NOTE

Differentiation of Diastereomeric Tertiarybutoxycarbonylprolylproline Ethyl Esters by Collision-Induced Dissociation of Sodium Adduct Ions in Fast Atom Bombardment Mass Spectrometry

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The formation and the fragmentation of the sodium adduct ions for tertiarybutoxycarbonyl-L-prolyl-L-proline ethyl ester (Boc-L-Pro-L-Pro-OEt) were compared with those for Boc-D-Pro-L-Pro-OEt on the addition of sodium chloride in positive-ion fast atom bombardment (FAB) mass spectrometry. The abundance of the $[M+Na]^+$ ion was about 2 times lower than that of the $[M+H]^+$ ion on the addition of sodium chloride in FAB mass spectrum for Boc-L-Pro-L-Pro-OEt, while the former was almost same as the latter in the spectrum for Boc-D-Pro-L-Pro-OEt, suggesting that Boc-D-Pro-L-Pro-OEt has a stronger Na^+ ion affinity than Boc-L-Pro-L-Pro-OEt. On the other hand, in the collision-induced dissociation (CID) mass spectra of the $[M+Na]^+$ ions, the abundance of the $[M+Na-C(CH_3)_3+H]^+$ ion, which is due to the loss of a tertiarybutyl group from the $[M+Na]^+$ ion for Boc-D-Pro-L-Pro-OEt, was higher than that for Boc-L-Pro-L-Pro-OEt. These results indicate that Boc-L-Pro-L-Pro-OEt was distinguished from Boc-D-Pro-L-Pro-OEt by the CID mass spectra of the $[M+Na]^+$ ions, and the difference of the cleavage patterns between Boc-L-Pro-L-Pro-OEt and Boc-D-Pro-L-Pro-OEt when the carboxyl-terminals were protected with ethyl ester, was smaller than that between tertiarybutoxycarbonyl-L-prolyl-L-proline (Boc-L-Pro-L-Pro) and Boc-D-Pro-L-Pro when they were free reported previously.¹⁾

1. Introduction

It was difficult to discriminate between a pair of diastereomeric peptides or their derivatives by mass spectrometry before the 1990's. A few papers were reported concerning the differentiation between a pair of diastereomeric di- or tripeptide derivatives after the development of fast atom bombardment (FAB) mass spectrometry.^{2), 3)} Tao and Cooks have recently reported the differentiation and enantiomeric quantification of dipeptides based on gas-phase dissociation of copper (II)-based complexes by electrospray ionization mass spectrometry.⁴⁾

We have reported that tertiarybutoxycarbonyl-L-prolyl-L-proline (Boc-L-Pro-L-Pro) is distinguished from Boc-D-Pro-L-Pro by the difference in the formation of the $[M+Na]^+$ ions and by collision-induced dissociation (CID) of these ions on the addition of sodium chloride in positive-ion FAB mass spectrometry.¹⁾ Our results also suggest difference in affinity for Na^+ ions and in the sodium ion binding sites between these diastereomers.

The chemical structure of the carboxyl-terminal (C-terminal) in Boc-protected prolylproline is important in the interaction of a Na^+ ion with these peptides. Then, a pair of diastereomeric tertiarybutoxycarbonylprolyl-

proline ethyl ester, in which the C-terminals were protected with ethyl ester, was synthesized and the cleavage patterns for them were compared with those for the diastereomeric tertiarybutoxycarbonylprolylproline.

In this paper, we report the difference in the formation of the $[M+Na]^+$ ions in positive-ion FAB mass spectrometry and in CID of the $[M+Na]^+$ ions between Boc-L-Pro-L-Pro-OEt and Boc-D-Pro-L-Pro-OEt on the addition of sodium chloride, and discuss the effect of the C-terminals of these peptides on the cleavage patterns of the $[M+Na]^+$ ions.

2. Experimental

2.1 Materials and methods

Boc-L-Pro and Boc-D-Pro were obtained from the Protein Research Foundation (Osaka, Japan). The ethyl ester hydrochloride of L-Pro (L-Pro-OEt HCl) was prepared using thionyl chloride and absolute ethanol.⁵⁾ All chemicals were of either analytical or reagent grade. The purity of each compound was checked by thin-layer chromatography on Kiesel-gel plates 60 PF₂₅₄ (Merck, Darmstadt, Germany) with chloroform-methanol-ethyl acetate (4:3:1, v/v) and chloroform-ethyl acetate (10:1, v/v) as developing systems. All melting points were uncorrected. The chemical structures of the synthesized compounds were confirmed with the aid of FAB mass spectrometry. ¹H-NMR spectra were measured at 500 MHz with a Varian UNITY-plus 500 system. The results of elemental analyses obtained for C, H, and N were within $\pm 0.3\%$ of the theoretical values.

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2.2 Syntheses of Boc-L-Pro-L-Pro-OEt and Boc-D-Pro-L-Pro-OEt

Boc-L-Pro-L-Pro-OEt was synthesized by the method reported previously.¹⁾ This product was recrystallized from dry ether. mp 71–72°C, *Anal.* Calcd for C₁₇H₂₈N₂O₅: C, 59.98; H, 8.29; N, 8.23. Found: C, 60.11; H, 8.26; N, 8.26. Positive-ion FAB mass spectrometry: m/z 341 ([M+H]⁺). In the ¹H-NMR, the positions of protons in Boc-L-Pro-L-Pro-OEt were indicated in Chart 1 using the alphabet (a–o). ¹H-NMR (DMSO-*d*₆) δ: 1.15 (3H, t, *J* = 7.1 Hz, H-o), 1.29–1.37 (9H, H-a), 1.70–1.86 (4H, m, H-e, f, j, k), 1.89–1.95 (2H, m, H-e, j), 2.08–2.23 (2H, m, H-f, k), 3.26–3.34 (2H, m, H-d, i), 3.47–3.66 (2H, m, H-d, i), 3.98–4.11 (2H, m, H-n), 4.29 (1H, dd, *J* = 8.5, 4.5 Hz, H-l), 4.37–4.43 (1H, m, dd, H-g).

Boc-D-Pro-L-Pro-OEt was prepared as described above for Boc-L-Pro-L-Pro-OEt. mp 84–85°C, *Anal.* Calcd for C₁₇H₂₈N₂O₅: C, 59.98; H, 8.29; N, 8.23. Found: C, 60.11; H, 8.27; N, 8.38. Positive-ion FAB mass spectrometry: m/z 341 ([M+H]⁺). ¹H-NMR (DMSO-*d*₆) δ: 1.17 (3H, t, *J* = 7.1 Hz, H-o), 1.30–1.48 (9H, H-a), 1.68–1.85 (4H, m, H-e, f, j, k), 1.88–1.99 (2H, m, H-e, j), 2.06–2.20 (2H, m, H-f, k), 3.24–3.35 (2H, m, H-d, i), 3.38–3.64 (2H, m, H-d, i), 3.99–4.18 (2H, m, H-n), 4.20 (1H, dd, *J* = 8.5, 4.0 Hz, H-l), 4.43 (1H, dd, *J* = 8.8, 3.0 Hz, H-g).

2.3 Measurement conditions for FAB and tandem mass spectrometry

All mass spectra were acquired with a JEOL SX/SX 102A tandem mass spectrometer of BEBE geometry, which was controlled by a JEOL DA-7000 data system (Tokyo, Japan). The positive-ion FAB mass spectra were obtained using only the first spectrometer. The samples were diluted in methanol at a concentration of 1 μg/μL. The solution (1 μL) was then subjected to analysis. *m*-Nitrobenzylalcohol (*m*-NBA) and *m*-NBA saturated with sodium chloride were used as a matrix. The ions were produced by bombardment with a neutral xenon atom at 5 kV. The mass range (m/z 1–1,000) was scanned for 5 s under an ion source accelerating potential of 10 kV, and the averaged peak intensities were recorded in decade scans. The molecular-related ions generated by FAB mass spectrometry were selected as precursor ions, and were then 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 CID spectra.

3. Results and Discussion

3.1 FAB and CID Mass Spectra for Boc-L-Pro-L-Pro-OEt and Boc-D-Pro-L-Pro-OEt

A positive-ion FAB mass spectrum for Boc-L-Pro-L-Pro-OEt was compared with that for Boc-D-Pro-L-Pro-OEt. Table 1 shows the mass spectrum for Boc-L-Pro-L-Pro-OEt. The fragment ion at m/z 241 ([M+H–Boc+H]⁺) due to the loss of the Boc group from the [M+H]⁺ ion was the base peak and a protonated molecule ion at m/z 341 was observed with high abundance. In addition, by the cleavage of the Boc group, an ion at m/z 285 due to the loss of a tertiary(*tert*)-butyl group and an ion at m/z 267 due to the loss of *tert*-butoxy group from the [M+H]⁺ ion were observed with fairly high abun-

Table 1. Relative Abundance (%) of the [M+H]⁺, [M+Na]⁺ and Fragment Ions for Boc-L-Pro-L-Pro-OEt and Boc-D-Pro-L-Pro-OEt in Positive-Ion FAB/MS

m/z	Compound	
	Boc-L-Pro-L-Pro-OEt	Boc-D-Pro-L-Pro-OEt
363 ([M+Na] ⁺)	2	2
341 ([M+H] ⁺)	62	41
285	15	17
267	27	26
241	100	100
144	23	25
114	24	20
70	45	42
57	17	14

Chart 1

dance. The abundance of the ion at m/z 363 ([M+Na]⁺) was very low. The other major fragment ions were at m/z 144 ([N–CH₂CH₂CH₂CHCO₂C₂H₅+2H]⁺), 114 ([O(CO)NCH₂CH₂CH₂CH+H]⁺), 70 ([N–CH₂CH₂CH₂CH+H]⁺) and 57 ([C(CH₃)₃]⁺). In addition, in the CID mass spectrum of the [M+H]⁺ ion for Boc-L-Pro-L-Pro-OEt, an ion at m/z 285 was the base peak and an ion at m/z 241 was observed with fairly high abundance. These results indicate that these fragment ions were formed from the [M+H]⁺ ion for this compound. On the other hand, the FAB and CID mass spectra for Boc-D-Pro-L-Pro-OEt was similar to those for Boc-L-Pro-L-Pro-OEt (Table 1), indicating that no significant difference was found in the FAB and tandem mass spectra of the [M+H]⁺ ions between these two diastereomers.

3.2 FAB and CID mass spectra for Boc-L-Pro-L-Pro-OEt and Boc-D-Pro-L-Pro-OEt on the addition of sodium ion

The formation of the [M+Na]⁺ ion for Boc-L-Pro-L-Pro-OEt was compared with that for Boc-D-Pro-L-Pro-OEt on the addition of sodium chloride. In the positive-ion FAB mass spectrum for Boc-L-Pro-L-Pro-OEt, the ion at m/z 241 was the base peak and the abundance of the ion at m/z 341 ([M+H]⁺) was about twice as high as that of the ion at m/z 363 ([M+Na]⁺) (Fig. 1(a)). The ion at m/z 263, which is assigned as a [241+Na–H]⁺ ion, was also observed. On the other hand, in the spectrum for Boc-D-Pro-L-Pro-OEt, the [M+Na]⁺ ion was observed with almost the same abundance as that of the [M+H]⁺ ion (Fig. 1(b)). The abundance of the fragment ion at m/z 263 was about twice as high as that for Boc-L-Pro-L-Pro-OEt. These results imply that the affinity of the Na⁺ ion for Boc-D-Pro-L-Pro-OEt is

Fig. 1. Positive-ion FAB mass spectra for (a) Boc-L-Pro-L-Pro-OEt and (b) Boc-D-Pro-L-Pro-OEt on the addition of sodium chloride.

Fig. 2. FAB CID tandem mass spectra of the $[M+Na]^+$ ions for (a) Boc-L-Pro-L-Pro-OEt and (b) Boc-D-Pro-L-Pro-OEt.

stronger than that for Boc-L-Pro-L-Pro-OEt. We have reported that the $[M+Na]^+$ ion was the base peak and the abundance of the $[M+H]^+$ ion was about 20% in the FAB mass spectrum for Boc-D-Pro-L-Pro on the addition of sodium chloride, while it was almost same as that of the $[M+H]^+$ ion for Boc-L-Pro-L-Pro.¹⁾ It is therefore thought that the difference of the formation of the $[M+Na]^+$ ion between Boc-L-Pro-L-Pro-OEt and Boc-D-Pro-L-Pro-OEt when the C-terminals were protected with ethyl ester, was smaller than that between Boc-L-Pro-L-Pro and Boc-D-Pro-L-Pro when they were free. Our results imply that the chemical structure of the C-terminals of the dipeptide derivatives have an effect on the interaction between a Na^+ ion and the diastereomeric prolylproline derivatives.

In addition, in the CID mass spectrum of the $[M+Na]^+$ ion for Boc-D-Pro-L-Pro-OEt, the ion at m/z 307 ($[M+Na-C(CH_3)_3+H]^+$), due to the loss of the *tert*-butyl group from the $[M+Na]^+$ ion, was observed in fairly high abundance, while in the spectrum for Boc-L-Pro-L-Pro-OEt, the abundance of this ion was very low (Figs. 2(a) and (b)). These results indicate that the elimination of the *tert*-butyl group from the $[M+Na]^+$ ion for Boc-D-Pro-L-Pro-OEt is easier than that for Boc-L-Pro-L-Pro-OEt in the gas phase.

4. Conclusion

Boc-L-Pro-L-Pro-OEt was discriminated from Boc-D-Pro-L-Pro-OEt by the CID of the sodium adduct ions in positive-ion FAB mass spectrometry. The difference of the cleavage patterns of the sodium adduct ions between Boc-L-Pro-L-Pro-OEt and Boc-D-Pro-L-Pro-OEt when the C-terminals of the dipeptides were protected with ethyl esters, was smaller than that between Boc-L-Pro-L-Pro and Boc-D-Pro-L-Pro when they were free.

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