Field Desorption Mass Spectrometry of Antibiotics

II. Peptide Antibiotics

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Field desorption mass spectra of peptide antibiotics of various kinds are presented. In each case, a M^+ or $(M+H)^+$ peak of the antibiotics is obtained. Fragmentations available for an analysis of amino acid sequence occur in straight-chain peptide antibiotics.

1. Introduction

Mass spectra of antibiotics have been studied less than mass spectra of many other classes of natural products. This is partly because many antibiotics are nonvolatile compounds of high molecular weight.

Field desorption mass spectra, having now become a routine procedure, lead us to expect wide-spread fields of application. The utilization of field desorption mass spectrometry (FDMS) in the study of antibiotic field has been reported¹⁻⁹. It can be seen in these reports that FDMS is the excellent method in determining molecular weights of a number of nonvolatile or thermally unstable antibiotics. Peptide and amino acid related antibiotics of various kinds have been isolated and some of them

used as chemotherapeutics or biochemical tools. Mass spectrometry is one of the most rapid and sensitive techniques available for deducing molecular structure. In recent years mass spectrometry has often been shown to be the method of choice for determining the amino acid composition and sequence of various peptides⁸⁻⁹. From the point of view of an analysis of fragmentation and detection of molecular ion, we have studied the FDMS of a number of underived peptide antibiotics which exhibit a wide range of structural variation and molecular weight.

Experimental

Antibiotics.

All antibiotics were homogeneous on thin-layer chromatography. They are as follows.

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Amino acid related antibiotics: acidomycin (1), actinoboline (2), azaserine (3), cycloserine (4), 6-diazo-5-oxo-L-norleucine (5), enteromycin (6), and primocarcin (7).

Peptide antibiotics: leupeptin Ac (8), althiomycin (9), pepstatin A (10), edeine B₁ (11), mikamycin B (12), etamycin (13), valinomycin (14), bassianolide (15) and actinomycin D (16).

Other peptide antibiotics: griseoviridin (17) and astechrome (18).

Apparatus.

The FD mass spectra were obtained by the use of a double focusing JEOL-O1SG-2 instrument fitted with a combined FD/FI/EI ion source and a JMA 2000 data analysis system.

Mass spectrometry.

The mass scales and instrument conditions must be changed according to molecular weight of compounds. Antibiotics were divided according to molecular weight into three groups, less than 1,000, between 1,000 and 1,200, and between 1,200 and 1,500.

In the first group, the mass scale was established by comparison with the EI spectrum of perfluorokerosene obtained under identical scanning conditions. Especially in the case of (11), (12), (13) and (15), because of their high molecular weight, the calibration was performed by using of both perfluorokerosene and n-heptyl-s-triazine. Instrument condition included: accelerating voltage of +10 KV; cathode voltage of -2 KV.

In the second group (valinomycin and astechrome), n-heptyl-s-triazine was used

as standard material for calibration and mass spectra were obtained under the following instrumental conditions: accelerating voltage of +6 KV; cathode voltage of -4 KV.

In the last group (actinomycin D), n-nonyl-s-triazine was used for the same purpose as mentioned above and mass spectra were obtained under the following conditions: accelerating voltage of +5 KV; cathode voltage of -4 KV. Scanning rate was 10 sec throughout the entire spectra. High temperature activated tungsten wire emitters were used and samples (approximately several μg) were applied to the emitter by means of glass capillary technique.

3. Results and discussion

(I) Amino acid related antibiotics.

It has been shown that the FD method is an excellent technique for the determination of molecular weight of amino acids. It can be seen in these reports that in the FD mass spectra of the α -amino acids a quasi-molecular ion QM^+ , $(\text{M}+1)^+$, instead of an M^+ was usually detected¹⁰⁻¹¹.

In order to compare with informations obtained from general amino acids, FD mass spectra of seven amino acid related antibiotics of various kinds were measured. The antibiotics examined are presented with the emitter currents at which the spectra were recorded followed by m/e values and relative intensities in Fig. 1.

From this figure, it is clear that the prominent $(\text{M}+1)^+$ or M^+ ions are given in all antibiotics at the best anode temperature (BAT) and, in similar manner as general amino acids, $(\text{M}+1)^+$ ions are

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Fig. 1. Emitter currents and relative intensities in FD mass spectra of amino acid related antibiotics

preferentially observed to M^+ in most cases. Although two antibiotics, (3) and (5), produced the fragment ions (m/e 129 and 127, respectively) due to loss of $COOH$ from the $(M+1)^+$, there were no significant fragment ions in other cases. These data show that FDMS is very useful for determining the molecular weight of wide variety of naturally occurring amino acids.

(II) Peptide antibiotics

It is common technique to all antibiotics that mass spectra are obtained for each 1 mA rise in emitter current after BAT.

Leupeptin Ac (8)

Leupeptins, in which arginal is contained as carboxy terminal amino acid, are a series of enzyme inhibitors. Fig. 2 shows the FD spectra and chemical structure of leupeptin Ac. Fig. 2c is the spectrum that was averaged of seven spectra which were obtained at each emitter current from 17 to 23 mA. At the BAT (Fig. 2a), 17 mA, the $(M+1)^+$ at m/e 427 is observed as base peak together with some fragment ions. At 21 mA (Fig. 2b) the abundance of $(M+1)^+$ ion is low, and the ion at m/e 241 provides the base peak, with the peak at m/e 286 being the second most intense.

Fig. 2. FD mass spectra of Leupeptin Ac

As can be seen in these spectra, a cluster of peak is centered at m/e 427 ($M+1^+$). In FDMS, mass peaks in the molecular ion region occur in clusters due to the addition of a hydrogen. In ions with high carbon count the $(M+1)^+$, $(M+2)^+$ and $(M+3)^+$ arise partially from ^{13}C contributions and partially from hydrogen transfer to the M^+ and/or $(M+1)^+$ ions. The addition of hydrogen is due to surface reactions at the anode¹²⁾. Less frequently peaks occur with hydrogen atoms abstracted from the ion during the surface reaction¹³⁾. As a consequence of these surface reactions, ion masses cannot be predicted with a certainty of more than plus or minus two mass units.

Peaks occurring at m/e 409($M+1-H_2O$),
399 ($397+2H$), 286 ($284+2H$), 269 ($268+H$)

and 241 in these spectra of leupeptin Ac may be related to the sequence of leupeptin Ac. The meaningful peaks were not observed in the region below m/e 150.

Althiomycin (9)

Althiomycin is a peptide antibiotic composed of novel amino acids. Fig. 3 shows the FD spectra and chemical structure of althiomycin. At the BAT, 13mA, the intensive (M+1)⁺ ion, m/e 440, was observed as base peak together with three fragment ions. The abundances of fragment ions increase with increasing emitter current (Fig. 3b and 3c). Sequence peaks due to cleavages in the backbone of the peptide occur at m/e 328(327+H), 310(312-2H), 300(299+H) and 282(284-2H). Other two fragment ions with moderate high intensity,

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Fig. 3. FD mass spectra of Althiomycin

Fig. 4. FD mass spectra of Pepstatin A

m/e 359 and 340, cannot be assigned.

Pepstatin A (10)

Fig. 4 shows the FD spectra and chemical structure of pepstatin A. At the BAT (Fig. 4a) the prominent $(M+1)^+$ ion, m/e 686, was appeared with the cluster ion $(M+Na)^+$, m/e 708. At 22mA, although the $(M+1)^+$ ion provides still base peak, many meaningful fragment ions are detected. Sequence peaks due to the cleavage of the peptide backbone occur at m/e 640 and 483. Further sequence peaks are assigned at m/e 597 (596+H), 527 (526+H), 441 (440+H) and 369 (368+H). Sequence ions assigned to fragments containing two abstracted protons occur at m/e 624 (626-2H) and 410 (412-2H).

Edeine B₁ (11)

Fig. 5 shows the FD spectra and chemical structure of edeine B₁. At the BAT (Fig. 5a), the $(M+1)^+$ ion at m/e 798 was observed as base peak with the cluster

ion, $(M+Na)^+$, at m/e 820. At 18.5 mA (Fig. 5b) the ion at m/e 756 provides base peak and three other fragment ions are observed. However, these are not the sequence peaks due to cleavage in the backbone of edeine B₁.

Mikamycin B (12), Etamycin (13)

Fig. 6 and 7 show the FD spectra and the chemical structure of mikamycin B and etamycin, respectively. They are similar cyclic peptide antibiotics. At the BAT (Fig. 6a and 7), both antibiotics gave the prominent $(M+1)^+$ ion at m/e 867 and 879, respectively. In etamycin such mass spectra as shown in Fig. 13 were obtained at more high emitter currents and fragmentations scarcely observed.

On the other hand, some reproducible fragment ions were observed at 18.5 mA in mikamycin B (Fig. 12b), but neither of them can be assigned to the sequence peaks.

Fig. 5. FD mass spectra of Edeine B₁

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Fig. 6. FD mass spectra of Mikamycin B

Fig. 7. FD mass spectrum of Etamycin

Valinomycin (14), Bassianolide (15)

Fig. 8 and 9 show the FD spectra at the BAT and chemical structure of valinomycin and bassianolide¹⁴, respectively. As can be seen from the structure of both antibiotics, they are cyclic depsipeptide with high molecular weight. It can be seen

in these figures that the intensive M^+ ions, m/e 1,110 and 908, were observed as base peak in both cases and the fragmentations scarcely occurred. This evidence means that cyclic peptide become to be thermally stable and less polar.

Fig. 8. FD mass spectrum of Valinomycin

Fig. 9. FD mass spectrum of Bassianolide

Fig. 10. FD mass spectra of Actinomycin D

Actinomycin D (16)

Fig. 10 shows the FD spectra and chemical structure of actinomycin D. Actinomycin D is an antibiotic which is constituted from two identical peptide lactone rings and the desepitido-actinomycin chromophore. At the BAT (Fig. 10a), the intensive (M+1)⁺ ion at m/e 1255 is observed as base peak. At 20mA (Fig. 10b) many fragment ions appeared, but neither of them can be assigned.

(III) Other peptide antibiotics.

Griseoviridin (17)

Fig. 11 shows the FD spectra and

chemical structure of griseoviridin. Griseoviridin possesses a novel cyclic structure. At the BAT the prominent M⁺ ion at m/e 477 is observed together with the cluster ion, (M+Na)⁺, at m/e 500 (Fig. 11a). Significant fragment ions were not obtained in all spectra measured at other different emitter current.

Astechrome (18)

Fig. 12 shows the FD spectrum and chemical structure of astechrome, which is a chelated fungal metabolite. The structure of astechrome has been estimated to be like as Fig. 12 by chemical studies¹⁰⁾. The

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Fig. 11. FD mass spectra of Griseoviridin

Fig. 12. FD mass spectrum of Astechrome

molecular ion was not observed by EIMS. However, FDMS give the prominent M^+ ion at m/e 1,111. This result leads to the conclusion that the estimated structure for astechrome is correct.

4. Conclusion

In mass spectral studies of peptides, it is the main purpose to determine the molecular weight and the amino acid sequence. The most severe obstacle in these studies of peptides has been the notoriously low volatility of these compounds. EI and CIMS of peptides are normally obtained from the chemically modified material, since the

compounds themselves usually show weak or no molecular ions.

From our data described here, it is clear that FDMS is a helpful technique in the peptide field. In the first place, from the spectra shown in Figs. 1–12, it should be emphasized that FDMS is the excellent method for determining the molecular weights of unmodified peptide and amino acid related antibiotics of various kinds. All cited antibiotics exhibited the intensive M^+ or $(M+1)^+$ as base peak at BAT. This typical pattern of FDMS is especially recognized in the cases of cyclic peptides and chelated compounds.

Recently mass spectrometry has been used as one of the standard techniques for amino acid sequence analysis of peptides. For this purpose various methods of ionization, EI or CI, have been applied and made to develop a general method. It was one of the purposes of this report to show that at appropriate anode temperatures the molecular ion could be obtained with sufficient fragment ions to allow in principle the sequencing of small peptides.

Sequence peaks in the EI mass spectra of peptides arise from ions formed by cleavages on either side of the carbonyl group of the peptide backbone⁽⁸⁾.

A similar fragmentation pattern is seen in the straight-chain peptide antibiotics, (8), (9) and (10), investigated by FDMS. It is also shown in our data that a large number of fragment ions were obtained including the portions of peptide which contained not the carboxy terminal but N-terminal amino acid. However, fragmentation of these peptides was insufficient to allow a sequence determination.

Furthermore, there are such undefined problems as the addition or abstraction of hydrogens in surface reaction in FDMS. In order to determine the amino acid sequence of peptides by FDMS, it is necessary to establish more certain general rule derived from the accumulation of many data. It is clear that FDMS is a good complement to EI and CIMS, therefore, combined use of these FDMS findings and informations available from other ionization methods such as EI and/or CI, will provide more effective and certain means for the sequence determination of peptides.

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Keywords

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