A Relatively Sensitive Method for the Mass Spectrometric Sequencing of Peptides

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Summary In the mass spectrometric sequencing of peptides, a carrier peptide of similar volatility to the unknown peptide may be used to enhance the sensitivity of the method.

THE use of mass spectrometry in peptide sequencing is frequently limited by the amounts of material available. For example, in our own work, although it was possible to sequence a dipeptide on only 50 nmol of material,[1] the decrease of signal intensity with increasing *m/e* indicated that it would be difficult to sequence fully larger peptides on similar quantities. Therefore, we felt there was a need for a simple method which would allow spectra to be obtained when only small quantities of peptide are available. We have taken advantage of the ability of mass spectrometry to sequence a mixture of peptides, and used a carrier molecule to allow spectra to be recorded using only small samples of peptides. The novelty of the work lies in the use of a carrier molecule.

Peptides were obtained by proteolytic digestion of *Pseudomonas aeruginosa* amidase.[2] Acetylation and permethylation of various peptides were carried out as previously described.[1] Mass spectra were obtained on an AEI MS 902 instrument, using an electron beam energy

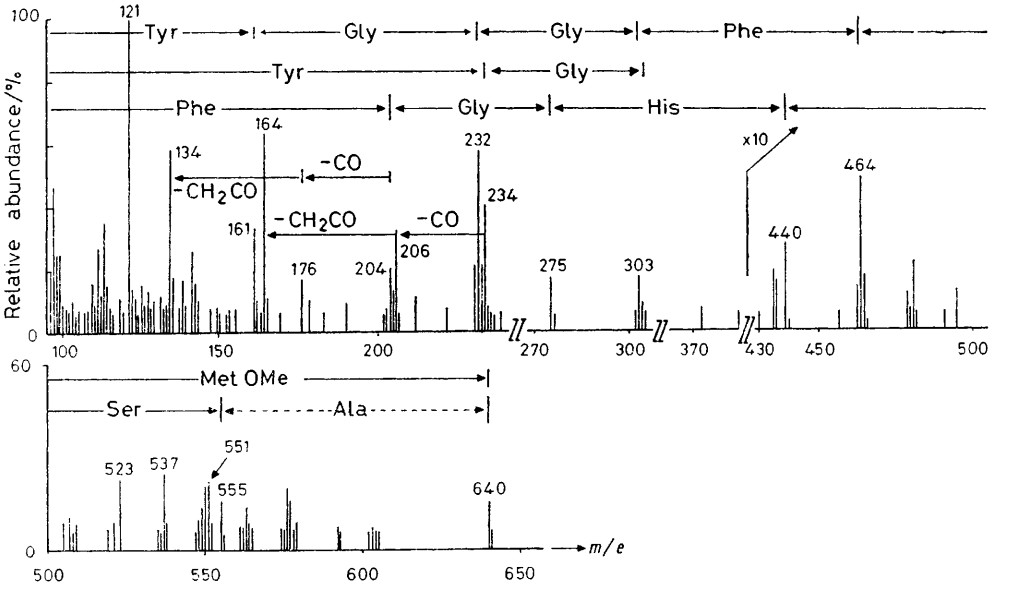

FIGURE. Partial mass spectrum of acetylated and permethylated met-enkephalin (100 nmol) and peptide PVH35 bi (10 nmol) at a source temperature of 215 °C (peaks at *m/e* 523, 537, and 551 are associated with an impurity, present in the spectrometer before the sample was introduced).

of 70 eV and an accelerating voltage of 8 kV. The mass spectrum of a derivatised mixture of met-enkephalin (Tyr–Gly–Gly–Phe–Met) (100 nmol) and peptide PVH35 bi (Phe–Gly–His–Ser–Ala; 10 nmol) is reproduced in the Figure. A peak at *m/e* 671, which would indicate *C*-terminal alanine in the 10 nmol sample, was too weak to be reliable. The peak at *m/e* 640 can arise both from the carrier met-enkephalin (as shown) and from the sample by loss of 31 mass units (OMe) from a molecular ion (*m/e* 671). Thus, at the 10 nmol level, only four (Phe–Gly–His–Ser. . .) of the five residues are reliably sequenced.

Experiments have been carried out in which the carrier peptide was omitted, but derivatisation was effected in a silanized tube and/or a silanized probe tip was used in the mass spectrometer. These experiments did not give useful sequence information on the sample at the 10 nmol level. Similarly, experiments using an involatile carrier (*e.g.* Soya Bean Trypsin inhibitor, a small protein) also failed. Thus, the success of the technique is not simply a matter of avoidance of absorption of the sample on to unspecified sites on the equipment.

It is important that the carrier molecule volatilises in the same temperature range as the sample. Using a pentapeptide sample (10 nmol) with a more volatile carrier (*e.g.* a dipeptide), or a less volatile carrier (an underivatised but different pentapeptide), it was possible to record only very poor spectra which were unsatisfactory for the sequencing of an unknown sample.

The reported method of sequencing small amounts of peptides by mass spectrometry is comparable in sensitivity to classical sequencing techniques. The identification of four residues on 10 nmol of material is equivalent to 2·5 nmol per cycle of dansyl-Edman degradation. Using 10 nmol of peptide, we have successfully sequenced four out of five residues of PVH35 bi, and four residues of peptide PVH-37a (Ala–Asp–Lys–Gln–Gln).

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[1] A. D. Auffret, D. H. Williams, and D. R. Thatcher, *FEBS Letters*, 1978, **90**, 324.
[2] R. P. Ambler, unpublished work.