DETERMINATION OF AMINO ACID SEQUENCES IN PEPTIDES BY MASS SPECTROMETRY. DESULFURIZATION OF SULFUR-CONTAINING PEPTIDES*

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1. Introduction

The mass spectrometric technique for the sequence determination of amino acids in peptides has received a new impetus since procedures for *O*, *N*-permethylation of peptide derivatives have been described [1, 2; for a review, see ref. 3]. However, difficulties were encountered in applying the permethylation technique to peptides comprising basic amino acids (arginine, histidine, etc.) as well as sulfur-containing amino acids (methionine, cystine, cysteine, etc.) since during methylation (using methyl iodide) quaternary ammonium or sulfonium salts of low volatility are formed rendering them unsuitable for mass spectrometric analysis. Methionine peptides are also reported [4] to give undesirable by-products during methylation with Ag_2O, CH_3I.

The problem of an arginine residue in a peptide could be solved [5] by its conversion to an ornithine residue on treatment with hydrazine [6] prior to *N*-acetylation and permethylation.

To overcome the difficulties with histidine-containing peptides, the cleavage of the imidazole ring by exhaustive treatment with diethyl pyrocarbonate, before applying the methylation procedure, has recently been suggested [7].

For sulfur-containing peptides, it became obvious that the formation of sulfonium salts could be avoided by desulfurization of such peptides before permethylation. Moreover, because of the complex mass spectral fragmentation pattern of sulfur-containing peptides [8], it was thought that desulfurization would give simpler mass spectra thus facilitating sequence determination. Desulfurization [9–12] of cystine/cysteine and methionine with Raney nickel catalyst leads to alanine and α-aminobutyric acid (= Abu), respectively. Earlier, with a methionine-containing hexapeptide we showed the usefulness of this reagent for the desulfurization of sulfur-containing peptides [5]. Desulfurization was carried out by refluxing the *N*-acetyl peptide for four hours in ethanol with freshly prepared Raney nickel. Our subsequent experience has shown, however, that refluxing in ethanolic, and particularly in methanolic solution often causes extensive degradation of the peptides and desulfurization can be incomplete. This procedure was therefore abandoned. In order to find a more reliable procedure we have re-investigated the conditions for desulfurization of several cysteine and methionine-containing peptides as models. Satisfactory results have been obtained by adopting the procedure described below.

2. Experimental

Raney nickel catalyst was prepared as described in ref. [13]. In the preparation of the catalyst the digestion time was shortened from 12 hr to 1 hr.

Complete desulfurization of peptides having cystine, cysteine or methionine residues has been

* Preceding communication, see ref. [2].

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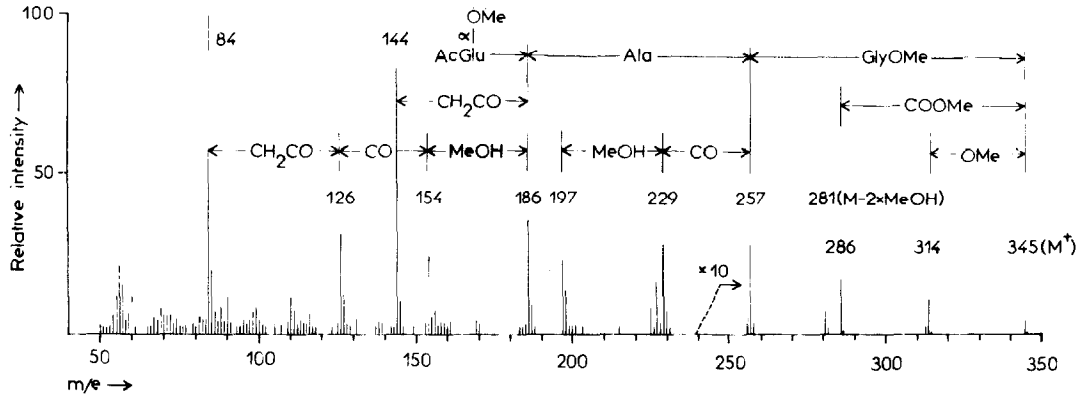

Fig. 1. Mass spectrum of peptide (4).

accomplished by stirring an aqueous solution of the free peptide (5–10 mg) at pH 7–7.5 in the presence of Raney nickel catalyst (ten times by weight of peptide) for 3 hr at room temperature followed by another 1 hr at 40°. When the peptide was insoluble in water, an ethanolic solution of the *N*-acetyl peptide was used. After the reaction the catalyst was filtered off, the solvent evaporated, the desulfurized peptides were *N*-acetylated with acetic anhydride in methanol [5], and then esterified with diazomethane. The *N*-acetyl methyl esters were then analysed by mass spectrometry.

Mass spectra were obtained with an AEI MS9 mass spectrometer operating at 70 eV.

3. Results and discussion

Cysteine, *S*-benzyl cysteine, methionine, glutathione (both in the reduced and oxidized forms) and the following peptides have been successfully desulfurized by employing the above method.

Met.Met.OH (1)
Gly.Met.Gly.OH (2)
Met.Phe.Met.OH (3)

Peptide (3) was not soluble in water but its *N*-acetyl derivative was completely desulfurized in ethanolic solution. Undesirable side reactions and peptide-bond splitting are almost negligible under the above conditions. Cysteine-containing peptides

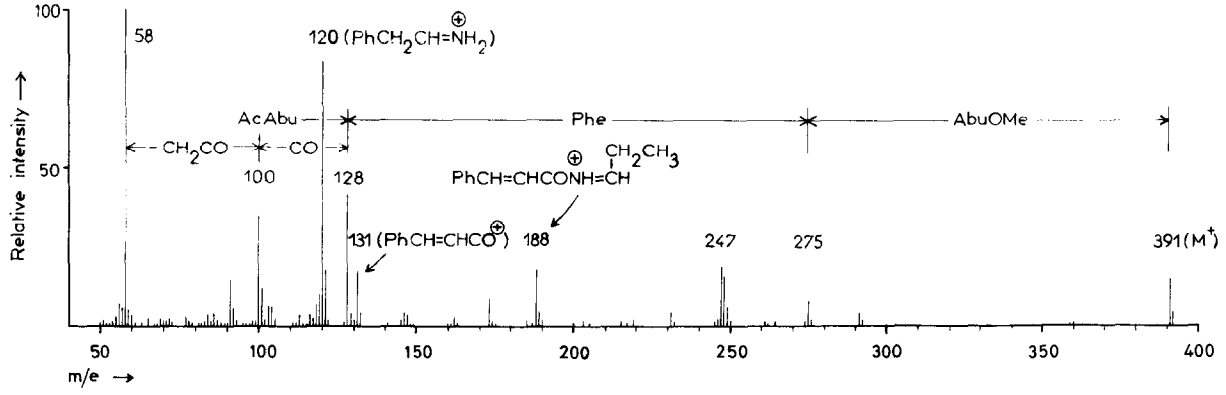

Fig. 2. Mass spectrum of peptide (7). The peak at *m/e* 247 may correspond to the fragment PhCH=CHCO. Abu.OMe; alternatively, it may be due to loss of CO from *m/e* 275.

desulfurize more easily since good results could be obtained with short reaction times, whereas longer heating time was found useful for the complete desulfurization of methionine-containing peptides (for example, peptide (3) was heated at 40–45° for 4 hr). Glutathione (reduced and oxidized forms), and peptides (1), (2) and (3) on desulfurization followed by *N*-acetylation and esterification with diazomethane gave respectively derivatives (4), (5), (6) and (7), the mass spectra of which are consistent with their structures.

α-OMe

Ac.Glu.Ala.Gly.OMe (4)
Ac.Abu.Abu.OMe (5)
Ac.Gly.Abu.Gly.OMe (6)
Ac.Abu.Phe.Abu.OMe (7)

Figs. 1 and 2 represent the mass spectra of peptides (4) and (7), respectively.

In the course of the present work several desulfurization techniques have been investigated. Complete desulfurization could also be effected by treating *N*-acetylpeptide methyl esters in dry ethanol or isopropanol (2 ml) with Raney nickel* (25-fold excess) at 100° for 48 hr in a sealed tube with magnetic stirring. However, the products were trans-esterified and some splitting of the peptide bonds occurred giving rise to compounds of lower molecular weights. Ac.Met.Phe.Met.OMe, for example, when treated with Raney nickel under these conditions in isopropanol solution gave a product which on mass spectrometric analysis was found to be a mixture of the following compounds:

Ac.Abu.Phe.Abu.OiPr (iPr = isopropyl)
Ac.Abu.Phe.OiPr
Ac.Abu.OiPr

With *N*-acetylated methyl esters of *S*-alkylated cysteine-containing peptides (e.g., *S*-methyl glutathione) however, complete desulfurization was obtained at 80°, and there was negligible peptide-bond cleavage, although partial trans-esterification still occurred.

Recently, Kiryushkin et al. [14] have carried out desulfurization of peptides with Raney nickel in dimethylformamide at 20° for 2 days. Although good results were reported to be obtained by this method it suffers from the disadvantage of a 2-day time requirement. The procedure adopted by us is much shorter and has so far given reproducible results.

* In these experiments the Raney nickel was made at 50°.

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