# FIELD DESORPTION MASS SPECTROMETRY OF PEPTIDES

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SUMMARY

Various peptides are measured with the field desorption technique and compared with the field ionization mass spectra. Field desorption mass spectrometry is a proper method to analyse substances of low volatility, for example free peptides, and even those containing unprotected arginine and histidine which do not yield useful electron or chemical ionization mass spectra.

## I. INTRODUCTION

Mass spectrometry is one of the standard techniques for amino acid sequence analysis of peptides and proteins. For this purpose using various methods of ionization attempts have been made to develop a general method for determining the amino acid sequence of small peptides. One of the main problems is to make the peptide sufficiently volatile. Derivatization of the polar termini of the peptide and permethylation of the amide bonds are the best approach to solve this problem[1,2,3]. On the other hand these methods require a fair amount of chemical processing and a certain minimum amount of substance. Another problem in mass spectrometric sequence determination is the complexity of the EI mass spectra with increasing peptide size. A way round this difficulty is the determination of the amino acid sequence in peptides by computer interpretation of their high resolution EI-mass spectra[4,5]. This method is as elegant as expensive, and for some biochemical laboratories for economic reasons not applicable. Other ionization methods used for amino acid sequence determination are field ionization (FI) and chemical ionization (CI). In

the case of field ionization a report of Brown and Pettit[6] demonstrates that this technique gives a molecular ion of enhanced intensity compared to the EI-mass spectra. On the other hand often the FI-mass spectra alone do not permit the derivation of the complete sequence. In recent reports Fales[7] and Gray[8] demonstrated the application of CI in peptide sequence analysis. The mass spectra given in their papers show increasing quasi-molecular ion $(M+1)^+$ intensities relative to the EI-mass spectra. The cleavage of peptide bonds occurs in two ways (at the N-terminal acyl carbonium ion and at the complementary C-terminal iminium ion) and the determination of the amino acid sequence of the peptides is possible. In the case of permethylated peptides the distribution of intensities of the ions arising from peptide bond cleavage is more uniform than with EI and therefore the CI mass spectra are very simple to interpret.

This paper deals with the results of mass spectrometric peptide analysis obtained by a special field desorption technique (FD), which was introduced by Beckey[9]. Field ionization mass spectra are compared also with those obtained by field desorption.

Field ionization of molecules occurs in the gas phase a few Å in front of the field anode, whereas field desorption of positive ions occurs with molecules adsorbed on the field anode itself. The term "FI" is used here for ionization of substances which are evaporated from a micro crucible onto the field anode, although not all of the incoming particles are ionized in the gas phase. The term "FD" is used for the case where the field emitter is dipped into a solution of the sample which is afterwards field desorbed, as described in the next section.

## II. TECHNIQUES

The mass spectra demonstrated in this paper were taken using a modified Varian MAT (former Atlas MAT) CH4 single focusing mass

spectrometer with electric detection and magnetic scan.

In the case of FI the samples were examined by evaporation from a crucible. Probe temperature was controlled by an electric heater.

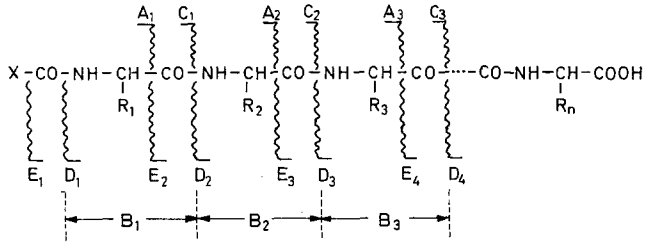
The FD-mass spectra are obtained by dipping the field emitter into a solution of the sample. As field emitter a tungsten wire of 10 μm diameter is used conditioned with benzonitrile[12]. More details of the technique have been presented elsewhere[10,11].

The main reason for the differences in the FI- and FD mass spectra is that in FI (and also in EI and CI) one is compelled to supply the whole lattice energy to evaporate the sample. In contrast, in the case of FD instead of the lattice energy one must supply the heat of desorption from the emitter, which is strongly reduced by the high electric field.

In the FD-mass spectra of free peptides no molecular ion was detected, but a strong $(M+1)^+$-peak was found. This protonation occurs on the amino groups originating either from the remaining solvent (in this case $H_2O$) or by an ion-molecule surface reaction[13].

## III. RESULTS

In scheme 1 the possible peptide bond cleavages are demonstrated schematically.




Scheme 1

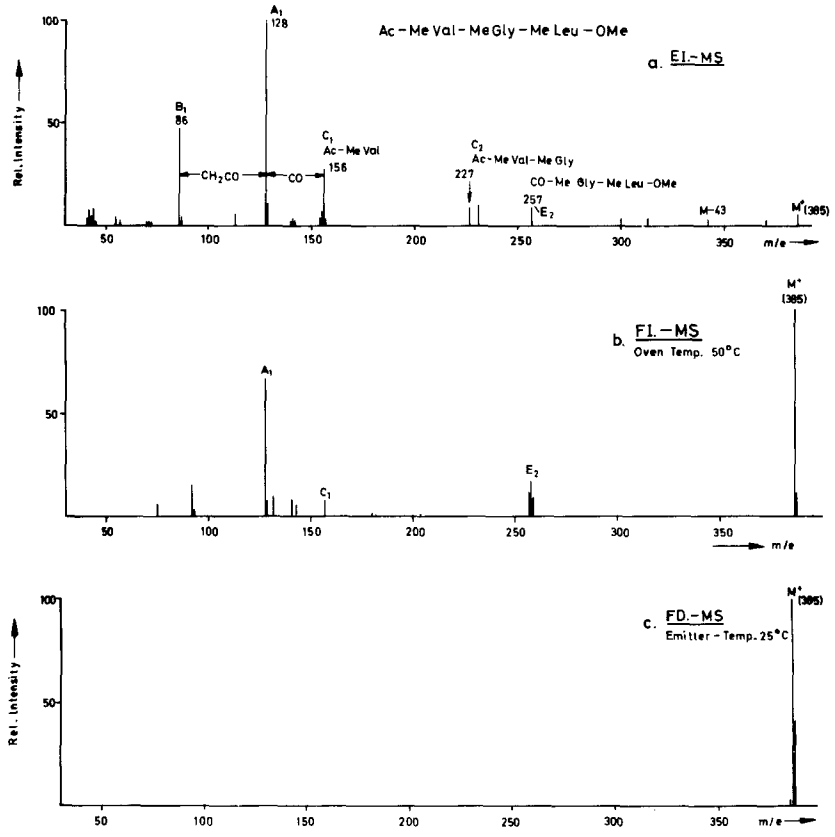



Fig. 1: EI-, FI-, FD-mass spectra of Ac-MeVal-MeGly-MeLeu-OMe

Fig. 1a shows the EI-mass spectrum (70 eV) for the permethylated tripeptide Ac-MeVal-MeGly-MeLeu-OMe. In addition to the small molecular ion peak there are characteristic fragment ion peaks which may be used for a sequence analysis. Fig. 1b shows the FI-mass spectrum of the same sample. The molecular ion peak is the base peak. The mass spectrum also exhibits some fragment ion peaks due to peptide bond cleavages. The FD-mass spectrum of the same compound taken at 25°C emitter temperature is shown in Fig. 1c. The FD-mass spectrum shows only the $M^+$-peak. In an attempt to obtain fragmentation, the emitter temperature was raised to about 50°C. At this temperature the substance was completely desorbed in a few seconds. Therefore it was not possible to run a complete mass spectrum. Later this will be possible, applying fast mass scans with the multi-scaling technique.

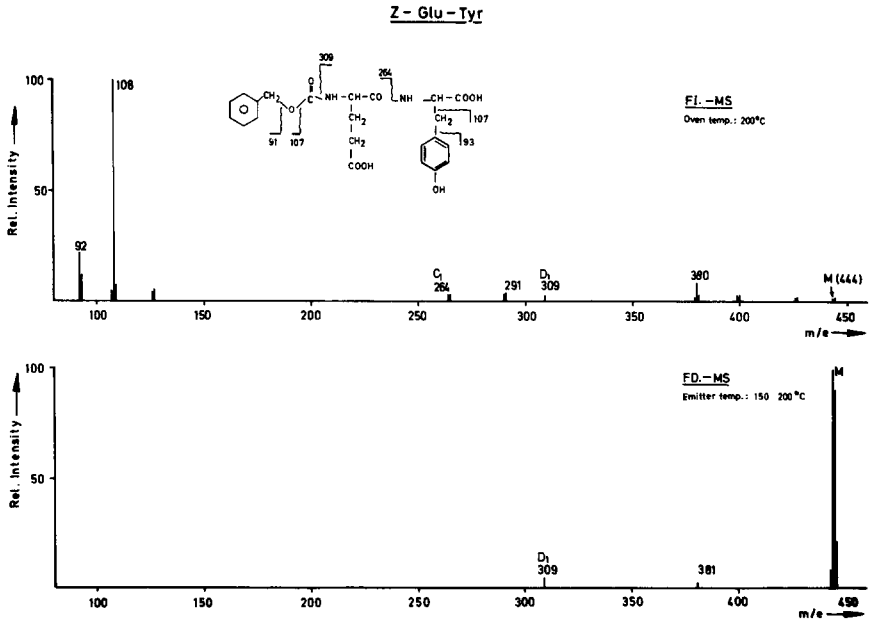



Fig. 2: FI- and FD-mass spectra of Z-Glu-Tyr

Fig. 2 compares the FI and FD mass spectra of Z-Glu-Tyr . Besides a very small molecular ion peak at m/e 444 in the FI mass spectrum some sequence determining fragment ions of weak intensity have been detected. The base peak at m/e 108 is due to a protonation of ions which are formed either by the loss of the side chain of tyrosineor by a cleavage of the oxybenzyl group. In contrast to the FI, the FD mass spectrum exhibits an intensive $M^+$ which is the base peak, and only small fragment ions at m/e 381 (loss of HCOOH + $H_2O$) from the molecular ion) and m/e = 309. The latter peak is due to a $D_1$-fragment. The FI and FD mass spectra complement each other because in the FI-mass spectrum, the molecular ion peak is not clearly identifiable.

Fig. 3 shows the FD mass spectrum of a substituted tetrapeptide containing two free arginins: Ac-Gly-Arg-Arg-Gly-OMe. The $(M+1)^+$ is the base peak. The two intense fragments ions at m/e 245 and 257 correspond to protonated ions due to the peptide cleavage

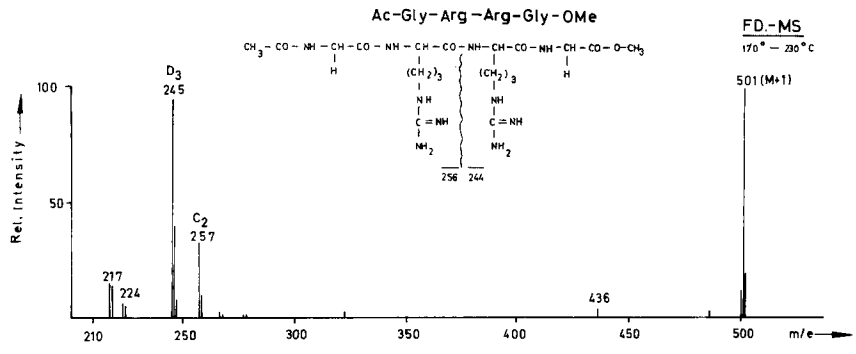



Fig. 3: FD-mass spectrum of Ac-Gly-Arg-Arg-Gly-OMe

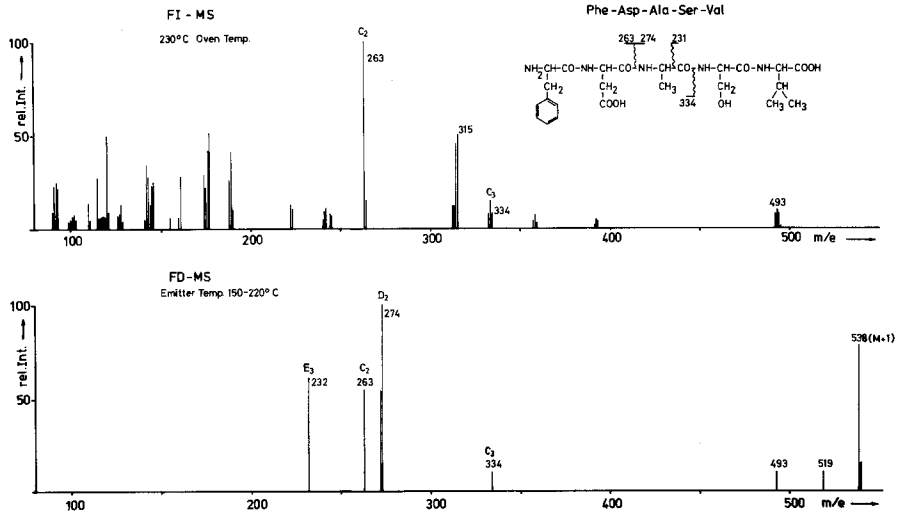



Fig. 4: FI- and FD-mass spectrum of Phe-Asp-Ala-Ser-Val

between the two arginines. To the best of the authors' knowledge it is not possible to detect molecular ions of peptides containing two free arginines with the EI or the CI techniques. Finally Fig. 4a and b show the FI-FD mass spectra of a free pentapeptide: Phe-Asp-Ala-Ser-Val. The FI-mass spectrum contains no molecular ion; but a strong fragmentation due to peptide bond cleavages and rearrangement reactions is found. In the FD-mass spectrum the $(M+1)^+$ ion gives a very intense peak. The fragment

+ Ac.MeLeu.MeAla.$Me_2$Lys(TFA).MeVal.MeAla.$Me_2$Tyr.MeVal.$Me_2$Tyr.-$Me_2$Lys(TFA).ProOMe

ions are all due to peptide cleavages (the sequence peaks $C_2$, $C_3$, $D_2$, $E_3$ are present), or to a loss of COOH (m/e 493)or $H_2O$ (m/e 519) from the molecular ion; but the derivation of the sequence is not easy because the $A_1$, $C_1$ and $D_1$ sequence peaks are absent in the FD-spectrum or not clearly distinguishable from the neighboring peaks in the FI-mass spectrum. Combination of FI and FD mass spectra with EI or CI mass spectra is advisable in this case. As compared to the EI mass spectra the C-terminal peaks (type D) are relatively strong, and this is a common feature of FI, FD and CI mass spectra.

## IV. DISCUSSION

The mass spectrum of the permethylated tripeptide demonstrated that for this volatile substance the sequence information is incomplete in the FI spectrum and fragment peaks are entirely absent in the FD-spectrum. Normally the EI and CI mass spectra of small permethylated peptides can be easily interpreted because fragmentation is almost exclusively at the peptide bonds. Although permethylated peptides containing ten[+,2)] or more residues have been sucessfully sequenced by EI mass spectrometry, the molecular ion peak of the cited peptide is of very low abundance (< 0.01 % of the base peak) and in the higher range the mass spectrum is very complex compared to those of smaller peptides. With protected peptides the sequence information from FI mass spectra is sometimes incomplete[6)], so that FD mass spectrometry is a good complement to EI and CI mass spectrometry. The FD mass spectra of <u>unprotected</u> peptides exhibit, in contrast to those obtained by FI, EI and CI, the molecular ion peak or the quasi-molecular ion $(M+1)^+$. Therefore FD is a very suitable method for analyzing very involatile compounds. A chemical treatment such as substitution by protecting groups would be unnecssary even for

peptides containing arginine and histidine. With the FD-method the authors succeeded in obtaining an intense molecular ion peak of the unprotected nona-peptide Arg $Gly_3$ Pro $Gly_3$ Arg. In the FD-mass spectrum of Ala-His the intensity of the largest fragment peak was less than 10 % of the molecular ion peak.

Another advantage of the FD-technique is the high sensitivity: Only $10^{-8}$ g need to be adsorbed at the field anode wire for a mass spectrum. Normally, about $10^{-6}$ g of a sample are dissolved in about 50 µl of a solvent and then about 1 % of this amount is adsorbed on the field anode; because of the geometry of the emitter support, only a part of this sample is adsorbed on the emitter wire itself.

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