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FAST ATOM BOMBARDMENT : A NEW MASS SPECTROMETRIC METHOD FOR PEPTIDE SEQUENCE ANALYSIS

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ABSTRACT

We have studied a selection of peptides using a new mass spectrometric ionisation technique - fast atom bombardment (FAB). We define the fragmentation pathways observed and comment on the utility in sequence analysis. A simple acetylation experiment is shown to aid rapid sequence assignment.

INTRODUCTION

Protein sequence analysis remains an important and arduous task in many branches of biochemical research including enzymology and neurochemistry. The structure elucidation of biologically active peptides, the identification of post-synthetic modifications in proteins and structure/function correlations are a few examples demanding amino acid sequence analysis. Even where DNA sequencing is applicable, a rapid definition of partial protein sequence is invaluable in locating the reading frame or defining 'introns'.

The most widely used non-classical approach to protein and peptide sequencing has been the mass spectrometric analysis of N-acetyl N,O -permethyl peptides, coupled where necessary with a 'mixture analysis' approach to total

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Abbreviations

FAB: Fast atom bombardment.

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protein sequence (1). This strategy has proven successful in the structure elucidation of innumerable unknown peptides including the enkephalins (2), adipokinetic hormone (3), and proteins including dihydrofolate reductase and chloramphenicol transacetylase (4,5,6). The method has also proven invaluable in the identification of unusual structures; examples here include the identification of γ-COOH glumatic acid (Gla) in blood coagulation zymogens (7), and the structure elucidation of SRS-A a novel peptido-lipid released from the lungs of sensitised mammals on antigen challenge- a model of the asthma crisis (8).

All of the above work has involved making derivatives of the peptides, and particularly where unusual structural features were anticipated (e.g. the enkephalins, prothrombin and SRS-A) great care had to be taken to interpret the mass spectra and discount the possibility of modification of the basic structure upon derivative formation. Other problems arise because the electron impact mass spectra of peptide derivatives often do not extend to the molecular ion due to extensive fragmentation. This has prompted the study of peptides by the new "soft" ionisation techniques such as chemical ionisation and field desorption. More recently, a new soft ionisation method, fast atom bombardment (FAB), has been described and here we report research on the application of FAB to the study of peptide sequencing. We have examined the fragmentation pathways for a series of peptides (Table 1) representative of all the common amino acids. The results summarised below suggest an important role for this new technique in peptide sequence analysis. Further, we describe a simple method for aiding the rapid and unambiguous interpretation of the FAB mass spectra of unknown peptides.

MATERIALS AND METHODS

Free Peptides

Peptides (1-10 μg) were either natural products isolated during our studies on total protein sequence analysis or were synthetic commercially available samples. The latter were checked for purity by high voltage paper electrophoresis.

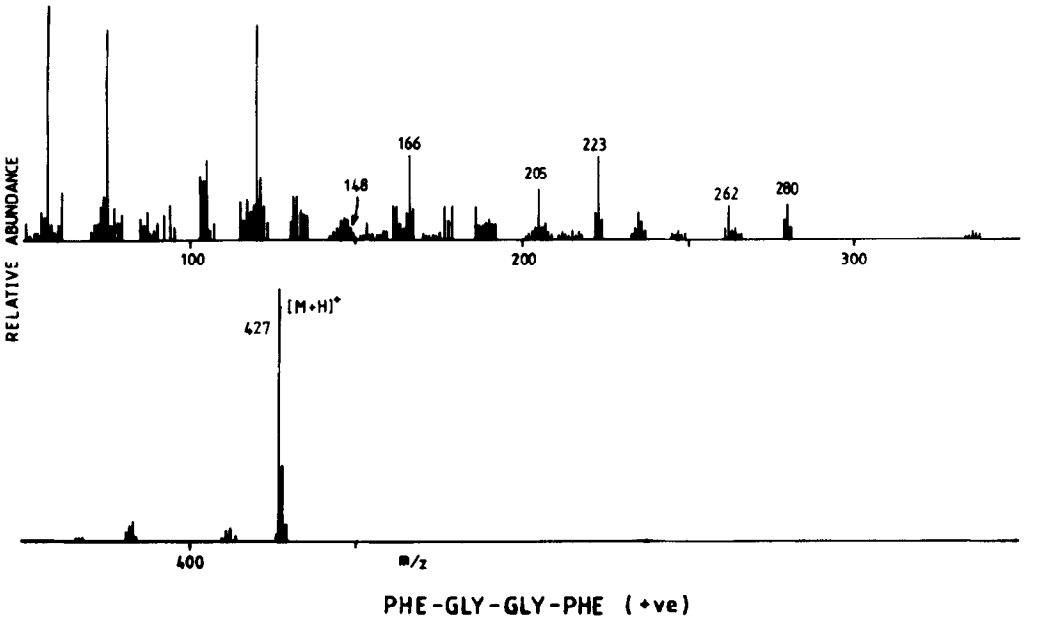


Figure 1

The positive FAB mass spectrum of Phe-Gly-Gly-Phe showing an intense quasimolecular ion $(M+H)^+$ at m/z 427, together with sequence ions which may readily be assigned - see the text.

Derivatives

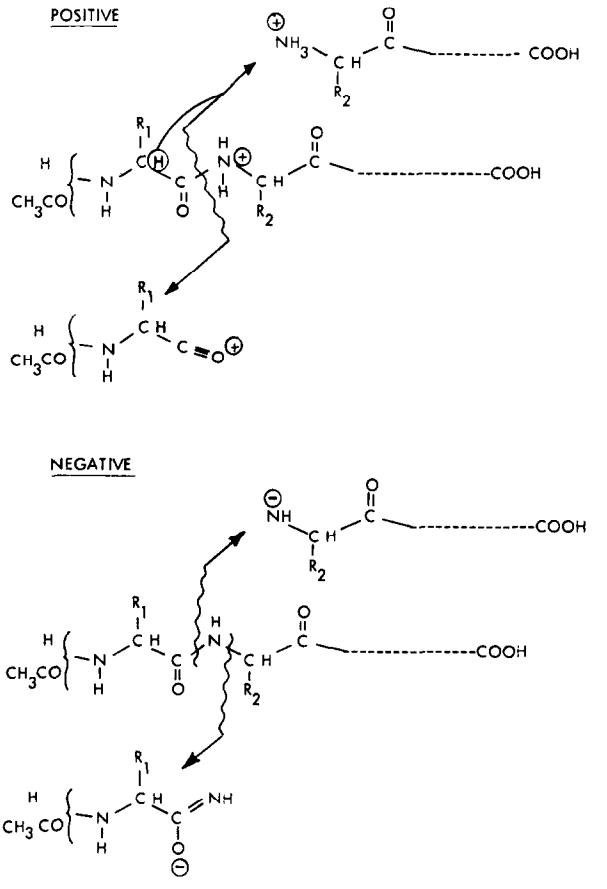
1:1 acetyl: ^2H-acetyl derivatives were prepared as described previously using a mixture of acetic anhydride and $^2H_6$-acetic anhydride in methanol (9, 10). It was found that short acetylation procedure (1 min.) could be conveniently carried out in the glycerol matrix used in sample loading; thus samples dissolved or suspended in glycerol were first examined in both positive and negative modes as free peptides. Subsequently the remaining material was acetylated either in the sample tube or on the probe tip prior to evaporation of reagents and re-examination.

Instrumentation

Spectra were recorded on VG ZAB mass spectrometers, both at VG Analytical, Altrincham, and at Imperial College, London, using FAB sources manufactured by VG Analytical and by M-Scan Ltd (on the Imperial College instrument). Samples were loaded onto the probe either by dissolution or suspension in a few µl of glycerol.

RESULTS AND DISCUSSION

Fig. 1 shows the positive mode mass spectrum of the free peptide Phe-Gly-Gly-Phe. The spectrum may be interpreted according to the chemical ionisation fragmentation mechanism shown in Scheme 1. In this Scheme we postulate protonation of the amide nitrogen of the peptide bond. This may then give rise to the two types of fragmentation observed i.e. formation of



Scheme 1

Fragmentation patterns observed in positive and negative modes of FAB. Positive sequence ions appear 2 m.u. above the corresponding negative ions if they arise from the C - terminus, and 15 m.u. lower if they are N- terminal.

acylium ions (N-terminal sequence ions) at m/z 205 and 262, and ammonium fragments (C-terminal sequence ions comprising the C-α rearranged H)at m/z 166, 223, and 280. Note that in common with our observations on the chemical ionisation fragmentation of peptides, the N-terminal acylium ion is often absent or undergoes facile loss of CO; other sequence ions may also lose CO.

In more complex peptides interpretation is greatly facilitated by preparing a 1:1 acetyl: ^{2}H-acetyl derivative. This fast procedure may be

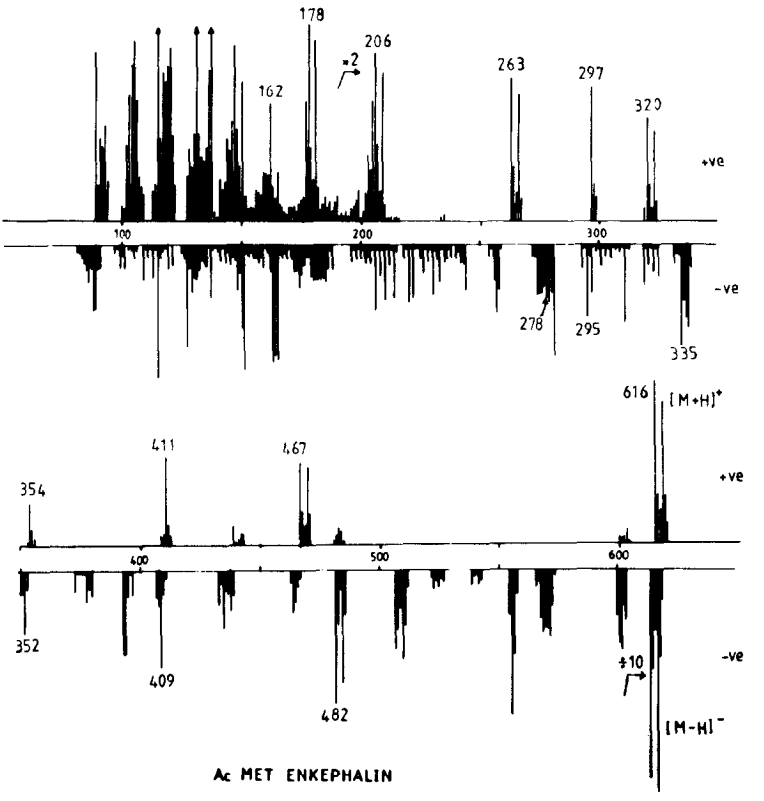


Figure 2

Positive and negative FAB mass spectra of N-(1:1 acetyl:^{2}H-acetyl) met- enkephalin; N - terminal fragments appear as 1:1 doublets, three mass units apart.

carried out either in the sample tube or in the glycerol matrix used to load the sample. The reaction labels only the amino terminal fragments or fragments containing Lys or Arg. This is well illustrated for the spectra of enkephalin whose structure was determined by electron impact mass spectrometry in 1975. The original work was greatly hampered by the expectation of unusual structural features and the absence of a molecular ion in the spectrum (2). The FAB mass spectrum of the acetyl derivative of enkephalin is shown in Fig. 2.

POSITIVE: N-terminal doublets are easily observed at m/z 206 (note the strong loss of CO, common at the amino terminus, at m/z 178) 263, 320 and 467 readily assignable to Tyr-, Tyr-Gly-, Tyr-Gly-Gly- and Tyr-Gly-Gly-Phe- respectively.

A strong doublet quasimolecular ion is then seen at m/z 616 completing the sequence. Note the C-terminal fragments are seen as singlets at m/z 297 (H_2-Phe-MetOH), 354 (H_2-Gly-Phe-MetOH) and 411 (H_2-Gly-Gly-Phe-MetOH) confirming the overall sequence.

NEGATIVE: The negative FAB spectrum is presented in inverse in Fig. 2. Note the very abundant $(M-H)^-$ (the signal is divided by 10 in the drawing) giving easy recognition of the molecular ion. Sequence ions are confirmed by looking for doublets 15m.u. higher than in the positive mode, and singlets (C-terminal signals) 2 m.u.lower in the negative spectrum according to the fragmentation shown in Scheme 1. This procedure "maps" m/z 335/338, 482/485 as N-terminal and m/z 295, 352 and 409 as C-terminal. We do not recommend using the negative spectrum for interpretation but rather to confirm positive spectrum assignments, in difficult examples, by looking for the +15 m.u (N-terminal) and -2 m.u. (C-terminal) shifts.

Fragmentation is not always observed in FAB mass spectrometry of peptides. Figure 3 shows the FAB spectra of a 1:1 acetyl:^{2}H-acetyl derivative of a peptide mixture isolated from a tryptic digest of pepsin. Only one component Ac-Val-Gly-Leu-Ala-Pro-Val-Ala gives both quasimolecular and sequence ions (N-and C- terminal fragments are readily assignable). Two other components PCA-Tyr-Tyr-Thr-Val-Phe-Asp-Arg and Ac-Gln-Tyr-Tyr-Thr-Val-Phe-Asp-Arg give quasimolecular ion signals but no fragmentation. A fourth component gives no signals at all. The free peptide mixture behaves similarly in both positive and negative FAB modes; methods for overcoming this problem will be discussed in detail elsewhere.

It should be noted that signals at m/z 1116 (doublet)and 1175 (triplet) in figure 3 correspond to partial acetylation of the guanidino group of Arg in the two octapeptides PCA-Tyr-Tyr..... and Ac-Gln-Tyr-Tyr.....; m/z 1074 (singlet) and 1133 (doublet) are quasimolecular ions of these peptides with a free guanidino group.

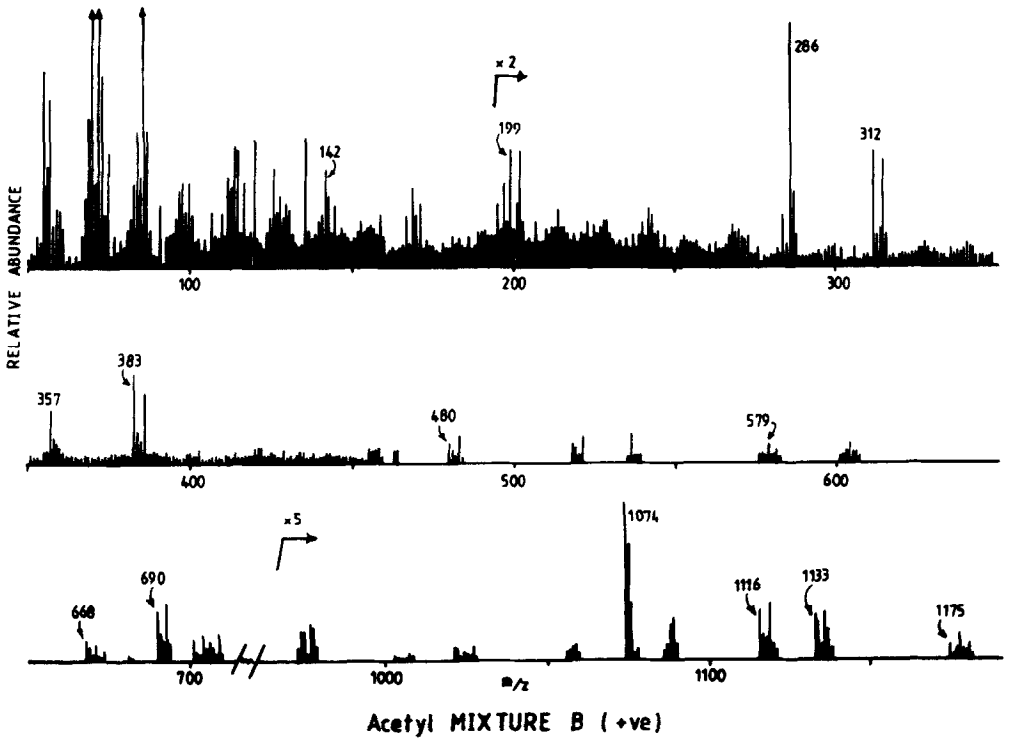


Figure 3

The FAB mass spectrum of a 1:1 acetyl:^{2}H acetyl peptide mixture. Component 1: Ac-Val-Gly-Leu-Ala-Pro-Val-Ala : quasimolecular doublet ions at $(M+H)^+$, m/z 668: $(M+Na)^+$, m/z 690: Sequence ions (e.g. m/z 286 (singlet), H_2-Pro-Val-Ala: m/z 312 (doublet), Ac-Val-Gly-Leu: m/z 383 doublet, Ac Val Gly Leu Ala)
Component 2: PCA-Tyr-Tyr-Thr-Val-Phe-Asp-Arg: quasimolecular ion m/z 1074 (singlet)
Component 3: Ac-Gln-Tyr-Tyr-Thr-Val-Phe-Asp-Arg : quasimolecular ion m/z 1133 (doublet)
No sequence ions were observed for components "2" or "3"; ions at m/z 1116 (doublet) and 1175 (triplet) correspond to partial acetylation of Arg in these peptides.

SUMMARY

We have studied a number of peptides representative of all common amino acids as free compounds and as simple acetyl derivatives by FAB-M.S.

1. We observe good quasimolecular ions (giving the molecular weight) in all samples (both positive and negative modes).

2. We find that where fragmentation takes place it follows a pathway of chemical ionisation, (Scheme 1): N-C or McLafferty rearrangements are not observed.

Table 1

PEPTIDES EXAMINED BY FAB
γ -Glu-Cys-Gly
Ac.Ala-Ser-Phe
Phe-Gly-Gly-Phe
Pro-Phe-Gly-Lys
Ala-Asn-Asn-Lys
Trp-Met-Asp-Phe-NH_2
Tyr-Leu-Pro-Glu-Phe
Tyr-Leu-Gly-Glu-Phe
Tyr-Gly-Gly-Phe-Met
Lys-Phe-Ile-Gly-Leu-Met-NH_2
Val-Gly-Leu-Ala-Pro-Val-Ala
Gln-Tyr-Tyr-Thr-Val-Phe-Asp-Arg
Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg
Val-Val-Tyr-Pro-Trp-Thr-Gln-Arg-Phe
Ac.Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val-NH_2
Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-Gln-Thr-Pro-Leu-Val-Thr

Peptides studied by FAB-MS both as free substances and as 1:1 acetyl: ^{2}H-acetyl derivatives. These include naturally blocked (both N- and C - terminal) and high molecular weight peptides. Both α-MSH (MW 1663) and α- endorphin (MW 1744) yield abundant quasimolecular ions and partial sequence information.

3. In some of the examples studied no fragmentation was observed, and in others complete sequence could be readily determined by this simple method of analysis.

4. Negative ion spectra, when compared to their positive counterparts, allow ready assignment even of quite complex spectra by looking for signals 15 m.u. apart (higher in -ve) -N-terminal, and 2 m.u apart (lower in -ve) - C-terminal.

5. A simple high yield acetylation experiment, which may be carried out in the glycerol matrix, greatly facilitates interpretation, particularly of unknown sequences.

6. The spectra may be obtained on very small quantities of peptide - low nanomole and even picomole levels have been detected (Morris, Dell, Panico, Barber, Bordoli, Sedgwick and Tyler: submitted for publication).

In conclusion, we note that the combination of high field magnet technology (11) with fast atom bombardment, which enabled the studies on α-MSH (MW 1663) and α- endorphin (MW 1744) - Table 1, should open the way for fruitful work on peptides within the 3,000 mass range.

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