BIOLOGICAL MASS SPECTROMETRY, VOL. 22, 703–706 (1993)

Fragmentations of Flurbiprofen-L-Arginine Diastereomers in Negative Ion Fast Atom Bombardment Mass Spectrometry

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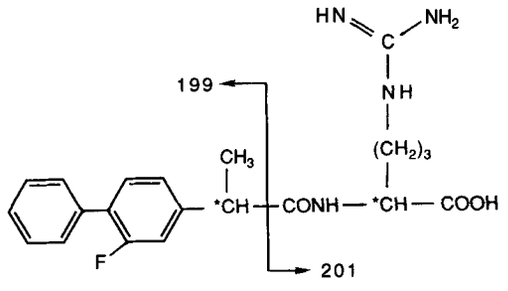
Fragmentations between flurbiprofen-L-arginine diastereomers, flurbiprofen(*S*)-L-arginine and flurbiprofen (*R*)-L-arginine, were examined in both fast atom bombardment (FAB) and field desorption mass spectrometry. In negative ion FAB mass spectrometry using glycerol as a matrix, the cleavage pattern for flurbiprofen(*S*)-L-arginine was very different from that for flurbiprofen(*R*)-L-arginine, and the former changed depending on the amount of xenon collisional energy. In positive ion FAB and field desorption mass spectrometry, no significant differences were found between the cleavage patterns of these two diastereomers. Our results indicate that the selection of the mode of ionization, the matrix and the FAB gun voltage were important in order to distinguish accurately between flurbiprofen-L-arginine diastereomers.

INTRODUCTION

It is well known that fast atom Bombardment (FAB) and field desorption (FD) mass spectrometry are useful for structural and analytical studies of thermolabile and non-volatile organic compounds. It has been generally assumed that it has been difficult to discriminate between the various enantiomeric compounds by mass spectrometry up until a few years ago. However, several recent reports have been made concerning the differentiation of steric isomers modified both chemically and physically by FAB mass spectrometry in combination with tandem mass spectrometry,[1–3] FAB mass spectrometry[4] and electron impact ionization.[5] Discrimination between the steric isomers by mass spectrometry promises to be very useful.

We have previously reported that significant differences were observed in negative ion FAB mass spectrometry among the intensities of the three fragment ions, $[M-91]^-$ (loss of the benzyl group), $[M-109]^-$ (loss of the benzyloxy group followed by dehydrogenation) and $[M-135]^-$ (loss of the benzyloxycarbonyl (Z) group), formed by the cleavage of the Z-group depending on the number and positions of the prolyl residues in the Z-protected tripeptide esters.[6,7] Similar observations were also made on fragmentations of the peptide derivatives in FD mass spectrometry.[8] Our results imply that the fragmentations of the Z-protected peptide derivatives depend on the conformational differences in the derivatives due to the existence of proline, while the cleavage of the Z-group moiety reflects the conformational difference of the peptide derivatives. This fact therefore suggests the possibility that various steric isomers could be distinguished by the fragmentations in mass spectrometry.

Flurbiprofen (FP) is a non-steroidal anti-inflammatory drug belonging to the group of 2-arylpropionic acids having a chiral centre at the α-carbon atom. This drug is used clinically as a racemate, although only the *S*-enantiomer possesses significant anti-inflammatory activity. To develop the optically active water-soluble prodrug for FP, we synthesized the racemic flurbiprofen-L-arginine (FP-Arg-OH) and separated the diastereomers of this compound by high-performance liquid chromatography (HPLC). When we confirmed the chemical structures of these diastereomers by mass spectrometry, we noticed that the fragmentations for only the *S*-enantiomer were affected by the xenon collisional energy (FAB gun voltage) in negative ion FAB mass spectrometry using glycerol as a matrix. We therefore examined the effect of several matrices and xenon collisional energy on the fragmentations for these diastereomers. As a result, we determined that the selection of the matrix and FAB gun voltage was important for discriminating between FP-Arg-OH diastereomers in negative ion FAB mass spectrometry.



FP-Arg-OH

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1052–9306/93/120703–04 $07.00

Received 24 May 1993
Revised 29 July 1993

EXPERIMENTAL

Materials

Racemic FP was purchased from Sigma (St. Louis, Missouri, USA). Bovine trypsin (lyophilized, salt-free; lot 10460524-66) was purchased from Boehringer Mannheim-Yamanouchi Co. (Tokyo, Japan). Carboxypeptidase Y (lot no. 102) was obtained from Takara-Shuzou Co. (Kyoto, Japan). L-Arginine ethyl ester (Arg-OEt) dihydrochloride was prepared by using thionyl chloride and absolute ethanol. The purity of each compound was checked by thin-layer chromatography on Kieselgel 60 PF_{254} plates (E. Merck, Darmstadt, Germany) with chloroform–methanol–ethyl acetate (4:3:1, v/v), and 1-butanol–acetic acid–water (4:1:1, v/v) as developing systems. The chemical structures of the synthetic compounds were determined with the aid of FAB and FD mass spectrometry. The results of the elemental analysis obtained for C, H and N were within $\pm 0.3\%$ of the theoretical values. All other chemicals were of analytical or reagent grades.

Preparation of FP-Arg-OH diastereomers

Racemic FP-Arg-OEt was synthesized by using dicyclohexylcarbodiimide and 1-hydroxybenzotriazole. The separation of the diastereomers of this compound was performed by HPLC. The HPLC system (Shimadzu LC 6A) consisted of an ultraviolet (UV) spectrometric detector (SPD-6B), chromatopac (C-R6A) and system controller (SCL-6B) (Kyoto, Japan). The UV detector was set at 280 nm. The column was Chemcopak C18 (250 × 10 mm i.d.). The mobile phase was methanol–acetonitrile–0.1 M sodium acetate (pH 7.4) (48:32:20, v/v) and the flow rate was 2.5 ml min^{-1} at 40 °C. The retention times of FP(*R*)-Arg-OEt and FP(*S*)-Arg-OEt were 15.8 min and 18.4 min, respectively. The ethyl ester of each diastereomer separated by HPLC was hydrolysed by bovine trypsin (8.59×10^{-9} M) in Tris-HCl buffer (0.05 M, pH 7.85) at 25 °C. FP(*R*)-Arg-OH and FP(*S*)-Arg-OH were purified by using a Dowex 50 (NH_4^+ form) column. To ascertain the optical purity of FP, each diastereomer was hydrolysed by carboxypeptidase Y in Tris-HCl buffer (0.025 M, pH 7.65) containing 0.1 M NaCl at 37 °C, and the optical purities of FP released by this enzyme were checked by HPLC (Sumichiral OA-2500). The optical purities for FP(*R*) and FP(*S*) were found to be more than 99%. The exact method of preparing FP-Arg-OH distereomers will be reported in detail elsewhere.

Measurement conditions for FAB mass spectrometry

The mass spectrometer employed was a JEOL JMS-DX 300 double-focusing model equipped with a FAB ion source and was interfaced with a JEOL JMA-3000 data system (Tokyo, Japan). A methanol solution of the sample was mixed with the matrix on a stainless steel target and subjected to FAB mass spectrometry. As a matrix, glycerol, thioglycerol, diethanolamine, triethanolamine and magic bullet were used for both positive and negative ion modes, while *m*-nitrobenzyl alcohol was used for the positive ion mode and triethylene glycol for negative ion mode. The target was bombarded with beams of neutral xenon atoms accelerated to an energy of 4–6 kV. The ions produced in this process were accelerated with a voltage of 3 kV and then analysed by scanning the mass range from *m/z* 1 to 1000 in 5 s.

Measurement conditions for FD mass spectrometry

Ion source accelerating potential was 3 kV for the field anode and −5 kV for the slotted cathode plate. A methanol solution of the sample was applied to the carbon emitter. The emitter heating current (e.h.c.) was manipulated between 15 and 22 mA, and the optimum e.h.c. for the detection of $[M + H]^+$ ions was between 19 and 20 mA.

RESULTS AND DISCUSSION

Negative ion FAB mass spectra for FP(*S*)-Arg-OH and FP(*R*)-Arg-OH

The characteristic fragmentations depending on the xenon collisional energy were observed in the negative ion FAB mass spectra for FP(*S*)-Arg-OH, when glycerol was used as a matrix. Figure 1 shows the negative ion FAB mass spectra for FP(*S*)-Arg-OH, in which the FAB gun voltage was changed from 4 to 6 kV. When the FAB gun voltage was 4 kV, a fragment ion at *m/z* 199, which is assigned as $[C_6H_5{-}C_6H_3F{-}CH(CH_3)]^-$, was the base peak, and a molecular ion $[M - H]^-$ at *m/z* 399 was observed with a strong intensity (67%). As the FAB gun voltage was increased to 5 kV, the intensity of the ion at *m/z* 199 was decreased, while that of the $[M - H]^-$ ion was increased and this ion became the base peak (Fig. 1(b)). At 6 kV, the molecular ion was the base peak and the intensity (57%) of the fragment ion at *m/z* 199 was further decreased (Fig. 1(c)). This phenomenon was observed to be independent of the sample concentrations. These results indicate that the fragmentations for FP(*S*)-Arg-OH depend on the xenon collisional energy and the molecular ion tends to be formed at the higher xenon collisional energy in negative ion FAB mass spectrometry. It is thus generally assumed that the formation of the fragment ions of molecules increases as the collisional energy increases. However, our observations indicated the opposite phenomenon and thus produced unexpected results. It is generally recognized that positive and negative ions derived from the molecules are sputtered from the surface of the matrix into the vapour state. It is therefore thought that the interactions of molecules with the matrix are a very important issue in the fragmentations of molecules in the sputtering process. It thus appears that the molecular ion may be stabilized due to the presence of glycerol at the higher collisional energy in the ionizing process of FP(*S*)-Arg-OH.

On the other hand, in the negative ion mass spectra for FP(*R*)-Arg-OH, the $[M - H]^-$ ion was the base peak and the fragment ion at *m/z* 199 was detected with

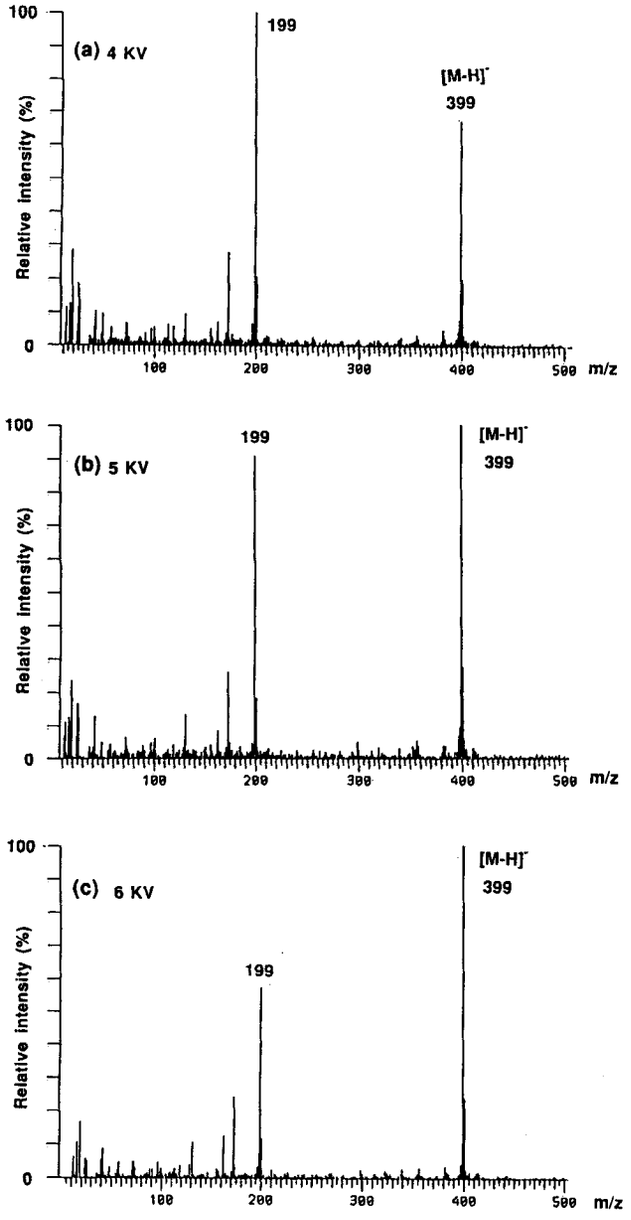

Figure 1. Negative ion FAB mass spectra for FP(*S*)-Arg-OH at (a) 4 kV, (b) 5 kV and (c) 6 kV of the FAB gun voltage. Glycerol was used as a matrix.

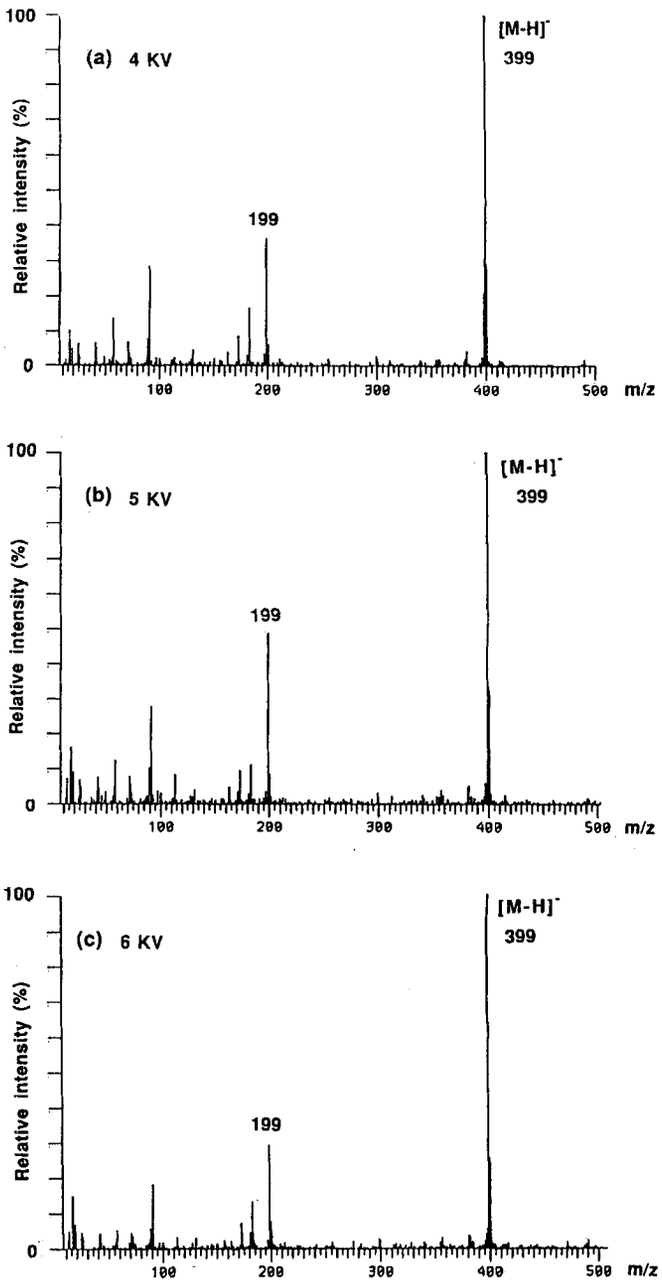

Figure 2. Negative ion FAB mass spectra for FP(*R*)-Arg-OH at (a) 4 kV, (b) 5 kV and (c) 6 kV of the FAB gun voltage. Glycerol was used as a matrix.

fairly strong intensity (37%) at 4 kV (Fig. 2(a)). However, the changes of the cleavage patterns depending on the xenon collisional energy were not observed in these mass spectra, described above for FP(*S*)-Arg-OH. In this case, the $[M - H]^-$ ion was the base peak and the intensities of the fragment ions at *m/z* 199 changed within the range of 30–50% at 4–6 kV of the FAB gun voltage (Fig. 2(a)–(c)). Then, when the FAB gun voltage was set at 4 kV and glycerol was used as a matrix, FP(*S*)-Arg-OH can be distinguished from FP(*R*)-Arg-OH by the cleavage patterns for these diastereomers in the negative ion mode. In such FAB mass conditions, the fragment ion at *m/z* 199 was the base peak in the spectrum for FP(*S*)-Arg-OH, while the molecular ion peak $[M - H]^-$ was the base peak for FP(*R*)-Arg-OH. The fragment ion at *m/z* 199 was formed by the fission of the asymmetric carbon–carbon bond in flurbiprofen. These results show that the formation of this fragment ion from FP(*S*)-Arg-OH was easier than that from FP(*R*)-Arg-OH in the presence of glycerol. It seems that glycerol promotes the fragmentations in FP(*S*)-Arg-OH in the FAB mass conditions. It is generally thought that the interaction of molecules with a matrix are different when the conformations of molecules are in a different state. Therefore, the interaction through the hydrogen bonding between FP(*S*)-Arg-OH and glycerol was different from that between FP(*R*)-Arg-OH and glycerol, and this gives rise to the difference in solvation of each diastereomer by glycerol. It is likely that this difference may have an effect on the fragmentations of each diastereomer in the sputtering process. Furthermore, one cannot rule out the possibility that the orientational difference of the hydropho-

bic biphenyl group which is bound to the asymmetric carbon in flurbiprofen may have an influence on the fragmentations of the two diastereomers.

To examine the effect of the matrix on the interaction between each diastereomer and matrix in the solution phase, several matrices were used instead of glycerol as a matrix. When thioglycerol, diethanolamine, triethanolamine and triethylene glycol were used as matrices, the $[M - H]^-$ ion at m/z 399 was the base peak and a fragment ion at m/z 199 was observed with intensities of about 40–60% for these two diastereomers of FP-Arg-OH at 4 kV. Similar results were also obtained when the FAB gun voltage was changed to 5–6 kV. These results indicate that no difference between the cleavage patterns for two FP-Arg-OH diastereomers was found when matrices other than glycerol were used. The cleavage patterns reflecting the conformational difference between two FP-Arg-OH diastereomers were observed only when glycerol was used as a matrix. Our results showed that the selection of the matrix and the xenon collisional energy were very important in distinguishing between FP-Arg-OH diastereomers by the fragmentations in FAB mass spectrometry.

Positive ion FAB mass spectra for FP(*S*)-Arg-OH and FP(*R*)-Arg-OH

In the positive ion mode, glycerol, thioglycerol, *m*-nitrobenzyl alcohol, diethanolamine, triethanolamine and magic bullet were used as matrices. In the mass spectra for both FP(*S*)-Arg-OH and FP(*R*)-Arg-OH, the molecular ion $[M + H]^+$ at m/z 401 was the base peak and a fragment ion at m/z 199 was detected with fairly strong intensities (30–50%). No changes in the cleavage patterns between these two diastereomers were observed when the matrices and the FAB gun voltage were changed.

FD mass spectra for FP(*S*)-Arg-OH and FP(*R*)-Arg-OH

In the mass spectra for these diastereomers, the molecular ion $[M + H]^+$ at m/z 401 was the base peak and the dimer ion $[2M + H]^+$ at m/z 801 was observed with weak intensities (about 10–20%). In addition, no significant differences were observed between the cleavage patterns for these two FP-Arg-OH diastereomers.

CONCLUSIONS

A significant difference was found between the cleavage patterns for FP-Arg-OH diastereomers by using glycerol as a matrix and changing the xenon collisional energy. Our research implies that the selection of the matrix and the FAB gun voltage together were important for accurately distinguishing between FP-Arg-OH diastereomers.

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