MASS SPECTRA OF UNDERIVATIZED PEPTIDE AMIDES RELATED TO SUBSTANCE P

by

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Summary

Mass spectra of underivatized hexa- and heptapeptide amides related to Substance P have been obtained with a conventional electron ionization mass spectrometer using sample vaporization from a tungsten wire by the technique of rapid heating, proton transfer ionization using ammonia, and photoplate recording of spectra. These spectra exhibit little evidence of sample pyrolysis and are readily interpreted to yield amino acid sequences.

In sequence-activity studies on analogs of the undecapeptide Substance P (Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-MetNH_2) (1-3), synthetic hexa- and heptapeptide (<Glu-Phe-Ile-Gly-Leu-MetNH_2 and <Glu-Gln-Phe-Phe-D-Leu-Leu-MetNH_2) were of particular interest because the hexapeptide is nearly as active (67%) as Substance P in the guinea pig ilium assay, and the heptapeptide is essentially inactive (<0.02%).

We have obtained mass spectra of these two analogs of Substance P, without resort to derivatization, which allow their amino acid sequences to be verified. These spectra and that of a tetrapeptide amide were obtained using a conventional electron ionization mass spectrometer employing (a) sample vaporization from a tungsten wire by the technique of rapid heating, (b) proton transfer ionization using ammonia, and (c) photoplate detection of ions produced. Significantly, these spectra exhibit little evidence of

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sample pyrolysis and compare favorably with those reported in previous studies of underivatized peptides by electron impact (4,5), chemical ionization (6-9), field desorption (10-12) and [^{252}Cf]-plasma desorption (13,14) mass spectrometries

Experimental

The mass spectrometer used was a CEC(DuPont)21-110B spectrometer with an electron ionization source maintained at 110-130°C. The ionization potentional was 70eV with 200 μA of electron current and an accelerating voltage of 6KV. The instrument resolution was set at ∿ 3000 and ions were collected on silver bromide vapor-deposited gel-free photoplates (Ionomet Co., Newton, Mass.).

Peptide samples of 50-100 μg in 1-2 μL of glacial acetic acid were deposited onto a loop of tungsten wire (approximately 0.1mm diameter) attached to the tip of a direct insertion probe. After the acetic acid solvent had evaporated the probe was inserted into the ion source and positioned such that the current flowing to the tungsten wire probe tip was 200-300 nA with the ionizing electron current set as indicated.

Ammonia was bled into the ion source at a rate sufficient to maintain the source ion gage at 2-3x10^{-5} Torr. For recording a spectrum, a preset current of 2.3 A was passed through the tungsten wire bearing the sample for periods of 1-5 sec. It was established that the probe wire reached a temperature of ∿ 1100°C (measured with an optical pyrometer) in ∿ 0.5 sec. under the conditions used.

Ion position and intensity data were obtained in digital form using a photomicrodensitometer (Grant Instruments, Inc., Berkeley, Calif., Model Mark III) interfaced to a PDP-11 computer and mass assignments were made using a conventional program. Relative intensities of ions (see Figs. 1-3) are of limited accuracy since in many instances, particularly those involving ions of low mass, the capacity of the photoplate was saturated.

The peptide amides <Glu-Phe-Ile-Gly-Leu-MetNH$_2$ and <Glu-Gln-Phe-Phe-D-Leu-Leu-MetNH$_2$ were synthesized and Trp-Met-Asp-Phe-NH$_2$ was obtained from Signa Chemical Co., St. Louis, Mo.

Results and Discussion

Figure 1 contains the spectrum of a tetrapeptide amide Trp-Met-Asp-PheNH$_2$ previously studied by Beuhler *et al.* (7,8). As expected for ionization *via* proton attachment[1], a pseudomolecular ion (^{m}/e 597) is observed and low intensity sequence ions (^{m}/e 433 and 318) derived by cleavages of the

1. The pressure of reactant gas in the source, probably 10^{-3}Torr, is much lower than that generally used for chemical ionization (1 Torr). However, because of the low volatilities of peptides, the relative pressures of sample and reactant gas may be comparable. In the absence of ammonia no molecular or pseudomolecular ions were observed for these peptide amides.

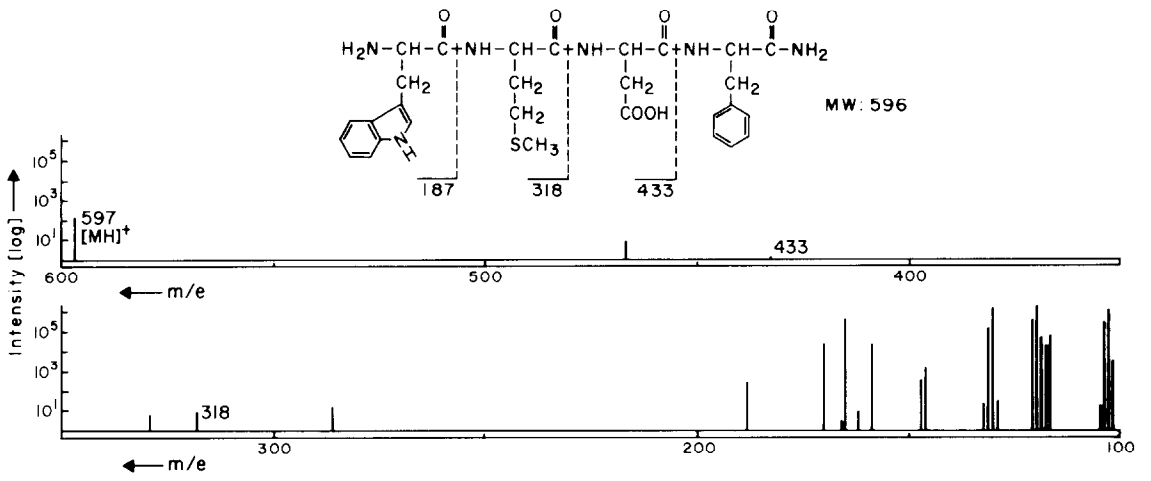

Figure 1. Mass spectrum of the tetrapeptide amide Trp-Met-Asp-PheNH_2.

peptide bonds are present. The third sequence ion (^{m}/e 187) is not present. An intense ion at ^{m}/e 165 is indicative of C-terminal phenylalanine amide (7-9). Loss of 3-methyleneindole from the tryptophan residue, either as a cation (preferred) or as a radical, is extremely facile, dominates the molecular fragmentation, and tends to repress more informative fragmentation processes.

Figure 2 contains the mass spectrum of the hexapeptide amide <Glu-Phe-Ile-Gly-Leu-MetNH_2. A pseudomolecular ion (^{m}/e 690), albeit of low abundance, is observed. Also, the spectrum exhibits ions at ^{m}/e 112, 259, 372, 429 and 542 derived by cleavage of each of the peptide bonds. The C-terminal amino acid Met-NH_2gives rise to an ion at ^{m}/e 149. These ions are readily assigned (given the amino acid composition of the peptide) and completely define the sequence.

As a final example, the spectrum of a heptapeptide amide <Glu-Gln-Phe-Phe-D-Leu-Leu-MetNH_2 is shown in Figure 3. In this case, we were unable to observe a pseudomolecular ion (^{m}/e 908). However, ions at ^{m}/e 112, 240, 387, 534 and 647 derived by cleavage of the first five peptide linkages from the N-terminus are observed. As in the previous example, the C-terminal amino acid (MetNH_2) is evident by the appearance of an ion at ^{m}/e 149; similarly,

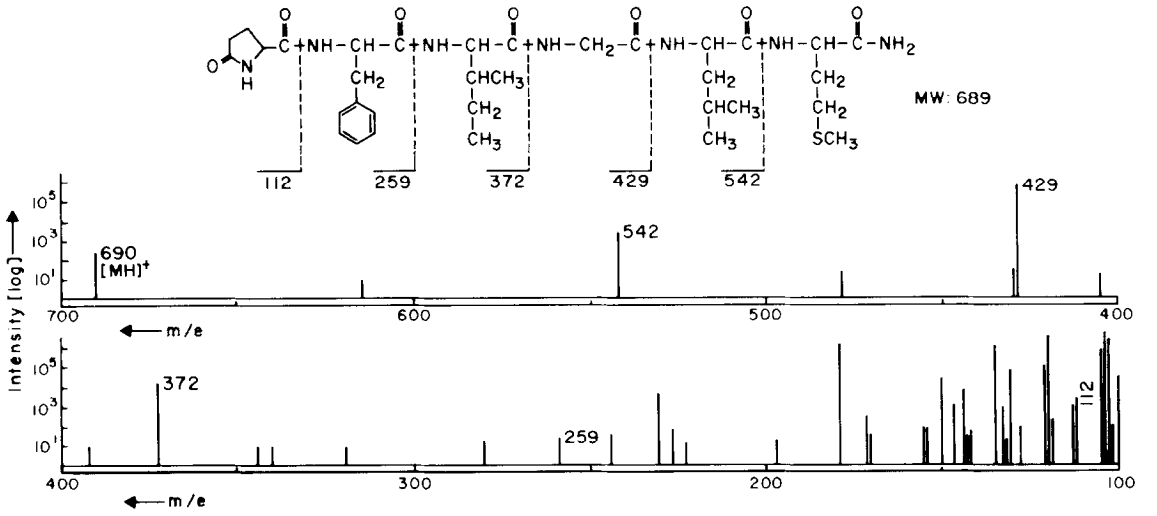

Figure 2. Mass spectrum of the hexapeptide amide <Glu-Phe-Ile-Gly-Leu-MetNH$_2$.

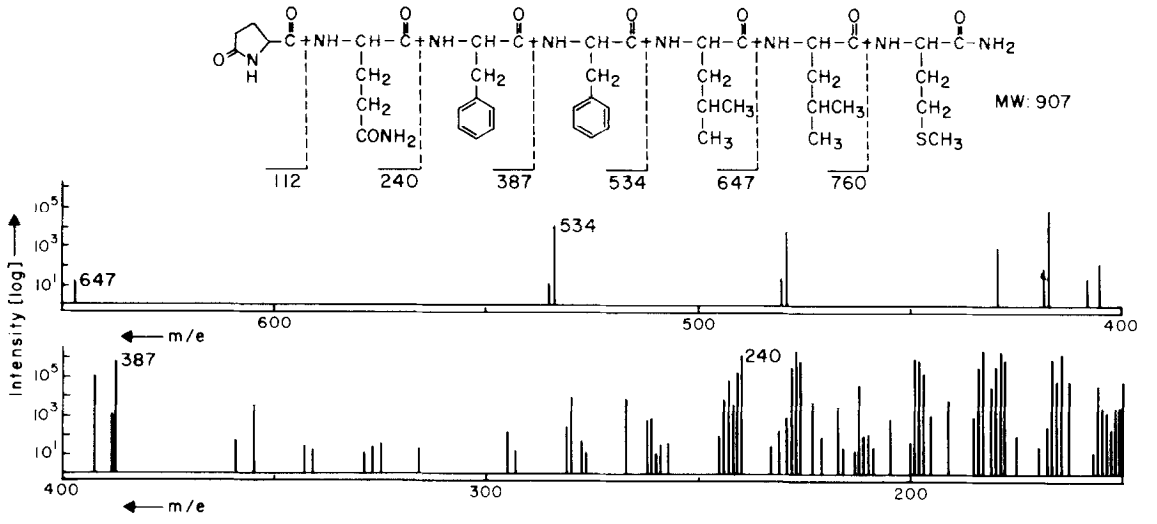

Figure 3. Mass spectrum of the heptapeptide amide <Glu-Gln-Phe-Phe-D-Leu-Leu-MetNH$_2$.

ions at $^{m}/e$ 261 and 262 are assignable to the C-terminal dipeptide leucinyl-methionylamide. Again, if the constituent amino acids are known, the proper sequence is readily established.

Somewhat surprising is the absence of ions resulting from losses of

small molecules (e.g. H_2O, NH_3, CO_2, HCOOH) typically observed in mass spectra of underivatized peptides (4-14) which may arise by thermal processes. This result is consistant with the concept advanced by Beuhler et al. (7,8) that rapid heating rates favor vaporization at the expense of pyrolysis.

Acknowledgment

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