Peptide Sequence Analysis by Laser Photodissociation Fourier Transform Mass Spectrometry

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Photodissociation of $(M + H)^+$ ions from a 10 pmol sample of a 15 residue tryptic peptide Phe-Gln-Glu-Thr-Phe-Glu-Asp-Val-Phe-Ser-Ala-Ser-Pro-Lxx-Arg (Lxx = Leu or Ile) from beef spleen, purple acid phosphatase affords a mass spectrum characteristic of the amino acid sequence of this molecule.

Methodology for the sequence analysis of proteins by tandem mass spectrometry has recently been described.[1] The approach involves enzymatic or chemical degradation of the protein to a collection of peptides that are then fractionated by h.p.l.c. Peptides in each fraction are sequenced, without further separation, by the combination of liquid secondary ion mass spectrometry and collision activated dissociation on a triple quadrupole mass spectrometer. Starting with 10 nmol of a protein of M_r *ca.* 50 000, it is usually possible to obtain sequence information covering 25—60% of the sample in the 4—5 days required to perform the above biochemical and instrumental manipulations.[1] Presently this approach is limited by the M_r 1800 range of the above instrument and by the sample quantity, 0.1—1.0 nmol of peptide, required for sequence analysis.

To reduce the sample size by an order of magnitude and to extend the methodology to mixtures of larger oligopeptides, new instrumentation, a tandem quadrupole Fourier transform mass spectrometer, has recently been constructed and is now routinely employed for characterizing the molecular weights of peptides in protein digests at the 10—50 pmol level in the mass range up to *m/z* 6000. Horse cytochrome-C at *m/z* 12 385 is the largest biological molecule observed to date on the new instrument.[2,3] Model studies on volatile di- and tri-peptide derivatives indicate the feasibility of using laser photodissociation to obtain structural information on peptide samples.[4,5] Here we report the use of laser photodissociation in a tandem quadrupole Fourier transform mass spectrometer to obtain the complete sequence of a tryptic peptide obtained from a protein of unknown primary sequence.

Shown in Figure 1 is the photodissociation mass spectrum generated from $(M + H)^+$ ions of a 15-residue tryptic peptide (M_r 1771). This particular peptide was obtained by digestion of beef spleen, purple acid phosphatase, a glycoprotein (M_r *ca.* 38 000) of unknown structure presently being sequenced in our laboratory. To obtain these data, sample at the 10 pmol level in 0.5 μl of glycerol–thioglycerol (1 : 1) is exposed to a beam of 10 keV Cs^+ ions for 4 ms. Sample ions sputtered from the matrix under these conditions are transferred through the fringing fields of a 7 T superconducting magnet with the aid of two sets of r.f.-only quadrupole rods and are then trapped in an elongated ion cyclotron resonance cell. A sweep-out pulse of r.f. is employed to eject most of the fragment ions below *m/z* 1600 from the cell. The remaining ions are then exposed to a single pulse of radiation at 193 nm from an argon fluoride excimer laser. Fragment ions, so produced, are excited by an r.f. pulse containing a range of frequencies that causes them to move coherently in large orbits at cyclotron frequencies characteristic of their individual masses. Image currents on two of the cell plates produced by this motion are amplified, digitized, and Fourier transformed to generate a mass spectrum. Data from 50 such experiments on the same 10 pmol sample were acquired and summed in less than 20 s to produce the data shown in Figure 1. All mass assignments are within 0.2 a.m.u. of the expected values.

Fragmentation observed in the present laser photodissociation spectrum is similar to that seen in collision activated dissociation experiments[1] and is discussed here according to the nomenclature rules proposed by Roepstorff and Fohlman.[6] Assignment of an unambiguous sequence to the tryptic

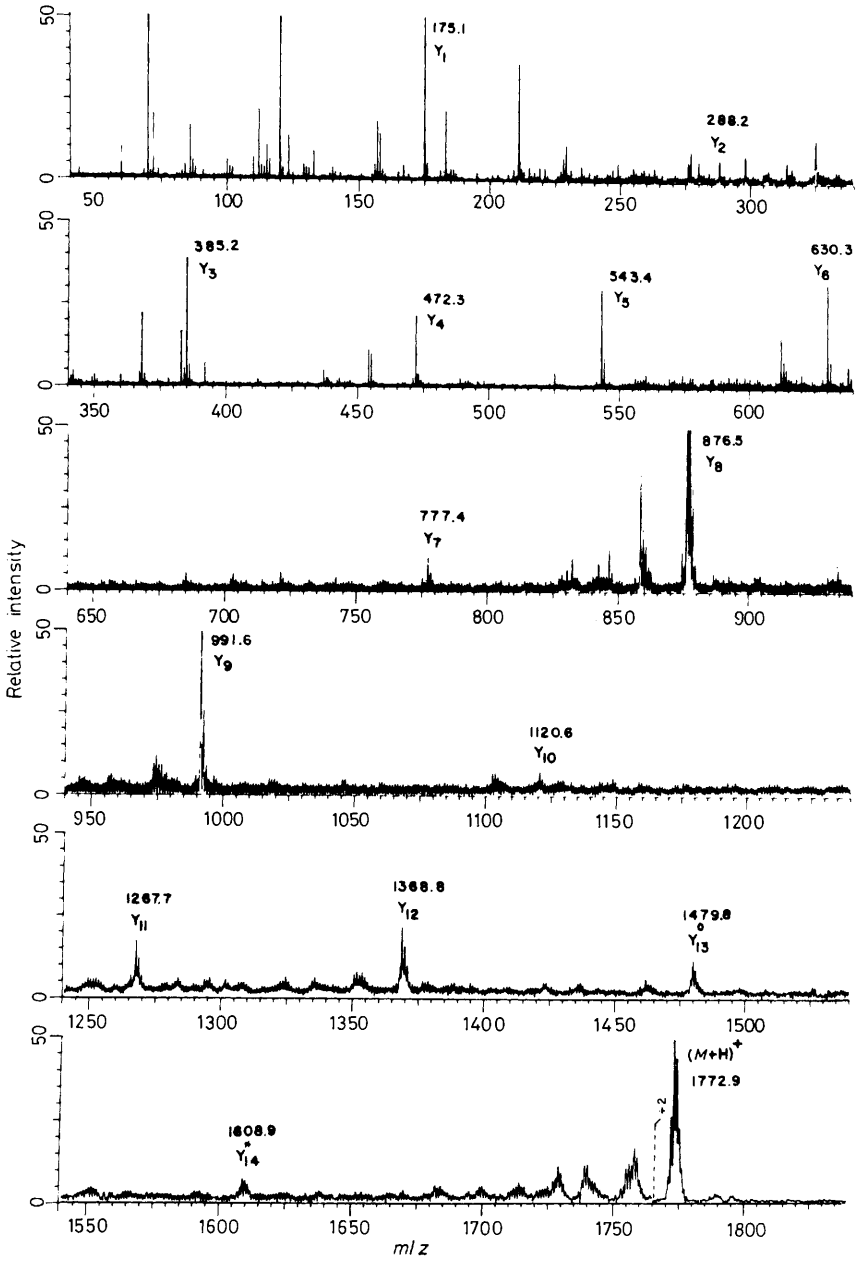
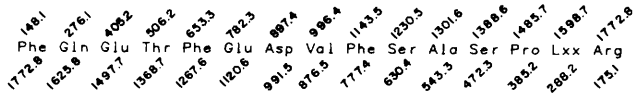


Figure 1. Laser photodissociation mass spectrum recorded on 10 pmol of the tryptic peptide Phe-Gln-Glu-Thr-Phe-Glu-Asp-Val-Phe-Ser-Ala-Ser-Pro-Lxx-Arg.



(A)

The numbers above the structure refer to the masses of ions of type B; those beneath refer to the masses of ions of type Y.

peptide is facilitated greatly by comparing the laser photodissociation spectrum in Figure 1 to that obtained on a 10 pmol sample of the corresponding methyl ester. The $(M + H)^+$ ion for the latter derivative is shifted to higher mass by 56 a.m.u., a number consistent with the presence of four carboxylic acid groups in the molecule. All major fragment ions in Figure 1 also shift to higher mass by multiples of 14 in the methyl ester spectrum and are thus assigned as being of type Y containing the C-terminus of the molecule. Observed mass shifts are as follows; $Y_1 - Y_8$, 14; Y_9, 28; $Y_{10} - Y_{12}$, 42; $Y_{13} - Y_{14}$, 56 a.m.u. Mass differences between the observed type Y_n fragment ions allow the sequence in (A) to be assigned to the present sample.

In structure (A) the penultimate residue is designated as Lxx to indicate that Leu and Ile cannot be differentiated by the present methodology. Ions at *m/z* 1479.8 and 1608.9 result from loss of water and ammonia, respectively, from ions of type Y containing Glu and Gln at the N-terminus. Mass analysis of peptides shortened by one amino acid as a result of

three separate Edman degradation steps confirmed the identity of the three *N*-terminal residues of the proposed sequence.

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