = Ala,\ A_2 = Gly \\ A_1 = Gly,\ A_2 = Ala \end{matrix} \right. \quad \left. \begin{matrix} [Ala\ Li^+]:[Gly\ Li^+] = 15:85 \\ [Ala\ Li^+]:[Gly\ Li^+] = 12:88 \end{matrix} \right\}$$

Scheme 6.

result can be interpreted in two ways: (i) formation of the y-type ions is more energetically demanding than formation of the b-type ions, or (ii) a new, entropically favorable (i.e., more direct) route to y-type ions opens up at higher energies. Teesch et al. [21] have argued in favor of the first explanation, but the second explanation is also consistent with the available data.

Table 2
Distribution between A_1Li^+ and A_2Li^+ products in the CID of the lithium complexes of A_1A_2 and A_2A_1[a]

Dipeptide pair (A_1/A_2)	A_1Li^+ (%)	
	A_1A_2	A_2A_1
Met/Leu	94	95
Ala/Leu	74	66
Ala/Pro	75	79
Phe/Ala	93	95
Trp/Gly	98	99
Gly/Leu	98	100
Ser/Gly	83	86
Gly/Ala	94	97

[a] Other products are formed.

To provide further evidence of this unusual lack of sequence specificity, we have studied the CID spectra of the lithium complexes of eight pairs of dipeptides (i.e., pairs with both sequences). For this work, we have used a quadrupole ion trap so the fragmentation is caused by multiple, low-energy collisions with a helium buffer gas. The compounds were chosen to give a variety of functional groups in the side chains as well a range of branching ratios from roughly 50:50 to greater than 99:1. The data are listed in Table 2 and shown in Fig. 5. It can be seen that in every case (including Ala/Leu), the sequenced-reversed pairs of dipeptides give almost identical product distributions. This is remarkable given the diversity of this set of dipeptides. It is important to point out that the spectra from the two sequences are not always identical, but the branching ratio between the $[b_1 + OH + Li]^+$ and $[y_1 + H + Li]^+$ ions is strikingly similar for every case. The Ala/Gly and Ser/Gly systems provide good examples of the two extreme types of behavior in this respect. For the Ala–Gly/Gly–Ala pair, the lithium complexes give very similar CID spectra (Fig. 6). In contrast, the lithium complexes of Ser–Gly and Gly–Ser give very different CID spectra (Fig. 7). However, if one only focuses on lithiated amino acid products (a very small yield in this case), the ratio of lithiated serine to lithiated glycine is nearly the same for both sequences. Therefore, even though the two sequences of some of the dipeptide systems (e.g., Ser/Gly) may have different product channels available, the pathways that lead to lithiated amino acid products are related and locked into identical product distributions. One also notices in Fig. 5 that the preferred product appears to be more dominant when that amino acid is at the C-terminus (i.e., there is usually a slightly greater yield of A_1Li^+ from the

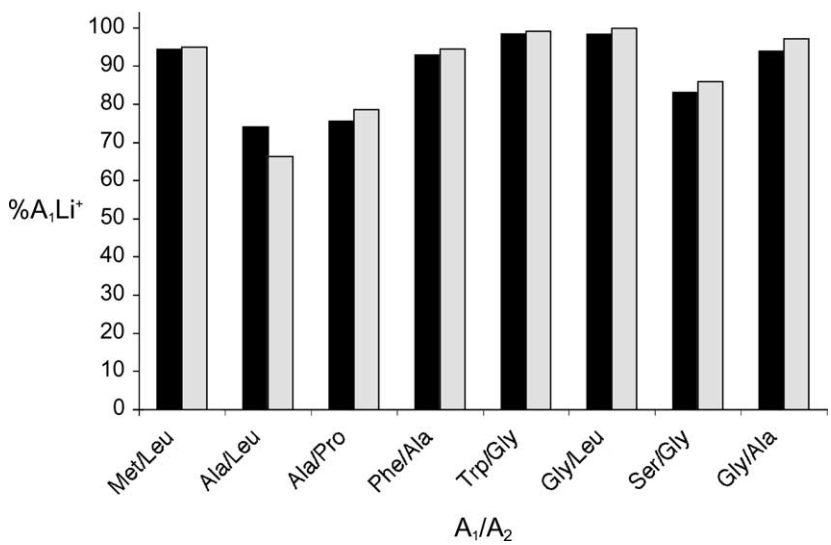


Fig. 5. Graphical representation of the product yields from the CID fragmentation of the lithiated dipeptides. Dark bars are for A_1/A_2 and light bars are for A_2/A_1.

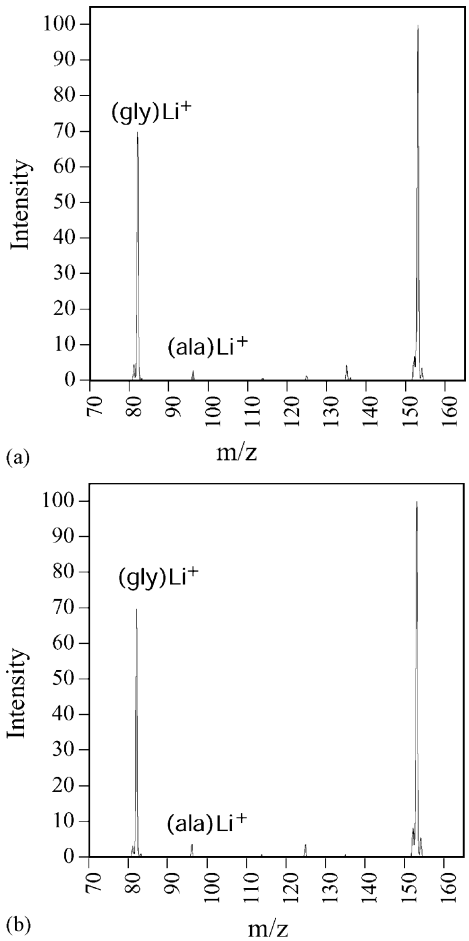


Fig. 6. (a) CID spectrum from the fragmentation of lithiated Gly–Ala. (b) CID spectrum from the fragmentation of lithiated Ala–Gly.

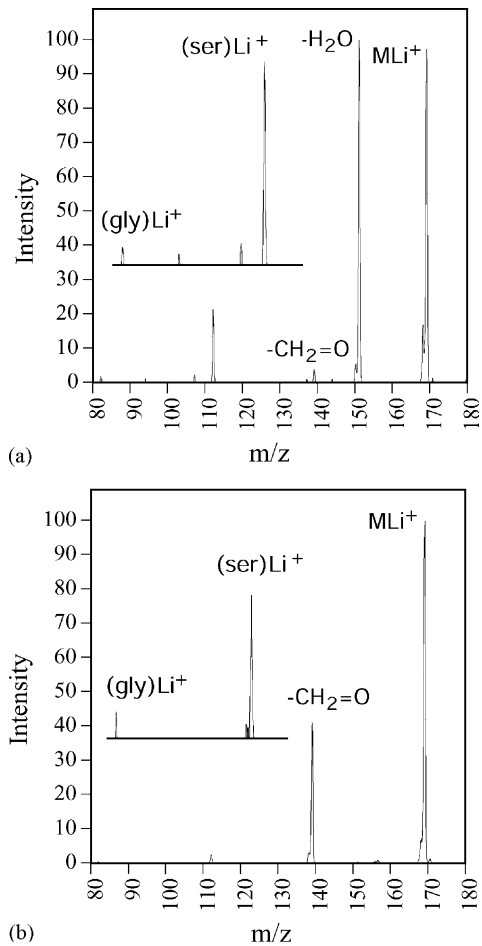


Fig. 7. (a) CID spectrum from the fragmentation of lithiated Gly–Ser. Inset expanded by a factor of ∼2.5. Unlabeled peaks at m/z = 94 and 107 represent $SerLi^+(–H_2O)$ and $Gly–SerLi^+(–H_2O, –CO_2)$, respectively. (b) CID spectrum from the fragmentation of lithiated Ser–Gly. Inset expanded by a factor of ∼15.

A_2A_1 sequence). This indicates that a very small amount of this product, $[y_1 + H + Li]^+$ ions, is being formed by a pathway where the amino acids do not become equivalent. This is consistent with Grese and Gross's high energy CID data and suggests that a direct cleavage pathway to the $[y_1 + H + Li]^+$ ions is available; however, it is not very competitive under the low-energy CID conditions of the ion trap.

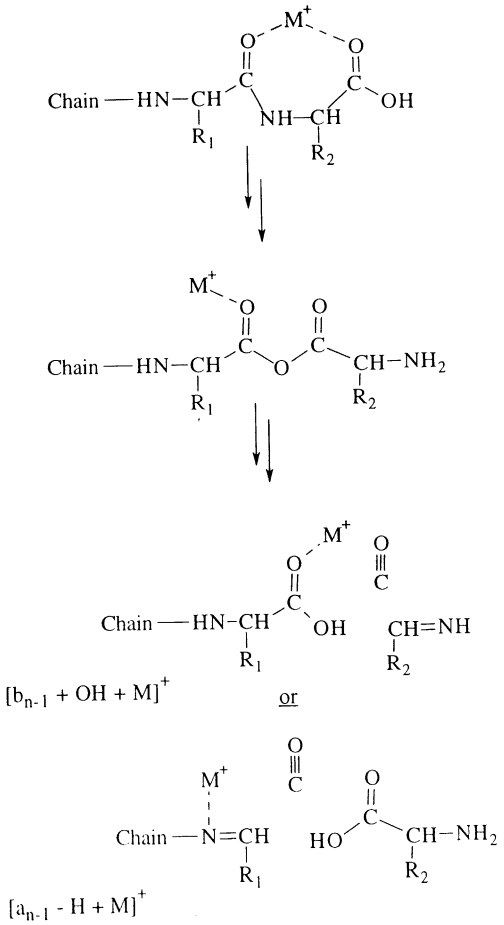
At the time, Grese and Gross [16] suggested that the y-type ions (i.e., A_2Li^+) were formed by a completely different mechanism than the b-type ions (i.e., A_1Li^+). Their mechanism for the formation of the y-type ions involved proton transfer from the N-terminal amino group to the amide nitrogen followed by fragmentation of the N-terminal residue to give CO and an imine. This seems unlikely. First, the amide nitrogen is only weakly basic and one would not expect it to initiate an elimination process by deprotonating an amine. Second, if the b- and y-type ions were formed by competing mechanisms, the above data would require that both mechanisms have

equivalent substituent effects. Consequently, the side chains would need to have the same effect (accelerating or decelerating) on the formation of b-type ions when they are at the N-terminal position as they have on the formation of y-type ions when they are at the C-terminal position. Although this is a possibility, it would be a remarkable coincidence given that the two mechanisms have little in common (other than the products) and that even side chains with varying properties (including steric, polar, and chelating) fit the pattern. The mechanism proposed here with an anhydride as the common intermediate seems like a much more likely explanation of the observed product ratios.

Farrugia and O'Hair [33] have observed analogous behavior in the fragmentation of protonated dipeptides that contain an arginine residue (i.e., both sequences give similar product distributions). Their studies are described in the accompanying paper. Based on their data, they have independently reached the same conclusion that an anhydride is the common intermediate in the fragmentation pathway.

Two issues from our experimental data deserve further comment. First, there are definite preferences in the product distributions, but the origin of these preferences is not always obvious. Some cases are easy to understand such as the Lys/Gly pair. Lysine has a much higher lithium affinity than glycine so it is not surprising that lithiated lysine is the dominant product. In Schemes 4 and 5, it can be seen that the lithium is associated with the residue that will become the lithiated amino acid product; consequently, one would expect that residues with high lithium affinities would dominate. This logic fails in cases such as the Gly/Ala pair. Here, the residue with the lowest lithium cation affinity (glycine) [47] clearly dominates the product mixture (~95% lithiated glycine is formed). In this case, the lithium affinities of the two residues are not very different, so another factor can come into play. The residue that is destroyed in the process goes through an acyl cation intermediate and eventually produces the imine product (Schemes 4 and 5). The acyl cation derived from glycine should be considerably less stable than the one from alanine and apparently this factor overwhelms the small difference in the lithium cation affinities of the two residues. In addition, neutral thermochemistry [48] indicates that the following reaction would be endothermic by 4.2 kcal/mol: glycine $+CH_3CH{=}NH$ => alanine + $CH_2{=}NH$. Bojesen et al. [47] have shown that the lithium affinity of alanine is about 1.6 kcal/mol greater than that of glycine. Combining these results, we can predict that in the fragmentation of lithiated Ala–Gly or Gly–Ala, formation of lithiated glycine will be favored by over 2 kcal/mol. The fact that the product distributions seem to be controlled by thermodynamics is also suggestive of a common intermediate. The second issue involves the reversibility of the amide => anhydride rearrangement process. It could be possible that this process is reversible and therefore, the dipeptides might eventually become completely scrambled. In other words, either sequence of the dipeptide could be converted to the same equilibrium mixture of the two sequences before fragmentation. However, if this were the case, then both sequences would give nearly identical CID spectra. In Fig. 7, it is clear that Ser–Gly and Gly–Ser give very different spectra during CID so sequence scrambling (i.e., amide => anhydride => amide) must be relatively slow relative to the dissociation processes.

In the case of a larger peptide, there are two important pathways to charged products (Scheme 7). The main chain of the peptide could be associated with the carboxylic acid product of the fragmentation and the result would be a C-terminal cleavage process to give a $[b_{n-1} + OH + M]^+$ ion. Alternatively, the main chain could be attached to the imine product. Because the main peptide chain (with its accompanying functional groups) should have a higher metal ion affinity than a single amino acid, the metal (and charge) would be on the imine product leading to an $[a_{n-1} - H + M]^+$ ion. These types of ions are common products in the CID of peptide/alkali metal ion complexes. For example, Grese et al. [15] give numerous examples of $[a_{n-1} - H + M]^+$ ions being formed in moderate yields from the lithium complexes of small peptides and we have shown that a series of heptapeptides give significant yields of $[a_6 - H + M]^+$ ions [49]. Although the a-type ions may be formed by the



Scheme 7.

pathway in Scheme 7, there is ample evidence that $[a_{n-1} - H + M]^+$ ions must be accessible through alternative mechanisms. For example, Adams and co-workers [21,22] has shown that alkali metal ion complexes of C-terminal esters can yield $[a_{n-1} - H + M]^+$ ions, an unlikely outcome from the mechanism proposed in Scheme 7. In addition, $[a_{n-1} - H + M]^+$ ions have been observed when the C-terminus has been converted to an amide [21] or a lithium carboxylate [13]. Given that the formation of $[a_{n-1} - H + M]^+$ ions is seemingly insensitive to the C-terminal functional group, there must be another route to these ions that does not significantly involve this functional group.

3.3. Mechanism of the C-terminal cleavage in charge-remote systems

Although C-terminal cleavage is not usually an important process in simple, protonated peptides [29,30], it has been observed in systems where the charge is sequestered on a site remote from the cleavage point. In the accompanying paper, Farrugia and O'Hair [33] describe the fragmentation in protonated peptides where the charge carrier is effectively localized on the side chain of a highly basic arginine residue [50]. Wysocki and co-workers [31] and Sadagopan and Watson [32] also have presented examples in which C-terminal cleavage is observed in systems where the charge is localized at a fixed group on the C-terminus (e.g., phosphonium cation). These results indicate that the metal does not play a vital role in "catalyzing" the C-terminal cleavage and that the process must be viable for essentially uncharged systems. In their work, Gaskell and co-workers [29], Wysocki and co-workers [31], and Farrugia and O'Hair [33] have suggested that the charge-remote process also involves an oxazolidin-5-one as the key intermediate.

To explore this point, we have examined the potential energy surface for C-terminal cleavage in neutral glycylglycine. In short, we have followed the same pathway outlined for the lithiated system, but simply omitted the metal ion from the calculations and re-optimized the structures. Because the system is smaller and fewer pathways were explored, a somewhat higher level of theory was used, MP2/6-31+G(d,p)//MP2/6-31+G(d) instead of MP2/6-31+G(d)//HF/6-31+G(d)). The results are given in Table 3 (structures are not shown). In general, the system follows nearly an identical path, but in some cases, two steps have collapsed into a single, concerted one because in the absence of the metal, some of the zwitterions are unstable. For example, the zwitterionic form of glycylglycine is only stable in a conformation where the ammonium and carboxylate are oriented so that they are far apart. This conformation has a high energy compared to ground-state glycylglycine (>40 kcal/mol). Conformations analogous to II collapse to ground-state glycylglycine. The cyclization

Table 3
Energies of intermediates and transition states in C-terminal cleavage process of neutral glycylglycine[a]

Species	HF/6-31+G(d)	MP2//HF/ 6-31+G(d)[b]	MP2//MP2/ 6-31+G(d,p)[c]	ZPE[d]	Relative energies		
					HF	MP2//HF[b]	MP2//MP2[c]
$\mathbf{I}_N$	−489.66223	−491.03438	−491.11107	0.14650	0	0	
$\mathbf{III}_N$	−489.51869	−490.91573	−490.99289	0.14285	88.0	72.4	72.1
$\mathbf{IV}_N$	−489.58194	−490.98364	−491.05789	0.14907	51.9	33.3	34.8
$\mathbf{V}_N$	−489.59293	−490.98935	[e]	0.14902	44.9	29.7	[e]
$\mathbf{VI}_N$	−489.56421	−490.97407	−491.04846	0.14492	60.6	36.9	38.4
$\mathbf{IX}_N$	−489.63484	−491.00832	−491.08487	0.14613	17.0	16.1	16.2
$\mathbf{XI}_N$	−489.55818	−490.93871	−491.01710	0.14168	62.5	57.3	56.2

[a] Absolute energies in hartree. Relative energies in kcal/mol. Labeled analagously to Table 1, but some structures are missing on the neutral potential energy surface.
[b] MP2/6-31+G(d)//HF/6-31+G(d) level of theory.
[c] MP2/6-31+G(d,p)//MP2/6-31+G(d) level of theory.
[d] Zero point vibrational energy (unscaled). Values scaled by 0.9135 [39] are used in the calculation of relative energies.
[e] Zwitterion collapses to conventional structure during MP2 optimization.

process goes through transition state $\mathbf{IV}_N$ with a barrier of 34.8 kcal/mol to produce the oxazolidin-5-one intermediate, $\mathbf{V}_N$. It is possible that other zwitterionic forms might lead to a lower barrier to this process (we are following a path analogous to the best one for the lithiated system), but the important result is that the barrier is relatively low and compatible with a CID process. Transition state $\mathbf{VI}_N$ is 38.4 kcal/mol less stable than $\mathbf{I}_N$ and involves a proton transfer to the ring nitrogen that appears to cause a spontaneous C–N bond cleavage with formation of the anhydride, $\mathbf{IX}_N$. In the lithiated system, a zwitterionic intermediate was identified (**VII**), but in the neutral system, this intermediate does not seem to exist (however, it is possible that it is in a very shallow well that we could not locate). This is not surprising because one might expect the lithium cation to preferentially stabilize zwitterions. Finally, the anhydride cleaves in one step to give the observed products through transition state $\mathbf{XI}_N$. This transition state is the highest on the surface and is 56.2 kcal/mol less stable than $\mathbf{I}_N$. This represents the high point on the potential energy surface (Fig. 8). Comparing the energetics of fragmentation in the lithiated and neutral systems (Fig. 4 vs. Fig. 8), one sees that for the early steps in the mechanism, the neutral system encounters lower energy barriers, but in the critical anhydride breakdown, the lithiated system is preferred by about 10 kcal/mol. This is easily rationalized because the heterolytic cleavage of the C–O bond in the anhydride leads to a very polar charge distribution that would be stabilized by the nearby cation. This also suggests that in the charge-remote systems, conformations that place the charge site near the anhydride would experience lower barriers due to a long-range attractive interaction with the large dipole moment of the transition state (6 debye at the HF level). To test this effect, we examined a model

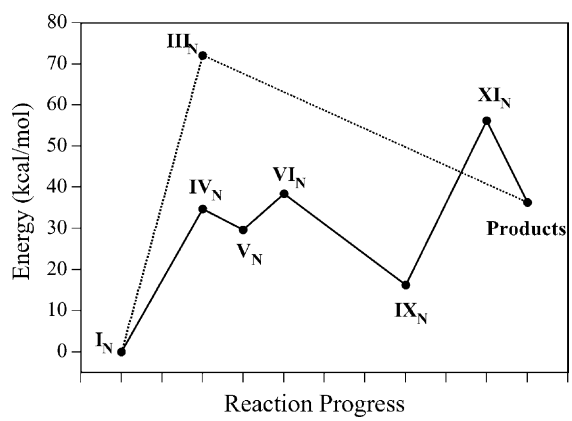


Fig. 8. Representation of the potential energy surfaces for the two pathways for fragmenting neutral glycylglycine. Only the stationary points that were located as a part of this study are included. The MP2//HF energy value is used for $\mathbf{V}_N$ (see Table 3).

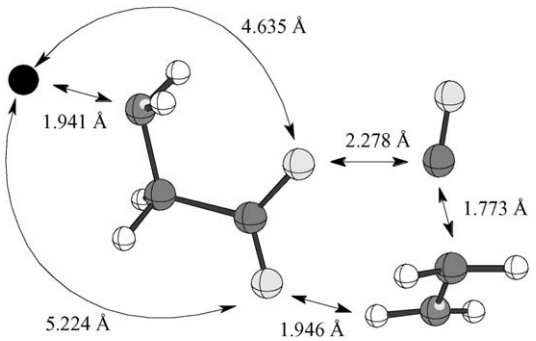


Fig. 9. Structure of the transition state in the charge-remote model of the anhydride fragmentation. Geometries at HF/6-31+G(d) level (carbon: dark gray; hydrogen: white; oxygen: light gray; nitrogen: patterned).

in which a cation (lithium) was forced to coordinate to one of the amino groups in the anhydride (the one that would eventually be in the glycine product), but not to either of the carbonyls (Fig. 9). This effectively provides a system with a remote-charge site (albeit nearby) and the lithium is more than 4.5 Å away from any of the oxygens. To assess the stability of this transition state, a lithium complex of glycylglycine with similar restrictions (i.e., lithium only interacts with N-terminal amino group) was constructed. The results show that the presence of the remote lithium cation reduces the barrier by about 10 kcal/mol (a simple charge/dipole model, positive charge 5 Å from a 6 debye dipole, gives a similar value, ∼13 kcal/mol). This model points out a novel feature of charge-remote processes in that it is possible for a localized charge center to catalyze a fragmentation by a through-space electrostatic interaction. As a result, it is very possible that the 56 kcal/mol barrier found for the neutral system fragmentation could be reduced by a long-range electrostatic interaction with the charge site. Finally, the transition state for a concerted fragmentation of the five-membered ring intermediate was located for the neutral system. The structure, $\mathbf{III}_N$, is very similar to the lithiated species (**III**) and is over 70 kcal/mol less stable than the $\mathbf{I}_N$. Clearly, the concerted cleavage pathway is not viable with or without the metal cation.

4. Summary

The experimental and computational studies presented here clearly indicate that some elements of the accepted mechanism for the C-terminal cleavage process are not viable. First, the fragmentation studies of the lithiated dipeptides offer compelling evidence that the process involves an intermediate where the two amino acids become equivalent. Second, the computations indicate that there are very large barriers for all of the pathways that involve the concerted cleavage of the oxazolidin-5-one intermediate. As an alternative, we believe that the C-terminal cleavage process involves the formation of an anhydride as an intermediate. This intermediate explains the lack of sequence specificity with the dipeptides and the computational work predicts that the barriers are much lower than for a concerted cleavage pathway. In addition, an analogous pathway has been suggested independently by Farrugia and O'Hair [33] for the fragmentation of protonated, arginine-containing peptides.

Although the C-terminal cleavage process has often been associated with alkali metal cation complexes of peptides, it appears that the metal plays only a small role in the process. This is in accord with previous results involving the fragmentation of peptides containing localized charge centers. Model computational studies with a neutral peptide show that fairly similar barriers are observed along the pathway with and without the metal cation. One exception is the final fragmentation of the anhydride because it involves a highly polar transition state that can be significantly stabilized by interaction with a charge center; however, this effect is purely electrostatic and a through-space interaction with a remote-charge center (i.e., close in space, but distant in terms of bond linkages) could catalyze this part of the cleavage process. The general inefficiency of C-terminal fragmentations in protonated peptides is probably a result of a "mobile" proton [51] opening up other, more facile reaction channels that overwhelm the C-terminal cleavage process.

Finally, the proposed mechanism involves several intermediates with zwitterionic charge distributions.

In recent years, intermediates of this type have often been put forward to explain fragmentation processes. However, the computations as well as earlier experimental work clearly indicate that these systems prefer conventional structures in their ground-states. As a result, it is important to remember that mechanistic evidence of zwitterionic intermediates is not a reliable indication that the system prefers a zwitterionic structure in its ground-state.

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References

[1] G. Siuzdak, Proc. Natl. Acad. Sci. U.S.A. 91 (1994) 11290.
[2] J.R. Yates, J. Mass Spectrom. 33 (1998) 1.
[3] E.J. Zaluzec, D.A. Gage, J.T. Watson, Protein Exp. Purif. 6 (1995) 109.
[4] T. Keough, R.S. Youngquist, M.P. Lacey, Proc. Natl. Acad. Sci. U.S.A. 96 (1999) 7131.
[5] G. Tsaprailis, H. Nair, A. Somogyi, V.H. Wysocki, W.Q. Zhong, J.H. Futrell, S.G. Summerfield, S.J. Gaskell, J. Am. Chem. Soc. 121 (1999) 5142.
[6] A. Marina, M.A. Garcia, J.P. Albar, J. Yague, J.A.L. de Castro, J. Vazquez, J. Mass Spectrom. 34 (1999) 17.
[7] J.A. Loo, H. Muenster, Rapid Commun. Mass Spectrom. 13 (1999) 54.
[8] W.P. Blackstock, M.P. Weir, Trends Biotechnol. 17 (1999) 121.
[9] V.M. Doroshenko, R.J. Cotter, Anal. Chem. 67 (1995) 2180.
[10] K. Biemann, Biomed. Environ. Mass Spectrom. 16 (1988) 99.
[11] J.A. Castoro, C.L. Wilkins, A.S. Woods, R.J. Cotter, J. Mass Spectrom. 30 (1995) 94.
[12] S.D. Patterson, Anal. Biochem. 221 (1994) 1.
[13] J.A. Leary, T.D. Williams, G. Bott, Rapid Commun. Mass Spectrom. 3 (1989) 192.
[14] J.A. Leary, Z. Zhou, S.A. Ogden, T.D. Williams, J. Am. Soc. Mass Spectrom. 1 (1990) 473.
[15] R.P. Grese, R.L. Cerny, M.L. Gross, J. Am. Chem. Soc. 111 (1989) 2835.
[16] R.P. Grese, M.L. Gross, J. Am. Chem. Soc. 112 (1990) 5098.
[17] D.H. Russell, E.S. McGlohon, L.M. Mallis, Anal. Chem. 60 (1988) 1818.
[18] L.M. Mallis, D.H. Russell, Anal. Chem. 58 (1986) 1076.
[19] D. Renner, G. Spiteller, Biomed. Environ. Mass Spectrom. 15 (1988) 75.
[20] X. Tang, W. Ens, K.G. Standing, J.B. Westmore, Anal. Chem. 60 (1988) 1791.
[21] L.M. Teesch, R.C. Orlando, J. Adams, J. Am. Chem. Soc. 113 (1991) 3668.
[22] L.M. Teesch, J. Adams, J. Am. Chem. Soc. 113 (1991) 812.
[23] M. Fujioka, I. Sumino, S. Koda, T. Yasuda, Org. Mass Spectrom. 25 (1990) 83.
[24] M. Becchi, S. Rebuffat, J.Y. Dugast, S. Hlimi, B. Bodo, G. Molle, Rapid Commun. Mass Spectrom. 9 (1995) 37.
[25] S.W. Lee, H.S. Kim, J.L. Beauchamp, J. Am. Chem. Soc. 120 (1998) 3188.
[26] T. Lin, G.L. Glish, Anal. Chem. 70 (1998) 5162.
[27] M.J. Tomlinson, J.R. Scott, C.L. Wilkins, J.B. Wright, W.E. White, J. Mass Spectrom. 34 (1999) 958.
[28] S.G. Summerfield, V.C.M. Dale, D.D. Despeyroux, K.R. Jennings, Eur. Mass Spectrom. 1 (1995) 183.
[29] G.C. Thorne, K.D. Ballard, S.J. Gaskell, J. Am. Soc. Mass Spectrom. 1 (1990) 249.
[30] A.H. Payne, J.H. Chelf, G.L. Glish, Analyst 125 (2000) 635.
[31] C. Gu, G. Tsaprailis, L. Breci, V.H. Wysocki, Anal. Chem. 72 (2000) 5804.
[32] N. Sadagopan, J.T. Watson, J. Am. Soc. Mass Spectrom. 12 (2001) 399.
[33] J. Farrugia, R.A.J. O'Hair, Int. J. Mass Spectrom. 222 (2003) 229–242.
[34] S. Hoyau, K. Norrman, T.B. McMahon, G. Ohanessian, J. Am. Chem. Soc. 121 (1999) 8864.
[35] R.W. Taft, F. Anvia, J.-F. Gal, S. Walsh, M. Capron, M.C. Holmes, K. Hosn, G. Oloumi, R. Vasanwala, S. Yazdani, Pure Appl. Chem. 62 (1990) 17.
[36] P. Burk, I.A. Koppel, I. Koppel, R. Kurg, J.-F. Gal, P.-C. Maria, M. Herreros, R. Notario, J.-L.M. Abboud, F. Anvia, R.W. Taft, J. Phys. Chem. A 104 (2000) 2824.
[37] M.J. Frisch, G.W. Trucks, H.B. Schlegel, P.M.W. Gill, B.G. Johnson, M.A. Robb, J.R. Cheeseman, T. Keith, G.A. Petersson, J.A. Montgomery, K. Raghavachari, M.A. Al-Laham, V.G. Zakrzewski, J.V. Ortiz, J.B. Foresman, C.Y. Peng, P.Y. Ayala, W. Chen, M.W. Wong, J.L. Andres, E.S. Replogle, R. Gomperts, R.L. Martin, D.J. Fox, J.S. Binkley, D.J. Defrees, J. Baker, J.P. Stewart, M. Head-Gordon, C. Gonzalez, J.A. Pople, Gaussian 98 Revision B, Gaussian, Inc., Pittsburgh, PA, 1995.
[38] M.J. Frisch, G.W. Trucks, H.B. Schlegel, G.E. Scuseria, M.A. Robb, J.R. Cheeseman, V.G. Zakrzewski, J.A. Montgomery, R.E. Stratmann, J.C. Burant, S. Dapprich, J.M. Millam, A.D. Daniels, K.N. Kudin, M.C. Strain, O. Farkas, J. Tomasi, V. Barone, M. Cossi, R. Cammi, B. Mennucci, C. Pomelli, C. Adamo, S. Clifford, J. Ochterski, K. Morokuma, D.K. Malick, A.D. Rabuck, K. Raghavachari, J.B. Foresman, J. Cioslowski, J.V. Ortiz, B.B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. Gomperts, R.L. Martin, D.J. Fox, T. Keith, M.A. Al-Laham, C.Y. Peng, A. Nanayakkara, C. Gonzalez,

M. Challacombe, P.M.W. Gill, B.G. Johnson, W. Chen, M.W. Wong, J.L. Andres, M. Head-Gordon, E.S. Replogle, J.A. Pople, Gaussian 98 Revision A, Gaussian, Inc., Pittsburgh, PA, 1998.
[39] J.A. Pople, A.P. Scott, M.W. Wong, L. Radom, Isr. J. Chem. 33 (1993) 345.
[40] B.J. Deppmeier, A.J. Driessen, W.J. Hehre, J.A. Johnson, P.E. Kluzinger, L. Lou, J. Yu, Wavefunction Inc., Irvine, CA, 1996.
[41] T. Wyttenbach, M. Witt, M.T. Bowers, Int. J. Mass Spectrom. 182/183 (1999) 243.
[42] W.D. Price, R.A. Jockusch, E.R. Williams, J. Am. Chem. Soc. 119 (1997) 11988.
[43] W.Y. Feng, S. Gronert, C.B. Lebrilla, J. Am. Chem. Soc. 121 (1999) 1365.
[44] B.A. Cerda, S. Hoyau, G. Ohanessian, C. Wesdemiotis, J. Am. Chem. Soc. 120 (1998) 2437.
[45] C.F. Rodriquez, T. Shoeib, I.K. Chu, K.W.M. Siu, A.C. Hopkinson, J. Phys. Chem. A 104 (2000) 5335.
[46] D. Ray, D. Feller, M.B. More, E.D. Glendening, P.B. Armentrout, J. Phys. Chem. 100 (1996) 16116.
[47] B.G. Bojesen, T. Breindahl, U.N. Andersen, Org. Mass Spectrom. 28 (1993) 1448.
[48] H.Y. Afeefy, J.F. Liebman, S.E. Stein, in: W.G. Mallard, P.J. Linstrom (Eds.), NIST Chemistry WebBook, NIST Standard Reference Database Number 69 (http://webbook.nist.gov), National Institute of Standards and Technology, Gaithersburg, MD, November 1998.
[49] W.Y. Feng, S. Gronert, unpublished results.
[50] J. Gonzalez, V. Besada, H. Garay, O. Reyes, G. Padron, Y. Tambara, T. Takao, Y. Shimonishi, J. Mass Spectrom. 31 (1996) 150.
[51] V.H. Wysocki, G. Tsaprailis, L.L. Smith, L.A. Breci, J. Mass Spectrom. 35 (2000) 1399.