MASS SPECTROMETRY OF PERMETHYLATED PEPTIDE DERIVATIVES : EXTENSION OF THE TECHNIQUE TO PEPTIDES CONTAINING ASPARTIC ACID, GLUTAMIC ACID, OR TRYPTOPHANE *

David W. THOMAS

Institut de Chimie des Substances Naturelles,
C.N.R.S., 91-Gif-sur-Yvette, France.

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Recent publications have described the advantages and limitations of mass spectrometry of N-permethylated peptide derivatives for amino acid sequence determination (Das et al., 1967, 1968 ; Thomas et al., 1968a, 1968b; Agarwal et al., 1968). N-permethylation was accomplished by the use of methyl iodide silver oxide in dimethylformamide (for experimental details see Thomas et al., 1968a). Under these reaction conditions it was found that several amino acid residues give undesirable results.

Particular problems with arginine and sulfur-containing residues can be avoided by preliminary chemical modification of the peptides containing these residues (Thomas et al., 1968b). However, several others posed more severe problems :

a) Methylation of peptides containing aspartic acid gives complex mixtures of products, the exact nature of which are not yet well understood (Agarwal et al., 1968).

b) Glutamic acid residues are frequently "well-behaved" (e.g. as in compounds 1, 2 and 3), but some peptide derivatives undergo partial chain cleavage with formation of a pyroglutamine residue. The heptapeptide derivative 4 was particularly susceptible to this undesirable reaction (Agarwal et al., 1968), as we have also found.

* Part XV in the series "Determination of amino acid sequences in oligopeptides by mass spectrometry" ; part XIV, D.W.Thomas, E.Lederer, M.Bodanszky, K.Isdebski & I.Muramatsu, Nature, in the press.

1 R. Glu. Leu. Leu. Val. Asp. Leu. Leu. OH

2 Z. Ala. Gln. Ala. Phe. Pro. Leu. Glu. Phe. OH

3 Ac. $[Glu(OBu^t)]_5$. Ala. Tyr. Gly. OMe

4 Ac. Glu. Glu. Ala. Glu. Ala. Tyr. Gly. OMe

c) Tryptophane residues normally form a dimethyl derivative, as expected, after permethylation ; but we found that one exception was the tryptophane residue at position 9 of the gramicidins A (5) and B (6) (Sarges and Witkop, 1965) which gave an artifact 30 mass units heavier (Das, Géro, Thomas, unpublished data and Lederer, 5th Intern. Symp. Chemistry Natural Products, London, July 1968, Pure & Applied Chemistry, in the press).

HCO. Val. Gly. Ala. Leu. Ala. Val_3. Trp. Leu X. Leu. Trp. Leu. Trp. $NH(CH_2)_2OH$

5 : X = Trp

6 : X = Phe

The three exceptions stated above did not always prevent the successful application of the permethylation technique, but they created severe limitations for the correct interpretation of data.

With the example of a mycoside C, Vilkas and Lederer (1968) have demonstrated that the methylation method described by Hakomori (1964) for glycolipids (methyl iodide with a methylsulfinyl carbanion in dimethylsulfoxide) is equally applicable for the N-permethylation of peptide derivatives. We have now investigated the use of this technique* with a wider variety of peptides, and we find that it has all the advantages of the silver oxide method. In addition, this technique is more rapid, with complete methylation being obtained after a reaction time of one hour.

The most significant observation was that the "Hakomori-Vilkas" method may be successfully used for peptide derivatives containing aspartic

* Experimental conditions involve preparation of the reagent by heating 20 mg of NaH/oil dispersion (prerinsed three times with ether) in 0.2 ml of DMSO at 100° until hydrogen evolution ceases (about 5 min). The peptide derivative (3 mg) is added to this reagent at room temperature, followed by 0.3 ml of methyl iodide. After one hour the product is diluted with water and extracted with chloroform. Mass spectra of the crude products are determined with an A. E. I. model MS9 mass spectrometer.

acid, glutamic acid, or tryptophane without formation of undesired byproducts :

a) The aspartic acid residue in the pentapeptide derivative 7 was converted to a simple O, N-methyl residue by the Hakomori reaction. A mass spectrum of the product 8 consisted predominantly of the "sequence peaks" which result from amide bond cleavage. Previous methylation of this peptide derivative by the silver oxide method had given a complex spectrum in which it was impossible to recognize the two residues at the N-terminal end .

7 Ac.Asp.Lys(Ac).Ile.Val.Gly.OH

8 Ac.MeAsp(OMe).Me_2Lys(Ac).MeIle.MeVal.MeGly.OMe

Application of the Hakomori method to esperinic acid (Ito and Ogawa, 1959 ; Ovchinnikov et al., 1966), followed by mass spectrometry, enabled us to deduce the complete structure 1 (Thomas and Ito, in preparation) ; whereas methylation with silver oxide made possible the identification of only the three residues at the N-terminal end.

b) The transformation of glutamic acid residues to pyroglutamine in peptide 4, with accompanying cleavage of the peptide chain, was reduced to a negligible extent by use of the Hakomori methylation procedure.

c) Application of this method to the gramicidins A and B (5 and 6) gave the expected dimethyl derivative for all tryptophane residues (including the 9th residue which had previously given an artifact) ; the resulting mass spectra confirmed en entier the structures determined by chemical methods by Sarges and Witkop (1965).

It would thus seem that the use of the Hakomori methylation technique can render mass spectrometry more generally applicable to peptides, regardless of their amino acid composition.

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