Fast Atom Bombardment Tandem Mass Spectrometry of Benzyloxycarbonyl-Protected Tetrapeptide Derivatives Containing Proline

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Fragmentations of *N*-benzyloxycarbonyl-protected tetrapeptide ethyl esters containing L-proline and L-alanine in the gas phase were examined by the collisional-activated decomposition of the deprotonated molecule and the fragment ions produced by the cleavage of the tetrapeptide derivatives, in which changes in both the number and positions of the prolyl residues were observed, in negative-ion FAB-MS. The cleavage patterns of these ions in the collisional-activated decomposition mass spectra were observed to depend on the number and positions of prolyl residues in the peptide derivatives. These results indicate that the conformational difference in the tetrapeptide derivatives due to the existence of L-proline may be an important factor responsible for the fragmentations of peptide molecules in the gas phase.

Key words fragmentation; FAB-MS/MS; benzyloxycarbonyl-protected tetrapeptide; proline

Many biologically important peptide sequences contain L-proline (Pro). It plays an important role in peptide and protein structures.[1,2] Peptides containing Pro may have more bending structures than those not containing it, and the conformation of the peptide molecules may thus change depending on the content of Pro.

We previously reported significant differences in negative-ion FAB and collisional-activated decomposition (CAD) mass spectra among the intensities of the fragment ions formed by cleavage of the benzyloxycarbonyl (Z)-group from the Z-protected tripeptides containing Pro, depending on the number and positions of the prolyl residues.[3–5] That indicated that the conformational difference in the tripeptide derivatives due to the existence of Pro influences the fragmentations of the peptide molecules in the solution and gas phase in FAB-MS. We further reported that the fragmentations of the *N*-Z-protected tetrapeptide derivatives containing Pro and L-alanine (Ala) are dependent on the content of Pro in the peptides in negative-ion FAB-MS.[6] Our results prompted us to examine the fragmentations of the tetrapeptide derivatives in the gas phase.

In the negative-ion FAB mass spectra for *N*-Z-protected tetrapeptide derivatives, the deprotonated molecule $[M-H]^-$ and the main three fragment ions formed by the cleavage of the Z-group were observed: the $[M-91]^-$ ion (loss of the benzyl group), the $[M-H-108]^-$ ($[M']^-$) ion (loss of the benzyl alcohol from the $[M-H]^-$ ion) and the $[M-135]^-$ ion (loss of the Z-group).[6] The mechanism of the formation of the $[M']^-$ion and the fragmentation process of the $[M-91]^-$ ion to the $[M-135]^-$ ion, however, were not clear.

The combination of FAB and tandem mass spectrometry is believed able to provide some detailed information on the fragmentation processes and the structures of the ions and molecules. Characteristic fragmentations were reportedly observed in high-energy tandem mass spectrometry of peptides containing Pro.[7] This phenomenon was also found in low-energy tandem mass spectrometry of these peptides.[8–11]

In this paper, we describe the CAD of the deprotonated molecule and the fragment ions for the *N*-Z-protected tetrapeptide derivatives containing Pro and Ala, which are formed by the cleavage of the Z-group, and also discuss the effect on the fragmentations of the peptide derivatives due to the existence of Pro in the gas phase.

Experimental

Materials All amino acids used were L-enantiomers. *N*-Z-protected tetrapeptide ethyl esters containing Pro and Ala were prepared as previously described.[6] All other chemicals were of analytical or reagent grade.

Measurement Conditions for FAB MS/MS All mass spectra were acquired with a JEOL SX/SX102A tandem mass spectrometer of BEBE geometry, which was controlled by a JEOL DA-7000 data system (Tokyo, Japan). The positive- and negative-ion FAB mass spectra were obtained using only the first spectrometer. The samples were diluted in dimethylformamide at a concentration of 1 μg/μl. The solution (1 μl) was then subjected to analysis. Triethylene glycol was used as a matrix, because it produced a superior mass spectra in the cleavage patterns to other matrices.[6] Ions were produced by bombardment with a neutral xenon atom at 5 kV. The mass range (m/z 1—1000) was scanned at 5 s under an ion source accelerating potential of 10 kV, and then the averaged intensities in decade scans were recorded. The pseudo molecular ions generated by FAB-MS were selected as precursor ions and then were collided with argon molecules in the third field-free region. The argon pressure was sufficient to attenuate the primary ion beam by 50%. The fragment ions were dispersed by the second spectrometer and the spectra were recorded as the CAD spectra.

The nomenclature of Roepstorff and Fohlman[12] for peptide fragment ions was used for the sequence ions with the Bieman modification.[13]

Results and Discussion

CAD of $[M-H]^-$ Ions We have shown that the $[M-H-108]^-$ ($[M']^-$) ion was formed due to the loss of the benzyl alcohol molecule from the $[M-H]^-$ ion, and was assigned as $[M-H-C_6H_5CH_2OH]$ in the high energy CAD of the $[M-H]^-$ ion for the *N*-Z-protected tripeptide derivatives containing Pro and the neutral amino acids (Ala, Leu and Phe).[5] Schwartz *et al.*[10] also reported that the $[M+H-108]^+$ ion was formed due to the loss of the benzyl alcohol from the $[M+H]^+$ ion in a low energy FAB tandem mass spectrometric study. We also examined the high energy CAD spectra of the

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$[M-H^-]$ ion for the *N*-Z-protected tetrapeptide derivatives containing Pro and Ala (Table 1). The $[M']^-$ ion was detected as a base peak for **1**—**10**, **12** and **13**, whereas it was a fragment ion with a very low abundance for **14** and **15** and with a fairly high abundance for **11** and **16**. These results showed that the $[M']^-$ ion was produced from the $[M-H]^-$ ion. In the negative-ion FAB mass spectra for **11**, **14**, **15** and **16**, the $[M']^-$ ion was not detected at all. It therefore seems that the fission of $C_6H_5CH_2O$–C bond in **11** and **16** was suppressed due to the interaction between the peptide derivatives and the matrix in FAB condition. In addition, it is likely that the $C_6H_5CH_2O$–C bond in **14** and **15** was buried in the peptide molecules, because the $[M']^-$ ion was hardly detectable in the CAD mass spectra for these two peptide derivatives.

The $[M-H-46]^-$ ion, due to the loss of ethanol, was the base peak for **11**, **14**, **15** and **16**, while it was not detected at all or it was a fragment ion with a very low abundance for the other peptides. Thus, the loss of ethanol was easier than loss of benzyl alcohol for the peptides containing Pro at both the P_3 and P_4 sites. The $[M-H-154]^-$ ion, due to the loss of ethanol and benzyl alcohol from the $[M-H]^-$ ion, was not detected at all or was a weak fragment ion for these four peptides, suggesting that benzyl alcohol was not lost from the $[M-H-46]^-$ ion. The CAD spectrum for **16** was similar to that for Z–Pro–Pro–Pro–OEt,[5] but the abundances of the $[M-H-154]^-$ ion for **11**, **14** and **15**, which had the sequences of Z–Pro–Pro, were different from that for Z–Pro–Pro–Ala–OEt.[5] The abundance (88%) of this ion for Z–Pro–Pro–Ala–OEt was very high, but the ion was not detected at all for these three peptide derivatives. Therefore, the $[M-H-46]^-$ ion for the tetrapeptides was more stabilized than that for the tripeptides due to the existence of Pro at both the P_3 and P_4 sites.

On the other hand, in the positive-ion FAB CAD spectra of the $[M+H]^+$ ions for the *N*-Z-protected tetrapeptide derivatives containing Pro and Ala, the abundance for the $[M+H-108]^+$ ion rose, as the number of Pro near the Z-group increased (Table 2). This ion was detected as a fairly strong fragment ion for the tripeptide, Z–Pro–Pro–Ala–OEt and Z–Pro–Pro–Pro–OEt,[5] and it was also

Table 1. Negative-Ion FAB Tandem Mass Spectra of the $[M-H]^-$ Ions for the *N*-Z-Protected Tetrapeptide Ethyl Esters Containing Pro and Ala[a]

Compound	M.W.	$[M-H-46]^-$	$[M']^-$	$[M-H-154]^-$
Z–Ala–Ala–Ala–Ala–OEt (**1**)	464	417 (—[b])	355 (100)	309 (—[b])
Z–Ala–Ala–Ala–Pro–OEt (**2**)	490	443 (1)	381 (100)	335 (—[b])
Z–Ala–Ala–Pro–Ala–OEt (**3**)	490	443 (—[b])	381 (100)	335 (1)
Z–Ala–Pro–Ala–Ala–OEt (**4**)	490	443 (—[b])	381 (100)	335 (1)
Z–Pro–Ala–Ala–Ala–OEt (**5**)	490	443 (—[b])	381 (100)	335 (—[b])
Z–Ala–Ala–Pro–Pro–OEt (**6**)	516	469 (—[b])	407 (100)	361 (1)
Z–Ala–Pro–Ala–Pro–OEt (**7**)	516	469 (3)	407 (100)	361 (16)
Z–Pro–Ala–Ala–Pro–OEt (**8**)	516	469 (1)	407 (100)	361 (2)
Z–Ala–Pro–Pro–Ala–OEt (**9**)	516	469 (1)	407 (100)	361 (1)
Z–Pro–Ala–Pro–Ala–OEt (**10**)	516	469 (1)	407 (100)	361 (5)
Z–Pro–Pro–Ala–Ala–OEt (**11**)	516	469 (100)	407 (13)	361 (—[b])
Z–Ala–Pro–Pro–Pro–OEt (**12**)	542	495 (3)	433 (100)	387 (33)
Z–Pro–Ala–Pro–Pro–OEt (**13**)	542	495 (3)	433 (100)	387 (9)
Z–Pro–Pro–Ala–Pro–OEt (**14**)	542	495 (100)	433 (1)	387 (—[b])
Z–Pro–Pro–Pro–Ala–OEt (**15**)	542	495 (100)	433 (3)	387 (—[b])
Z–Pro–Pro–Pro–Pro–OEt (**16**)	568	521 (100)	459 (25)	413 (11)

a) Values given are *m/z* with relative intensities in parentheses. The latter are the values of the relative intensities when the ion with the highest abundance was set to the base peak. *b*) These fragment ions were not detected.

Table 2. Positive-Ion FAB Tandem Mass Spectra of the $[M+H]^+$ Ions for the *N*-Z-Protected Tetrapeptide Ethyl Esters Containing Pro and Ala[a]

Compound	M.W.	$[M+H-46]^+$	$[M+H-108]^+$	b_3	y_3	b_2	y_2	b_1	y_1
Z–Ala–Ala–Ala–Ala–OEt (**1**)	464	419 (50)	357 (—[b])	348 (100)	260 (3)	277 (50)	189 (33)	206 (5)	118 (7)
Z–Ala–Ala–Ala–Pro–OEt (**2**)	490	445 (1)	383 (—[b])	348 (100)	286 (—[b])	277 (54)	215 (23)	206 (4)	144 (75)
Z–Ala–Ala–Pro–Ala–OEt (**3**)	490	445 (9)	383 (—[b])	374 (13)	286 (3)	277 (5)	215 (100)	206 (3)	118 (—[b])
Z–Ala–Pro–Ala–Ala–OEt (**4**)	490	445 (60)	383 (5)	374 (100)	286 (34)	303 (52)	189 (—[b])	206 (—[b])	118 (2)
Z–Pro–Ala–Ala–Ala–OEt (**5**)	490	445 (51)	383 (—[b])	374 (100)	260 (—[b])	303 (69)	189 (22)	232 (6)	118 (4)
Z–Ala–Ala–Pro–Pro–OEt (**6**)	516	471 (—[b])	409 (—[b])	374 (21)	312 (2)	277 (5)	241 (100)	206 (2)	144 (4)
Z–Ala–Pro–Ala–Pro–OEt (**7**)	516	471 (2)	409 (6)	374 (100)	312 (10)	303 (45)	215 (2)	206 (—[b])	144 (46)
Z–Pro–Ala–Ala–Pro–OEt (**8**)	516	471 (1)	409 (—[b])	374 (100)	286 (—[b])	303 (40)	215 (14)	206 (4)	144 (34)
Z–Ala–Pro–Pro–Ala–OEt (**9**)	516	471 (20)	409 (7)	400 (45)	312 (92)	303 (100)	215 (11)	206 (3)	118 (—[b])
Z–Pro–Ala–Pro–Ala–OEt (**10**)	516	471 (6)	409 (—[b])	400 (14)	286 (—[b])	303 (15)	215 (100)	232 (5)	118 (—[b])
Z–Pro–Pro–Ala–Ala–OEt (**11**)	516	471 (78)	409 (12)	400 (100)	286 (2)	329 (80)	189 (—[b])	232 (15)	118 (2)
Z–Ala–Pro–Pro–Pro–OEt (**12**)	542	497 (2)	435 (11)	400 (61)	338 (100)	303 (54)	241 (33)	206 (2)	144 (3)
Z–Pro–Ala–Pro–Pro–OEt (**13**)	542	497 (—[b])	435 (—[b])	400 (16)	312 (—[b])	303 (9)	241 (100)	232 (3)	144 (5)
Z–Pro–Pro–Ala–Pro–OEt (**14**)	542	497 (—[b])	435 (3)	400 (100)	312 (—[b])	329 (36)	215 (—[b])	232 (6)	144 (18)
Z–Pro–Pro–Pro–Ala–OEt (**15**)	542	497 (12)	435 (18)	426 (24)	312 (2)	329 (100)	215 (2)	232 (22)	118 (—[b])
Z–Pro–Pro–Pro–Pro–OEt (**16**)	568	523 (3)	461 (55)	426 (100)	338 (5)	329 (98)	239 (6)	232 (29)	144 (3)

a, *b*) See Table 1.

detected for **15** and **16**, indicating that the $[M+H-108]^+$ ion was stabilized due to the existence of Pro near the Z-group.

CAD for $[M']^-$ Ions The CAD mass spectra for the $[M']^-$ ion for twelve *N*-Z-protected tetrapeptide derivatives were measured, in which this ion was observed in the negative-ion FAB mass spectra. (It was not detected at all for **11**, **14**, **15** or **16**.[6])

In the mass spectrum for **1**, the fragment ion at *m/z* 184 (c_2-H), was the base peak and many fragment ions were observed with fairly high abundance (Fig. 1a). The major fragment ions were at *m/z* 340 ($[355-CH_3]^-$), 327 ($[355-CO]^-$), 311 ($[355-CONH-H]^-$), 309 ($[355-C_2H_5OH]^-$), 284 ($[355-CONHCH(CH_3)]^-$), 282 ($[355-CO_2C_2H_5]^-$), 268 ($[355-CONHCH(CH_3)-CH_3-H]^-$), 213 ($x_2-2H$), 141 ($[Ala-Ala-H]^-$) and 113 ($c_1-H$). In the mass spectrum of **2** which contains Pro at the P_1 site, the fragment ion at *m/z* 167 ($[Ala-Pro-H]^-$) was the base peak and the cleavage pattern of this derivative was similar to that of **1** (Fig. 1b). However, in the spectra for **3**, **4** and **5**, the number and abundance of the fragment ions observed for **1** decreased (Fig. 1c, d and e). In addition, the ion at *m/z* 213 (x_2-2H) was the base peak for both **4** and **5**, but significant difference was found in the ion's abundance of the ion at *m/z* 353 ($[381-CO]^-$), 335 ($[381-C_2H_5OH]^-$), 284 (x_3-2H), 210 (c_2-H) and 139 (c_1-H). These results indicate that the decomposition of the $[M']^-$ ion in the gas phase was greatly influenced by the existence of one Pro in the tetrapeptide derivatives. This may be mainly due to the conformational difference of the tetrapeptide derivatives containing one Pro.

Figure 2 shows the CAD mass spectra for the peptides containing two Pro, in which the number of fragment ions

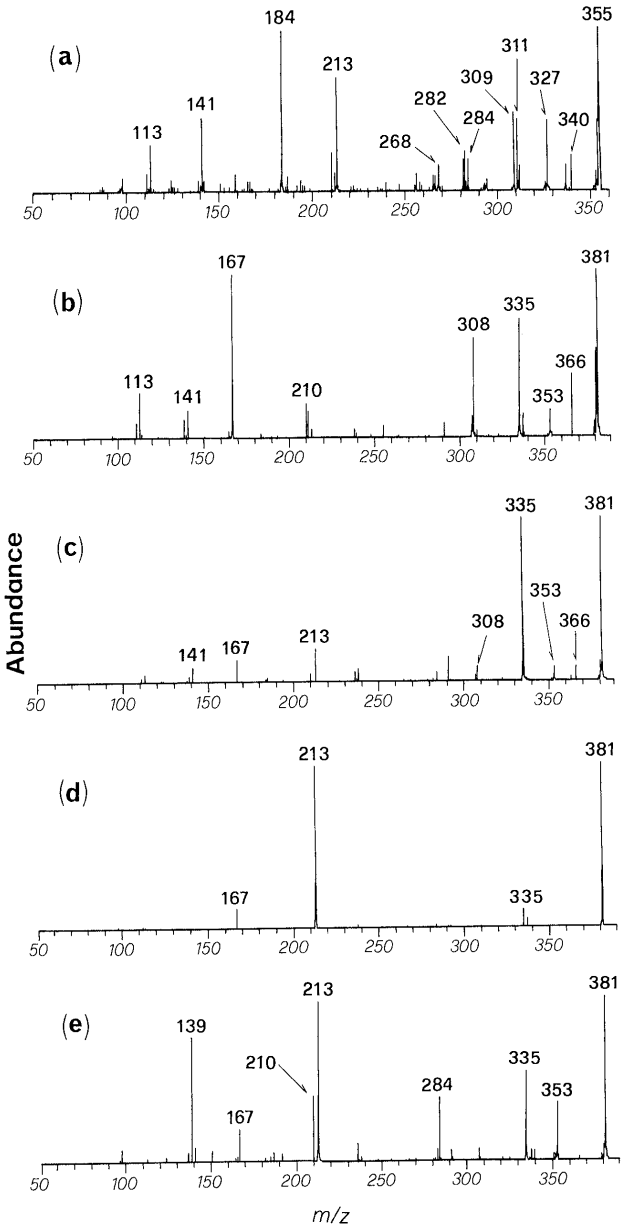

Fig. 1. FAB CAD Tandem Mass Spectra of the $[M']^-$ Ions for (a) Z-Ala-Ala-Ala-Ala-OEt (**1**), (b) Z-Ala-Ala-Ala-Pro-OEt (**2**), (c) Z-Ala-Ala-Pro-Ala-OEt (**3**), (d) Z-Ala-Pro-Ala-Ala-OEt (**4**) and (e) Z-Pro-Ala-Ala-Ala-OEt (**5**)

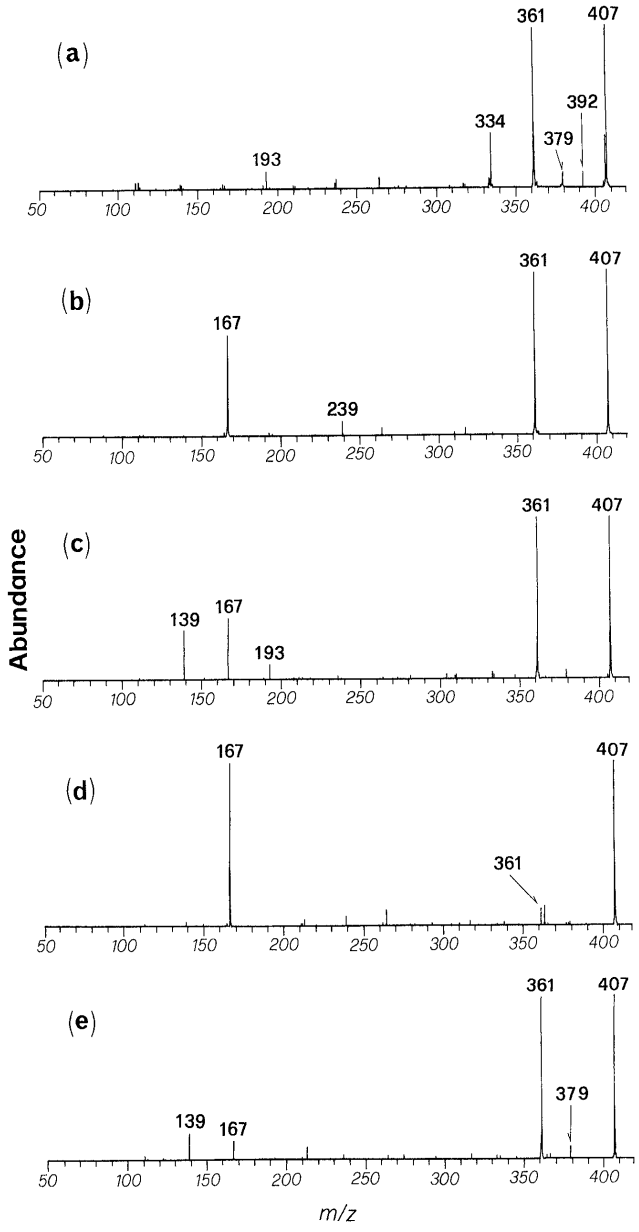

Fig. 2. FAB CAD Tandem Mass Spectra of the $[M']^-$ Ions for (a) Z-Ala-Ala-Pro-Pro-OEt (**6**), (b) Z-Ala-Pro-Ala-Pro-OEt (**7**), (c) Z-Pro-Ala-Ala-Pro-OEt (**8**), (d) Z-Ala-Pro-Pro-Ala-OEt (**9**) and (e) Z-Pro-Ala-Pro-Ala-OEt (**10**)

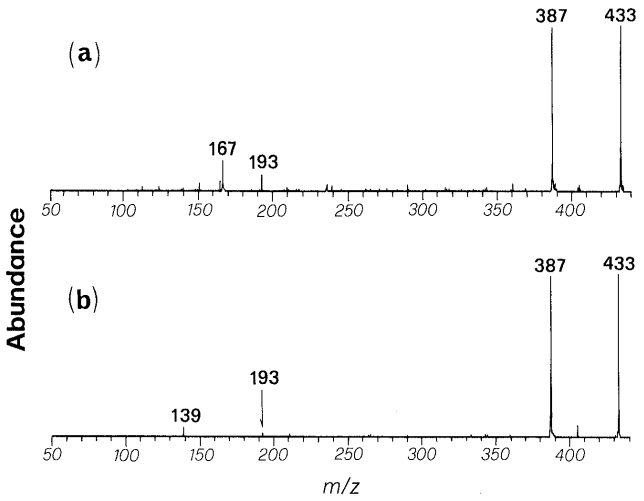

Fig. 3. FAB CAD Tandem Mass Spectra of the $[M']^-$ Ions for (a) Z–Ala–Pro–Pro–Pro–OEt (**12**) and (b) Z–Pro–Ala–Pro–Pro–OEt (**13**)

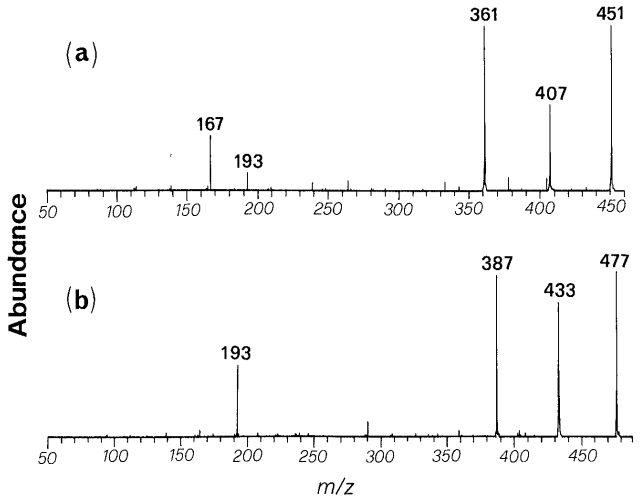

Fig. 4. FAB CAD Tandem Mass Spectra of the $[M-91]^-$ Ions for (a) Z–Ala–Pro–Pro–Pro–OEt (**12**) and (b) Z–Pro–Pro–Pro–Pro–OEt (**16**)

was lower than that for the peptides containing one Pro. The cleavages at both the amino- and carboxyl-terminals of the peptide derivatives thus tend not to occur with increase in the number of Pro in the peptides. The fragment ion at *m/z* 167 ($[Ala–Pro-H]^-$) was the base peak for **9**, while the ion at *m/z* 361 ($[407-C_2H_5OH]^-$) was the base peak for the other four peptides containing two Pro. However, significant difference was found in the abundance of the ions at *m/z* 193 ($[Pro–Pro-H]^-$), 167 and 139 for these four peptides.

In the tetrapeptides containing three Pro (**12**, **13**), the ion at *m/z* 387 ($[433-C_2H_5OH]^-$) was the base peak for these two peptides (Fig. 3a and b), but the abundance of the fragment ions at *m/z* 193, 167 and 139 for **12** differed from those for **13**.

The cleavage patterns for the $[M']^-$ ions in the gas phase were therefore very different depending on the number and positions of Pro in the tetrapeptide derivatives. It is likely that the conformational difference in these derivatives due to the existence of Pro plays an important role in determining the decompositions of the $[M']^-$ ion in the tetrapeptides in the gas phase.

CAD for $[M-91]^-$ Ions In the negative-ion FAB mass spectra for the tetrapeptides containing Pro and Ala, the $[M-91]^-$ ion was the base peak for **12** and **16**, but the abundance of this ion was low for the other peptides. Furthermore, the $[M-135]^-$ ion was not detected at all for **12** and **16**.[6] We reported similar results concerning the tripeptide derivatives, Z–Ala–Pro–Pro–OEt and Z–Pro–Pro–Pro–OEt in the negative-ion FAB-MS.[4] These results indicate that the $[M-91]^-$ ion was the base peak when the amino acid sequences were all Pro, or they are all Pro except for those adjacent to the Z-group. The $[M-135]^-$ ion was not produced by decarboxylation from the $[M-91]^-$ ion, because the latter ion formed from **12** and **16** existed as a stable fragment ion. The CAD mass spectra of the $[M-91]^-$ ion for **12** and **16** were then examined to confirm the observations described above.

Figure 4a shows the CAD mass spectra of the $[M-91]^-$ ion for **12**. The ion at *m/z* 361 ($[M-91-44-46]^-$) due to the loss of the carbon dioxide and the ethanol from the $[M-91]^-$ ion was the base peak, and the ion at *m/z* 407 ($[M-135]^-$) due to the loss of the carbon dioxide from the precursor ion was also observed in high abundance. The fragment ion at *m/z* 193 ($[Pro–Pro-H]^-$) and at *m/z* 167 ($[Ala–Pro-H]^-$) were also observed. A similar cleavage pattern was obtained in the CAD mass spectra of the $[M-91]^-$ ion for **16** (Fig. 4b). These results thus indicate that the $[M-135]^-$ ion was produced by decarboxylation from the $[M-91]^-$ ion in the gas phase in the tetrapeptide derivatives. We reported similar results for the *N*-Z-protected tripeptide derivatives.[5] It is therefore thought that decarboxylation of the $[M-91]^-$ ion did not occur in the solution phase for the tri- and tetrapeptide derivatives containing all Pro or Pro except for the site adjacent to the Z-group.

Our results suggest that the fragmentations of the deprotonated molecule and the fragment ions produced from the *N*-Z-protected tetrapeptide derivatives containing Pro were different depending on the content of Pro in the peptides. It is therefore thought that the fragmentations of the tetrapeptide derivatives containing Pro were controlled by Pro in the gas phase.

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