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FAST ATOM BOMBARDMENT MASS SPECTROMETRY OF SERYL- AND O-PHOSPHOSERYL-CONTAINING PEPTIDES

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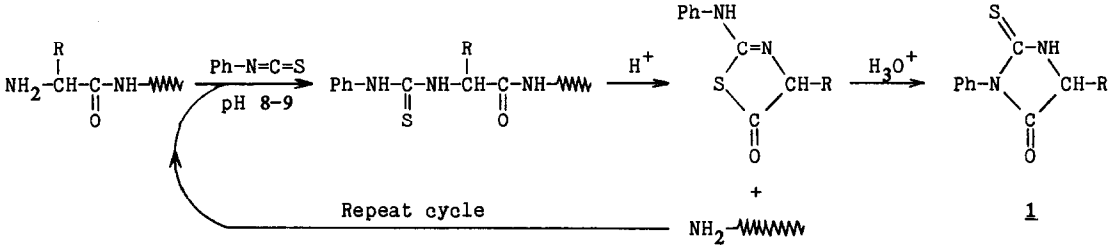
ABSTRACT: FAB-MS was found to be a mild technique for the rapid identification of O-phosphoseryl residues in peptides and the characterization of O-phosphoseryl-containing peptides.

Since 1980, it has become well established that the regulation of many cell processes is mediated by the phosphorylation of particular proteins and peptides. However, extensive biochemical and biological studies of such phosphorylated domains has previously been restricted by their lack of availability or difficulty in chemical synthesis. While we have largely overcome the synthetic difficulties[1-3], a continuing problem in this work lay in the sequencing and characterization of isolated or synthetic O-phosphoseryl-containing peptides.

The Edman degradation[4], which is currently the most widely used technique for sequence elucidation, is based on the removal of the N-terminal amino-acid residue from the protein or peptide followed by the subsequent identification of a stable phenylthiohydantoin (PTH) derivative 1; this derivative formed by the treatment of the peptide with phenyl isothiocyanate under basic conditions followed by acid treatments (Scheme 1). Despite its

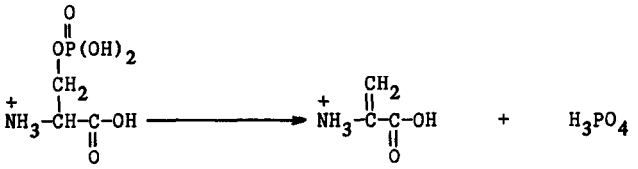
Scheme 1



wide applicability, a serious limitation of the Edman degradation is the failure to obtain[5] an identifiable PTH derivative when O-phosphoserine 2 (or O-phosphothreonine or O-phosphotyrosine) is encountered during peptide sequencing. While this difficulty can usually be overcome by indirect methods, these alternative procedures are tedious and time-consuming, especially in the case of peptides containing several O-phosphoseryl residues. As FAB-MS has previously been useful in peptide analysis[6], we therefore considered this mild technique would be of potential use in the examination of O-phosphoserine-containing peptides. In this communication, we report our initial work on the FAB-MS of PSer 2, PSer-Leu[1] 3 and a simple model tripeptide, Glu-PSer-Leu[1] 4.

In the first instance, positive ion FAB-MS[7] of O-phosphoserine 2 was examined by preparing the sample as a glycerol matrix containing acetic acid and using Argon as ionization gas. The mass spectrum of O-phosphoserine was encouraging as it displayed an intense m/z 186 ion (MH^+) as the base peak and three major ions at m/z 110 (29%), 88 (27%) and 61 (10%). The m/z 88 ion was significant as the difference of 98 mass units from the base peak indicated the loss of H_3PO_4, presumably via a β-elimination process (Scheme 2).

Scheme 2



A comparison of the mass spectrum for O-phosphoserine 2 (Fig. 1) and serine 5 (Fig. 2) showed three major features, firstly, that O-phosphoserine did not fragment to serine via direct P-O cleavage, secondly, there is a loss of 98 mass units (i.e., H_3PO_4, m/z 186→88), and thirdly, that O-phosphoserine and serine fragment via different pathways (i.e., serine gave rise to $H_2\overset{+}{N}=CH-CH_2-OH$ while O-phosphoserine did not give rise to the corresponding phosphate fragment, $H_2\overset{+}{N}=CH-CH_2-OPO_3H_2$). These three findings were significant as they indicated that there would be no ambiguity in the identification of a seryl or an O-phosphoseryl residue.

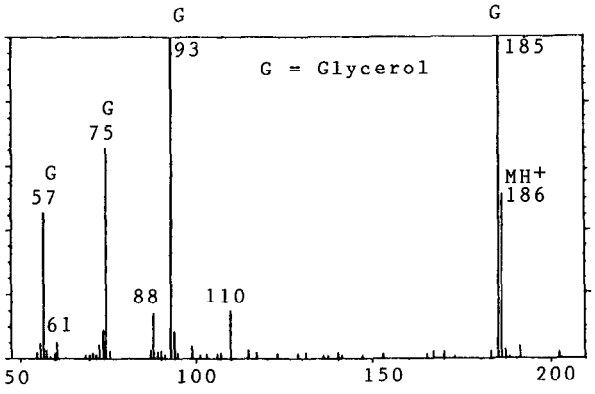


Fig. 1. FAB mass spectrum of O-phosphoserine 2

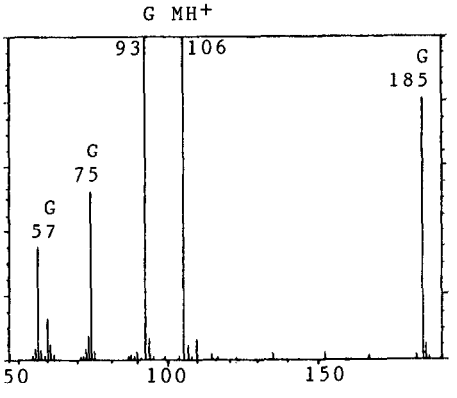
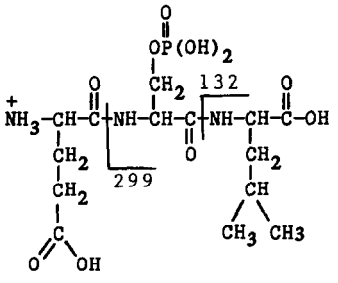


Fig. 2. FAB mass spectrum of serine 5

In an extension of these results, positive ion FAB-MS of Glu-PSer-Leu 4 was then examined. The mass spectrum of Glu-PSer-Leu 4 (Fig. 3) contained a strong ion at m/z 428 (MH^+) and ions at m/z 299 (PSer-Leu) and 132 (Leu). These latter fragment ions corresponded to successive amido cleavage of the N-terminal amino acid residue and thereby permitted ready characterization of the O-phosphopeptide and its sequence elucidation. A comparison of this



spectra with that obtained for Glu-Ser-Leu[8] 6 (Fig. 4) clearly showed that, while both peptides gave rise to fragment ions resulting from C-terminal cleavage, 4 gave a less complicated fragmentation and was not contaminated by any non-phosphorylated peptide.

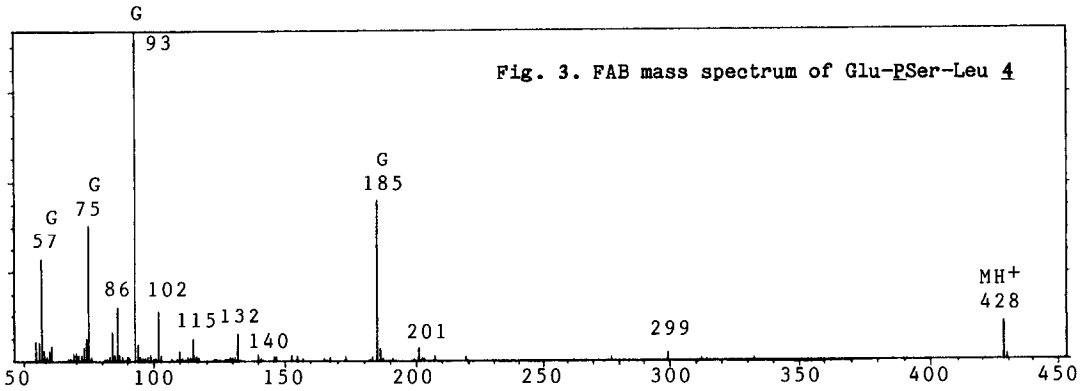


Fig. 3. FAB mass spectrum of Glu-PSer-Leu 4

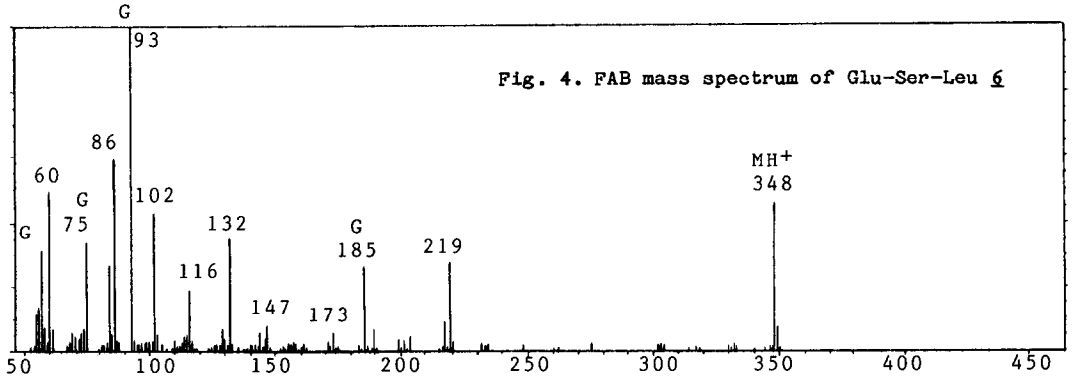
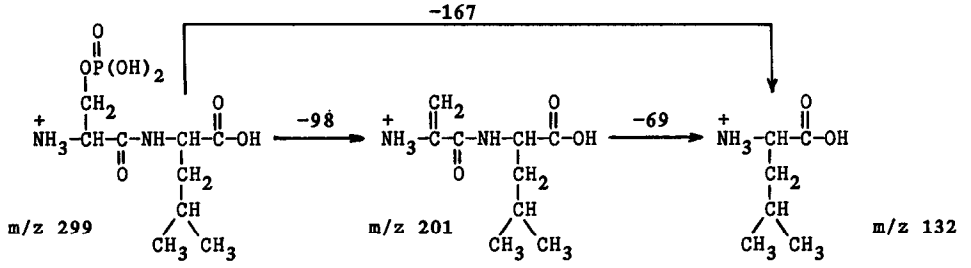


Fig. 4. FAB mass spectrum of Glu-Ser-Leu 6

In addition to these major fragmentations, the loss of 98 mass units (m/z 299→201) in the Glu-PSer-Leu 4 spectrum was also a major fragmentation pathway and presumably corresponds to β-elimination of H_3PO_4 from the dipeptide fragment. Hence, we postulate that the formation of the leucyl fragment (m/z 132) is possible via two possible fragmentations, (a) immediate cleavage of the PSer-Leu amide linkage and (b) β-elimination of H_3PO_4 from the dipeptide fragment followed by cleavage of the ΔAla-Leu amide linkage (Scheme 3).

Scheme 3



These proposed fragmentation assignments were substantiated by the fact that the FAB mass spectrum of PSer-Leu[1] 3 (Fig. 5) contained molecular ions indicative of the above fragmentations. In view of the consistent loss of 98 mass units for PSer 2, PSer-Leu 3 and Glu-PSer-Leu 4, it appears that this particular fragmentation is a diagnostic mass loss for an O-phosphoseryl residue, whether in its free form or incorporated in a peptide.

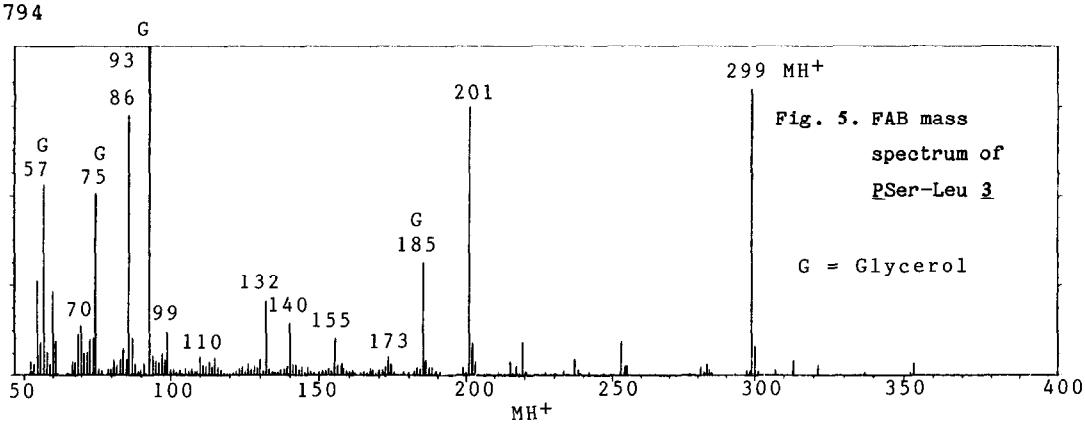


Fig. 5. FAB mass spectrum of PSer-Leu 3

G = Glycerol

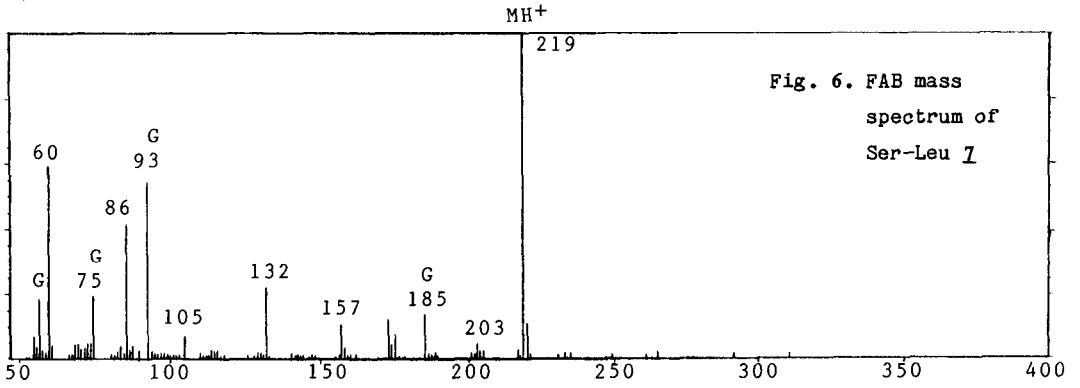


Fig. 6. FAB mass spectrum of Ser-Leu 7

In summary, these preliminary results indicate that FAB-MS is a useful technique for molecular weight determination, sequence elucidation and the characterization of O-phosphoseryl-containing peptides. In particular, a loss of 98 mass units was found to be a diagnostic test for the identification of an O-phosphoseryl residue incorporated in a peptide. The use of FAB-MS in the analysis of larger O-phosphoserine-containing peptides and multi-O-phosphoserine-containing peptides is currently in progress.

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NOTES AND REFERENCES

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7. Jeol DX-300 mass spectrometer equipped with a FAB source.
8. Prepared by hydrogenolysis of Z-Glu(OBzl)-Ser(Bzl)-Leu-OBzl in methanol.
9. Obtained from Vega Biochemicals.

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