MASS SPECTROMETRY OF PEPTIDE DERIVATIVES. TEMPORARY PROTECTION OF METHIONINE AS SULFOXIDE DURING PERMETHYLATION

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1. Introduction

Permethylation of peptide derivatives has increased the possibilities for mass spectrometric sequence determination of peptides [1]. However, certain amino acid residues cause difficulties due to the formation of onium salts during the permethylation [2] such as the methionyl residue [2, 3]. In order to overcome this difficulty it has been proposed to desulfurize the sulfur containing peptides before permethylation [1–3]. The same procedure has also been employed to avoid complex side chain fragmentation of methionine containing acyl-peptide esters [4]. This technique, however, demands large amounts of peptide (5–10 mg) and is too time-consuming. Furthermore, incomplete desulfurization may occur to such a degree that the interpretation of the mass spectra becomes impossible.

To avoid these problems the method described below has been worked out. The permethylation of methionine containing peptides has been performed after protection of the thioether function as sulfoxide [5], followed by deprotection of the thioether group by a rapid and quantitative [6] reduction [7] of the sulfoxide.

$$-\text{Met}-\xrightarrow[\text{HOAc}]{H_2O_2}-\text{Met(O)}-\xrightarrow[\text{DMF}]{\text{NaH},CH_3I}-\text{MeMet(O)}-$$

$$\xrightarrow[2)\ Na_2S_2O_3]{1)\ \text{KI},\ CH_3COCl}-\text{MeMet}-$$

2. Experimental

The method has been tested on the following three peptide derivatives: Ac-Val-Met-Pro-OMe (I), Ac-Ile-Gly-Leu-Met-OMe (II) and Ac-Ala-Met-Ala-Leu-Phe-Gly-OMe (III). The peptide derivatives were synthesized by the solid phase method of Merrifield [8]. The peptides were cleaved off the resin as methyl esters by treatment with methanol/10% triethylamine for 24 hr at ambient temperature. Acetylation was in the case of I and III carried out on the resin bound peptides before the cleavage as a normal coupling by replacing Boc*-amino acid with acetic acid. II was obtained by deprotection of the Boc-tetrapeptide methyl ester with trifluoroacetic acid followed by acetylation with acetic anhydride/methanol (1:3) for 30 min.

Typical experimental conditions for the oxidation permethylation-reduction as follows: III (0.8 mg, 1.2 μmole) was dissolved in 0.1 M hydrogen peroxide in glacial acetic acid (0.03 ml, 3 μmole) and left at room temperature for 1 hr. The conversion into the sulfoxide was followed by thin-layer chromatography (2-butanol-formic acid-water, 15:3:2). After evaporation of the solvent under reduced pressure, the permethylation was carried out with sodium hydride and methyl iodide in dimethylformamide (0.1 ml) [9]. After addition of acetic acid (0.1 ml) to eliminate the excess sodium hydride, the sulfoxide was reduced by stirring for 5 min with potassium iodide (5 mg)

Abbreviation: Boc: butyloxycarbonyl.

and one drop of acetyl chloride. Then hydrochloric acid (0.1 ml) was added and the liberated iodine reduced by the addition of a few drops of 0.1 N sodium thiosulfate solution followed by addition of chloroform and washing 3–4 times with water. After drying over anhydrous sodium sulfate and filtration, the chloroform was evaporated leaving the hexapeptide derivative as a viscous, yellowish oil.

The permethylated derivatives of I and II were prepared in a similar way. The permethylation of the sulfoxide derivative of I was also carried out satisfactorily by using sodium hydride-dimethylacetamide [10] and sodium hydride-dimethylsulfoxide [11] as base, in the latter experiment, however, it was necessary to evaporate the dimethylsulfoxide under reduced pressure after the addition of acetic acid but before the reduction of the methionylsulfoxide residue.

The mass spectra were obtained on a Perkin Elmer 270 mass spectrometer operating at 70 eV, The samples were introduced directly into the ion source, the temperature of the solids inlet probe is indicated on each figure. The ion source temperature was 150°.

3. Results

3.1. *Ac-Val-Met-Pro-OMe (I)*

The spectrum of I (fig. 1a) is dominated by a peak at *m/e* 327 which can be attributed to the loss of the side chain of methionine from the molecular ion by a McLafferty rearrangement. The sequence determining peaks at *m/e* 142 and 273 are of rather low intensity compared to other peaks which may be attributed to side chain fragmentation. The spectrum of permethylated I (fig. 1b) shows more dominant sequence determining peaks at *m/e* 156 and 301 and a less pronounced loss of the side chain of methionine (*m/e* 355) is observed.

3.2. *Ac-Ile-Gly-Leu-Met-OMe (II)*

The spectra of II (fig. 2a) and permethylated II (fig. 2b) both clearly show the first three sequence peaks at *m/e* 156, 213 and 326 respectively 170, 241 and 368 but the loss of the side chain of methionine is much more pronounced in fig. 2a (*m/e* 414) than in fig. 2b (*m/e* 470). Peaks at *m/e* 375 and 402 in fig. 2b may be attributed to the loss of Ac-Me-Ile and part of this from the *C*-terminal. At masses higher

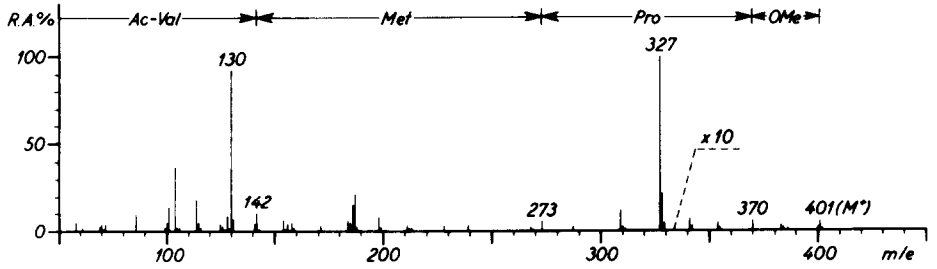

Fig. 1a. Mass spectrum of Ac-Val-Met-Pro-OMe, temp. 100°.

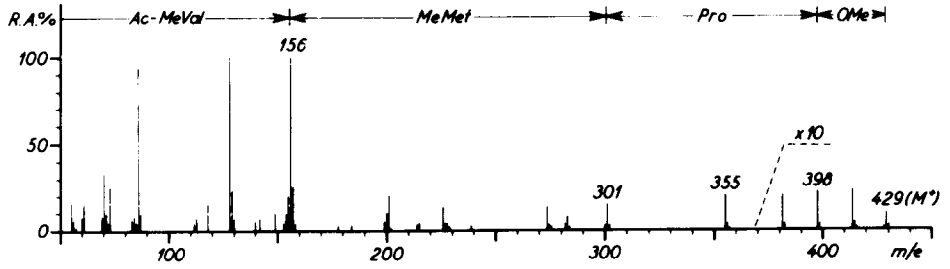

Fig. 1b. Mass spectrum of Ac-MeVal-MeMet-Pro-OMe, temp. 60°.

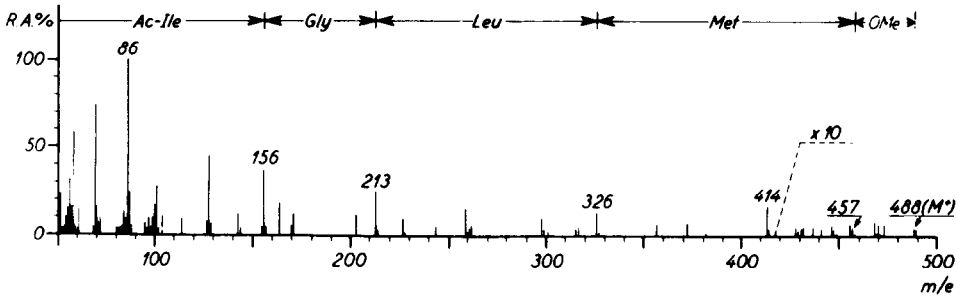

Fig. 2a. Mass spectrum of Ac-Ile-Gly-Leu-Met-OMe, Temperature 120°.

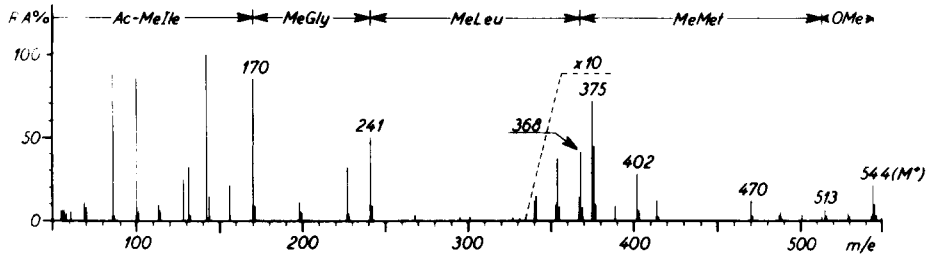

Fig. 2b. Mass spectrum of Ac-MeIle-MeGly-MeLeu-MeMet-OMe, Temp. 60°.

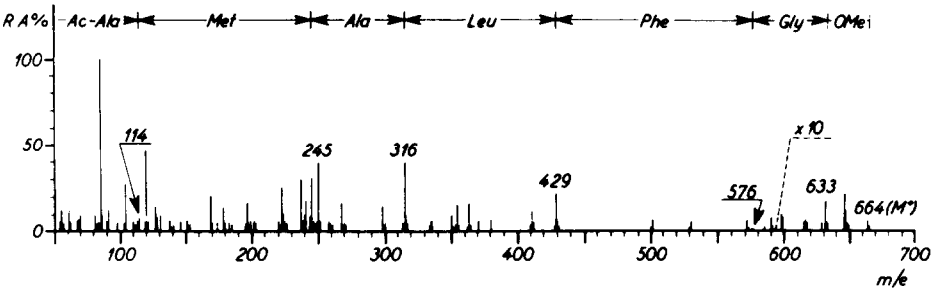

Fig. 3a. Mass spectrum of Ac-Ala-Met-Ala-Leu-Phe-Gly-OMe, temp. 220°.

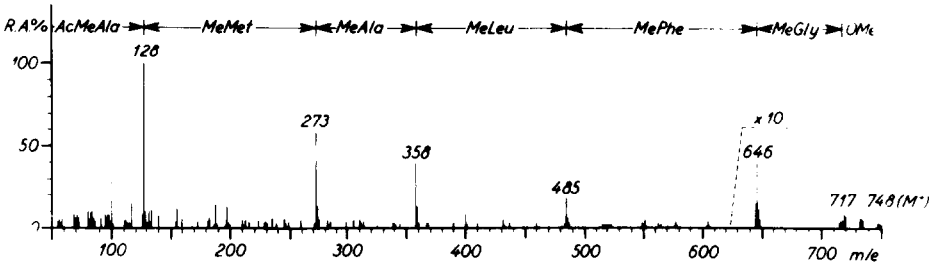

Fig. 3b. Mass spectrum of Ac-MeAla-MeMet-MeAla-MeLeu-MePhe-MeGly-OMe, temp. 175°.

than the third sequence determining peak, however, the permethylated product gives a "cleaner" spectrum than the non-permethylated.

3.3. *Ac-Ala-Met-Ala-Leu-Phe-Gly–OMe (III)*

The spectrum of III (fig. 3a) only shows three of the sequence determining peaks with a reasonable intensity at *m/e* 245, 316 and 429, while the first at *m/e* 114 and the fifth at *m/e* 576 are nearly negligible. Most of the other peaks in the spectrum can be explained by losses of side chains and rearrangement reactions. The spectrum of permethylated III (fig. 3b) on the other hand is a remarkably clear spectrum dominated only by the sequence determining peaks.

4. Discussion

The results show that the mass spectrometric sequence determination of methionine containing permethylated peptide derivatives is made possible by the method here described, while the sequence determination of the non-permethylated compounds is difficult or impossible. Comparison of the spectra a and b in figs. 1, 2 and 3 shows that the well-known advantages of the use of permethylated peptide derivatives is also valid for methionine containing peptides, i.e. increased volatility and simpler fragmentation pattern than obtained with the non-permethylated derivatives. In the case of the permethylated tripeptide I and tetrapeptide II, however, the fragmentation pattern, as is often observed for small peptides, is somewhat more complex than in the permethylated hexapeptide III. Compared to the desulfurization technique the method here described is more rapid and the sample demand considerably reduced. Work is in progress to expand the method to include cysteine containing peptides.

Acknowledgements

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