PERMETHYLATION OF METHIONINE-CONTAINING OLIGOPEPTIDES FOR SEQUENCE ANALYSIS BY MASS SPECTROMETRY

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Summary. Methionine-containing oligopeptides have been permethylated on the nanomolar level. Under the conditions developed, the formation of sulfonium iodide and cyclopropane derivatives during the permethylation reaction was avoided. The mass spectra presented here contain complete sequence information.

INTRODUCTION

Mass spectrometry has proven to be a valuable tool in obtaining the sequence of oligopeptides.[1-6] Much of the mass spectrometric work done up to this point has been carried out with milligrams of peptides containing mostly aliphatic amino acids.[4,6] These peptides have little in common with those extracted from natural sources. Natural peptides usually contain many polar groups. In addition, in most cases only micrograms of sample are available from natural sources.

For mass spectrometry, peptides must be derivatized in order to remove their zwitterionic character and to increase their volatility (lowered by hydrogen bonding). A variety of derivatives has been employed over the years. Permethylation was first employed on peptides by Das, et al.[7] and involves methyl iodide plus a silver oxide catalyst. The reaction was im-

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proved by the use of methylsulfinyl methide carbanion as a catalyst.[8-12] The methyl group is a good derivative because of the minimal increment in the molecular weight per replaceable hydrogen.

To avoid the formation of quaternized salts during permethylation, free amino groups in the peptide have to be (mono) acetylated first.[12] Two other functional groups had to be modified prior to methylation, the guanidino group of arginine and the thioether group of methionine. Thus far, reported mass spectra of permethylated peptides containing either an arginine or a methionine residue display sequence-determining peaks from the N-terminus up to, but neither including nor beyond that residue.[12] The permethylation of methionine residues yields sulfonium iodide[12] or cyclopropane[13] derivatives. Therefore, the only way to handle methionine-containing peptides for mass spectrometry has been to desulfurize the methionine residues prior to methylation.[12]

Polan, et al.[6] have successfully O,N,S-permethylated milligrams of cysteine-containing peptides and obtained partial mass spectra. They suggested that their technique might be applicable to methionine and histidine residues. The latter residue has been handled adequately recently.[14] Lenard, et al.[4] were the first to employ permethylation techniques to peptides (without methionine residues) on the nanomolar level. However, they used excessive amounts of reagents (more than 1,000 equivalents) which would quaternize histidine, etc. They obtained partial mass spectra on peptides containing mostly aliphatic amino acids.

Based upon the work of Polan, et al.,[6] we have investigated the stoichiometric requirements of the permethylation reaction on the microgram level.[15] Optimum results were obtained when the ratio of equivalents of carbanion:methyl iodide:peptide was about 10:10:1.

By application of this "equimolar, ten-fold excess" method on the microgram level, we succeeded in sequencing methionine-containing peptides. As model systems, we have chosen the tetrapeptide Met-Gly-Met-Met and the dipeptide Thr-Met.

MATERIALS AND METHODS

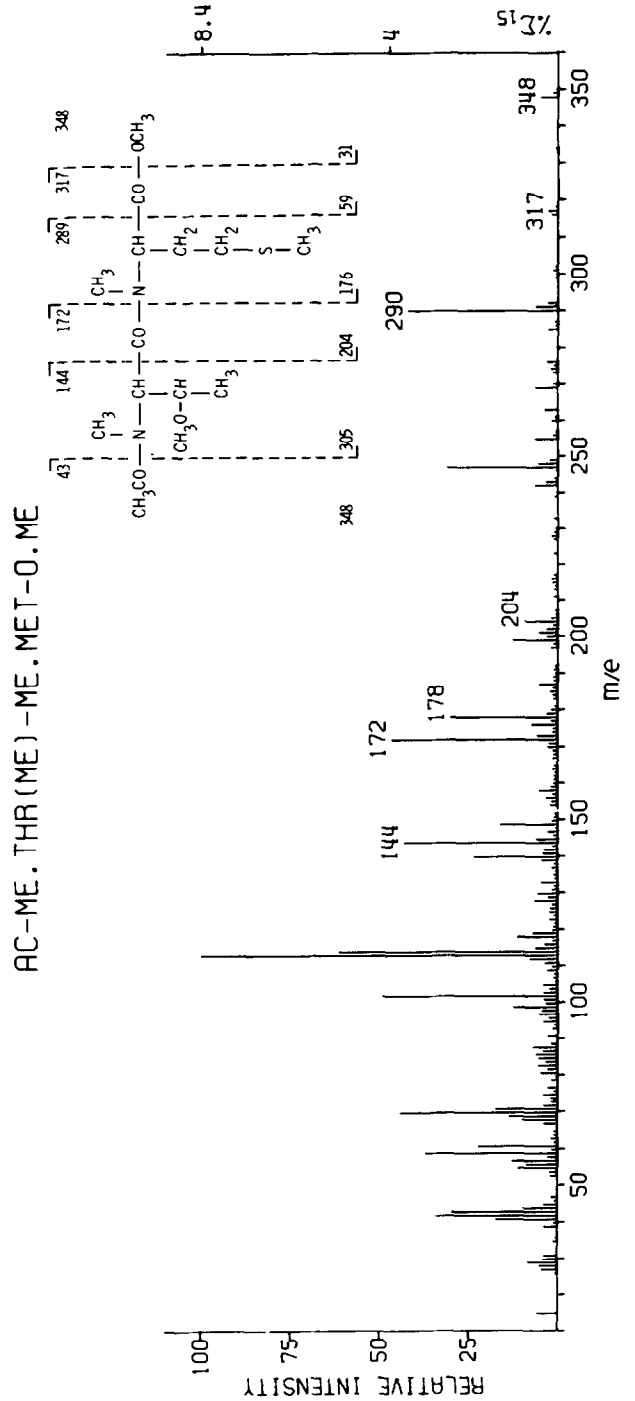
The peptides were obtained from Fox Chemical Co. (Los Angeles, Calif.). Each peptide (50 μg) was acetylated and permethylated according to our procedure.[15] Ultrasonic treatment was used throughout to facilitate dissolution and reaction.[16]

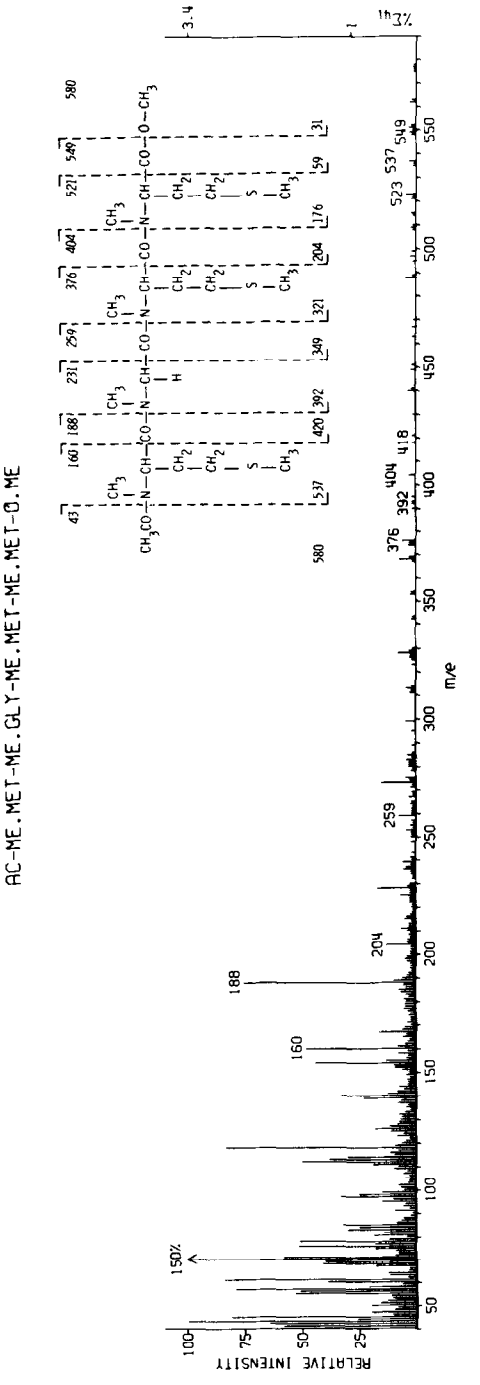
The peptides were acetylated in methanol:acetic acid anhydride (4:1)[12] at room temperature for three hours. The reagents were removed *in vacuo*. The acetylated peptides were dissolved in dimethylsulfoxide. Under nitrogen, 8 μl of a 1.0 M methylsulfinylmethide carbanion ($CH_3SOCH_2^-Na^+$) solution[8-11,15] was added, followed in 15 min by the addition of 0.5 μl methyl iodide. After one hour at room temperature, the reaction was terminated by adding water. The permethylated peptides were extracted into chloroform, washed with water, and the solutions concentrated.

The mass spectra were obtained from approximately 20 μg of each peptide. The samples were introduced into the ion source of a LKB 9000 mass spectrometer by means of a direct introduction probe. The probe temperature was 100° C for the dipeptide and 170° C for the tetrapeptide. The LKB 9000 was operated with a 70 eV ionizing voltage and a 60 μA trap current.

RESULTS

The mass spectrum and fragmentation pattern of acetylated and permethylated threonyl-methionine are given in Figure 1. All but one (*m/e* 289) of the sequence-determining ions from the *N*-terminus are found


AC-ME.THR(ME)-ME.MET-O.ME
RELATIVE INTENSITY
m/e
348
317
290
204
178
172
144


AC-ME.MET-ME.GLY-ME.MET-ME.MET-O.ME
RELATIVE INTENSITY
m/e
188
160
204
259

Captions for Figures

1. (Upper) Mass spectrum of permethylated N^{α}-acetyl threonyl-methionine (LKB 9000, direct introduction probe, probe temperature = 100° C.)

2. (Lower) Mass spectrum of permethylated N^{α}-acetyl methionyl-glycyl-methionyl-methionine. (LKB 9000, direct introduction probe, probe temperature = 170° C.)

(m/e values 144, 172, 317, and the molecular ion at 348). These masses are confirmed by accurate mass measurements using a CEC (Dupont) 21-110B high resolution mass spectrometer. In addition to the "sequence peaks", the loss of the threonyl side chain gave rise to a series of peaks at m/e 113, 247 and 290.

The mass spectrum and fragmentation pattern of acetylated and permethylated methionyl-glycyl-methionyl-methionine are given in Figure 2. The molecular ion (m/e 580) is not present due to the loss of the C-terminal methoxy group (CH_3O). This does not present a problem in establishing the molecular ion as we shall see in the Discussion.

All but one (m/e 231) of the remaining sequence-determining peaks are present (m/e values 160, 188, 259, 376, 404, 521, and 549). The loss of methyl mercaptan is shown by the peaks occurring at m/e 112, 140, 211, 328 and 488.

DISCUSSION

The mass spectra of both peptides clearly define the sequences. There is no indication for the formation of any side products from the thioether groups. The tetrapeptide mass spectrum is an excellent example due to the presence of an N-terminal methionine residue and a C-terminal methionyl-methionine dipeptide.

We have modified a computer program written by Biemann, et al.[17]

for the determination of the sequence of a peptide from the high resolution mass spectrum. We adapted the program to accept the low resolution mass spectral data and those amino acids known to be present in order to elucidate the unique sequence of the peptide. Application of this modified program to the dipeptide data yielded the two possible sequences. They are (with accumulated intensities of "sequence peaks" in parentheses): Thr-Met (1517), and Met-Thr (545). From the tetrapeptide spectrum, two of the possible fourteen tetrapeptide sequences were found. They were Met-Gly-Met-Met (1130) and Gly-Met-Met-Met (558).

In both the di- and the tetrapeptide, the correct sequence can be assigned to that peptide having the highest accumulated intensity value. While it is difficult to prove that the correct sequence will always yield the highest intensity value, we have found that this occurs in all of our examples. Therefore we are confident that this computer program can be depended upon when sequencing unknown oligopeptides.

Methionine is now included in the list of amino acids that can be permethylated in oligopeptides on the nanomolar level. A larger variety of oligopeptides of biological origin can therefore be sequenced by mass spectrometry. Arginine is the only remaining amino acid that causes difficulty in sequencing peptides by mass spectrometry. However, current results indicate that pyrimidyl ornithine derivatives[18] of arginine can be permethylated on the nanomolar level and the resulting peptide sequenced by mass spectrometry.[19]

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