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Partial Methanolysis and Fast Atom Bombardment Mass Spectrometry of Peptides Containing Sulphurated Amino Acids

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Sequence determination by partial methanolysis and fast atom bombardment (FAB) mass spectrometry of peptides containing cysteine and methionine was investigated. Cysteine-containing peptides require methylation of the sulphydryl group by methyl iodide to give a stable *S*-methylcysteinyl residue prior to partial methanolysis and mass spectrometry. Methionine-containing peptides undergo partially a methylation on sulphur during methanolysis, with formation of an *S*-methylsulphonium ion which under FAB conditions is extracted from the matrix and eliminates methyl sulphide in the gas phase. The presence of additional peaks due to chemical modifications or gas-phase fragmentations, however, does not interfere with the sequence information of the spectra.

INTRODUCTION

Several strategies have been reported for enhancing the usually insufficient[1,2] sequence information of fast atom bombardment (FAB) mass spectra of peptides. These include tandem mass spectrometric methodologies or a combination of chemical or enzymatic degradation with FAB mass spectrometry.[3–19]

Partial methanolysis of relatively low molecular weight peptides has proved to be valuable for increasing the sequence information of their FAB mass spectra.[20,21] The data collected, however, have also shown that, under the conditions of the methanolysis, modification of some amino acid residues occurs.

This paper reports an investigation of the behaviour of cysteine- and methionine-containing peptides using the partial methanolysis/FAB mass spectrometric procedure.

EXPERIMENTAL

Peptides, methyl iodide and mercaptoethanol were purchased from Sigma (St Louis, MO, USA). All the other chemicals were of the highest purity commercially available and were used without further purification unless stated otherwise.

Partial methanolysis

About 0.5 mg of each peptide was placed in a quartz vial and 150 μl of a freshly prepared 5 M solution of hydrochloric acid in dry methanol was added.

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The vial was firmly stoppered and the mixture was allowed to react at 37 °C for 6 h, after which the reaction was stopped by cooling the solution in liquid nitrogen. The solvent was then removed by vacuum evaporation. The residue was taken up with methanol and about one tenth of the solution was transferred to the FAB probe.

Preparation of the hydrochloric acid–methanol solution and subsequent partial methanolysis were carried out in quartz vials, since large clusters of glycerol with potassium and potassium chloride appeared in the spectra when glass vials were used.

Sulphydryl group methylation of glutathione

The reaction was carried out by a modification of a literature procedure.[22] A 1 mg (3.3 μmol) amount of glutathione was dissolved in 150 μl of a 0.1 M solution of Tris adjusted to pH 8.5 with hydrochloric acid. A 5-μl volume (80 μmol) of colourless methyl iodide was added and the solution was shaken repeatedly at room temperature for 15 min. After addition of 5 μl of mercaptoethanol the reaction mixture was cooled in liquid nitrogen and freeze-dried. The residue was directly subjected to methanolysis without further purification. The yield of *S*-methylglutathione was about 90% as determined by high-performance liquid chromatographic (HPLC) analysis of a separate sample.

FAB mass spectra

FAB mass spectra were recorded with a Kratos MS-50 instrument fitted with a standard FAB source.

The samples (about 5–10 nmol) were deposited by evaporation from methanol solution onto a copper probe tip and about 5 μl of glycerol were added. A

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beam of 7–9 keV of xenon atoms (Ion Tech gun) was impacted on the sample. The source operating pressure was typically 10^{-5} Torr (1 Torr = 133.3 Pa). Spectra were recorded on UV-sensitive paper and calibrated manually using glycerol clusters as reference peaks.

Nomenclature of the methanolytic fragments

The nomenclature for designating the methanolytic fragments is given in Fig. 1. The amino acid residues are numbered starting from the *N*-terminus.

Methyl ester fragments produced by cleavage at the carboxylic end of the first, second, ..., *n*th amino acid residue are indicated as CE1, CE2, ..., CE*n* if they contain the *C*-terminal amino acid, and as NE1, NE2, ..., NE*n* if they contain the *N*-terminal amino acid. These fragments are referred to as primary methanolytic fragments. Further methanolysis of these primary fragments would result in the formation of fragments which do not contain either the *C*- or the *N*-terminal amino acid of the original sequence. These fragments would correspond formally to cleavage of two peptide bonds in the original sequence and are indicated here as secondary methanolytic fragments.

RESULTS AND DISCUSSION

FAB mass spectra of peptides 1–3

The peptides investigated are shown in Table 1. The sequence ions appearing in their FAB mass spectra are reported in Table 2. Fragment ions are designated following Biemann's modification[8] of the Roepstorff and Fohlman notation.[23]

Table 1. Model peptides subjected to partial methanolysis

No.	Peptide	Relative molecular mass	Amino acid sequence
1	Glutathione	307	γ-Glu-Cys-Gly
2	Chemotactic peptide	409	Met-Leu-Phe
3	Methionine enkephalin	573	Tyr-Gly-Gly-Phe-Met

The relative intensities are reported only for those fragment ions which can be differentiated from the matrix background.

Examination of Table 2 shows that only a few sequence ions are found in the FAB spectra of peptides **1** and **2**. Ions *y* and *a* are observed for peptide **2**. In addition, the structural significance of the data in Table 2 is further lowered by interference from matrix peaks or isobaric fragments. Overall, it appears that sequence determination from the data in Table 2 is not possible.

FAB mass spectra of methanolytic mixture of peptides 1–3

When subjected to partial methanolysis, peptide **1** produced a reaction mixture that did not give any structure-related peak in the FAB mass spectrum.

This result was apparently related to the presence of the cysteine residue, since peptides containing glycine and glutamic acid are known to produce the expected methanolytic fragments.[20,21] Conversion of cysteine to *S*-methylcysteine prior to methanolysis was therefore tried in order to obtain a more stable derivative.

Treatment of glutathione with methyl iodide under carefully controlled pH conditions afforded *S*-methylglutathione in 90% yield, as determined by HPLC

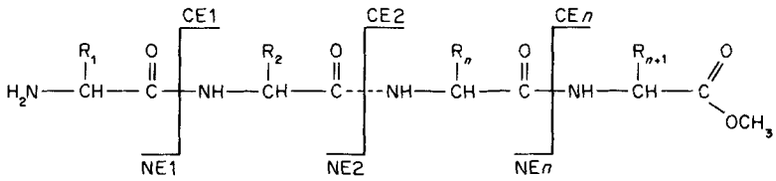


Figure 1. Nomenclature for methanolytic fragments.

Table 2. Relative abundance of sequence ions in the FAB mass spectra of peptides 1–3[a]

Peptide	MH^+	a_1	b_1	c_1	x_1	y_1	z_1	a_2	b_2
1[b]	308 (100)	102 (38)[c]	130 (—)	147 (45)[c]	102 (38)[c]	76 (—)	59 (—)	205 (—)	233 (12)
2	410 (100)	104 (36)	132 (—)	149 (20)[c]	192 (—)	166 (20)	149 (20)[c]	217 (3)	245 (22)
3	574 (100)	136 (80)	164 (—)	181 (—)	176 (—)	150 (—)	134 (—)	193 (—)	221 (—)

Peptide	c_2	x_2	y_2	z_2	a_3	b_3	c_3	x_3	y_3
1	250 (—)	205 (—)	179 (—)	162 (—)					
2	262 (7)[c]	305 (—)	279 (18)	262 (7)[c]					
3	238 (—)	323 (—)	297 (20)	280 (—)	250 (—)	278 (50)[c]	295 (8)	380 (—)	354 (9)

Peptide	z_3	a_4	b_4	c_4	x_4	y_4	z_4
1							
2							
3	337 (—)	397 (7)	425 (—)	442 (7)	437 (—)	411 (5)	394 (—)

[a] Relative intensities (in parentheses) are calculated taking 100 as the intensity of the MH^+; values are reported only for fragment ions distinguishable from the background.

[b] γ-Glutamyl moiety is taken as *N*-terminal residue; a_1 and x_1 fragments correspond to cleavage between γ-carboxyl group and adjacent CH_2.

[c] Possible contribution from another isobaric fragment.

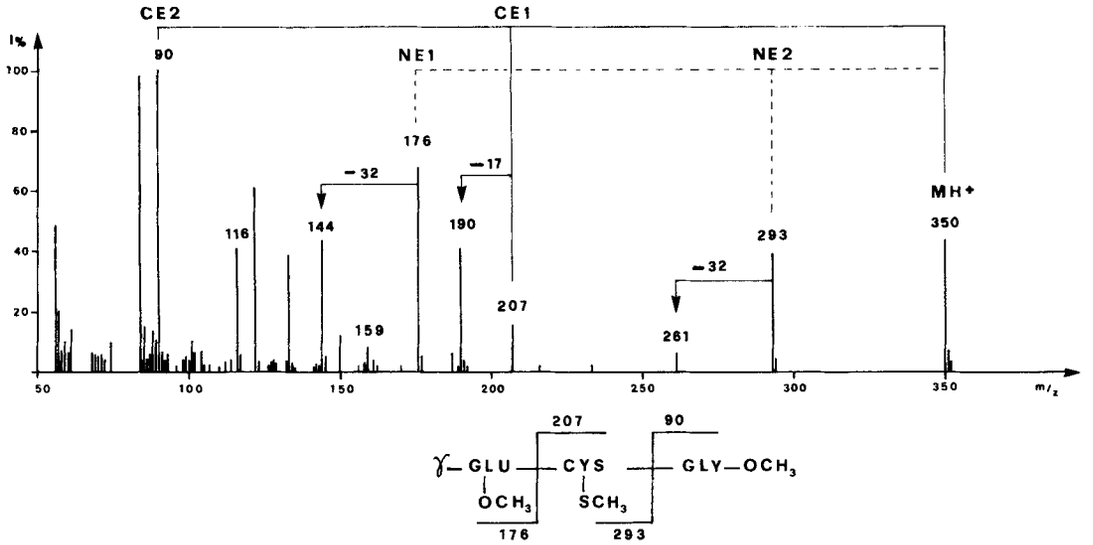


Figure 2. FAB mass spectrum of the mixture from partial methanolysis of *S*-methylglutathione.

analysis. After freeze-drying, the residue was directly subjected to methanolysis.

The FAB mass spectrum of the resulting mixture (Fig. 2) is characterized by the presence of abundant protonated structure-determining signals. It shows the abundant molecular ion of *S*-methylglutathione methyl ester at *m/z* 350. MH^+ signals for the CE-type methanolytic fragments are clearly distinguishable at *m/z* 207 and 90. The MH^+ ion at *m/z* 207 shows a loss of 17 u (supported by metastables) and the formation of an intense daughter ion at *m/z* 190 (Fig. 2). A minor fragmentation route of the *m/z* 207 ion, also confirmed by the corresponding metastable peak, is the loss of 48 u, with production of a weak peak at *m/z* 159. Elimination of NH_3 and CH_3SH, respectively, from the *N*-terminal *S*-methylcysteine accounts for these fragmentations (Scheme 1).

Methanolytic fragments of NE-type are present with large relative abundances at *m/z* 176 and 293 (Fig. 2). Both these ions show a loss of 32 u (supported by metastables), corresponding to a loss of methanol. This elimination has been observed previously in the FAB spectra of methanolytic mixtures of peptides containing glutamic acid 20 and can be rationalized by one of the two pathways shown in Scheme 2.

The FAB mass spectrum of the methanolytic mixture of the methionine-containing peptide **2** is shown in Fig. 3. It contains the MH^+ of the peptide methyl ester at *m/z* 424. MH^+ ions for the CE methanolytic fragments are present at *m/z* 293 and 180, respectively, the latter being very abundant. Signals for the NE methanolytic fragments cannot be distinguished from the background.

The FAB mass spectrum of the methanolysate of

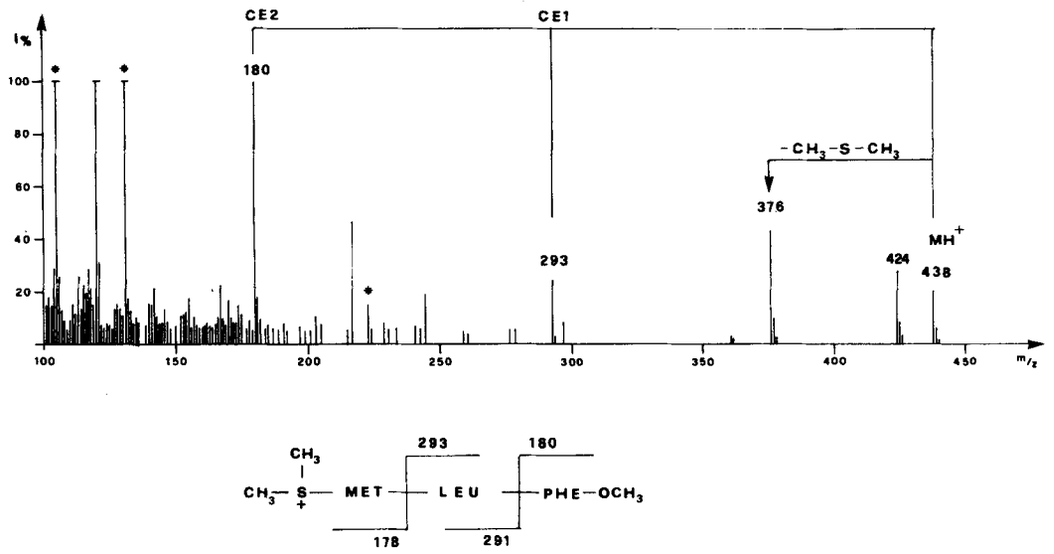
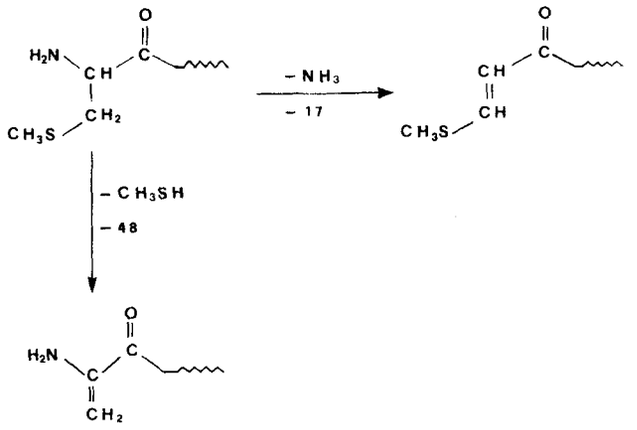


Figure 3. FAB mass spectrum of the mixture from partial methanolysis of peptide **2**.



Scheme 1

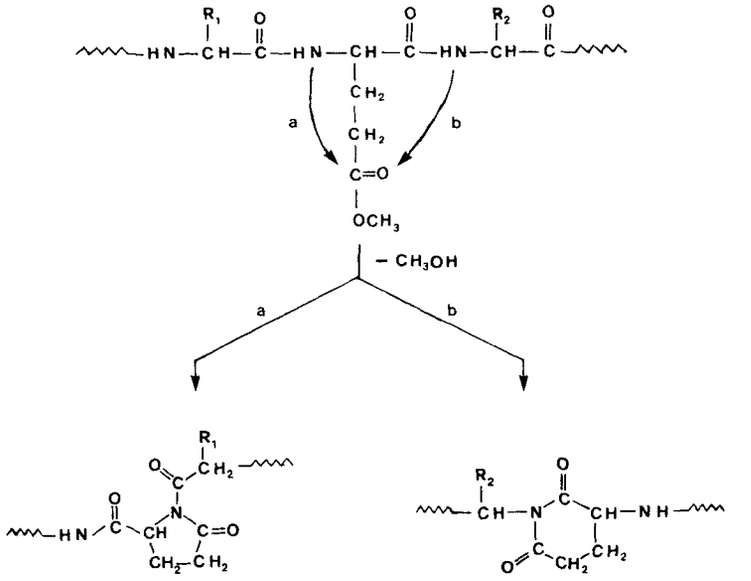
peptide **2** shows an additional signal in the molecular ion region at *m/z* 438 (424 + 14). An intense metastable peak indicates that this ion suffers a loss of 62 u to give an abundant daughter ion at *m/z* 376. The presence of these signals is unexpected, especially considering the behaviour of the *S*-methylcysteine described previously. The rationale for their formation is given in Scheme 3. According to this interpretation, the *m/z* 438 ion arises from methylation of the methionine residue during methanolysis. Under FAB conditions the pre-formed *S*-methylsulphonium ion is desorbed from the matrix and eliminates methyl sulphide through a rearrangement reaction to give a cyclic structure.

Methylation of the methionine residue with subsequent loss of methyl sulphide is also observed for peptide **3**. The FAB mass spectrum of its methanolytic mixture (Fig. 4) exhibits the MH^+ ion for the methyl ester of the peptide at *m/z* 588 and a second peak of comparable relative intensity at *m/z* 602, corresponding to the MH^+ of the *S*-methylated peptide. The *m/z* 602 ion gives a loss of 62 u with formation of a fragment ion at *m/z* 540 (supported by metastables). As methionine constitutes the *C*-terminus in this peptide, two series of CE fragments are formed, one containing the unmodified methionine residue and the other containing the methionine residue modified as a methylsulphonium group. Peaks at *m/z* 425, 368, 311 and 164 belong to the first group, whereas the second group includes signals at *m/z* 382, 325 and 178.

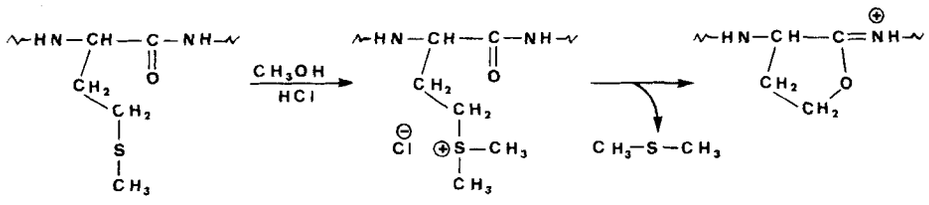
Only a weak MH^+ ion at *m/z* 253 is found for the NE methanolytic fragments.

CONCLUSION

It appears that using partial methanolysis/FAB mass spectrometry for sequence investigation, peptides containing cysteine require a stabilization of the free thiolic group. Methylation by methyl iodide can be conveniently used for this purpose. For peptides or methanolytic fragments in which cysteine constitutes the *N*-terminus, gas-phase decomposition with elimination of NH_3 and, to minor extent, CH_3SH from the *S*-



Scheme 2



Scheme 3

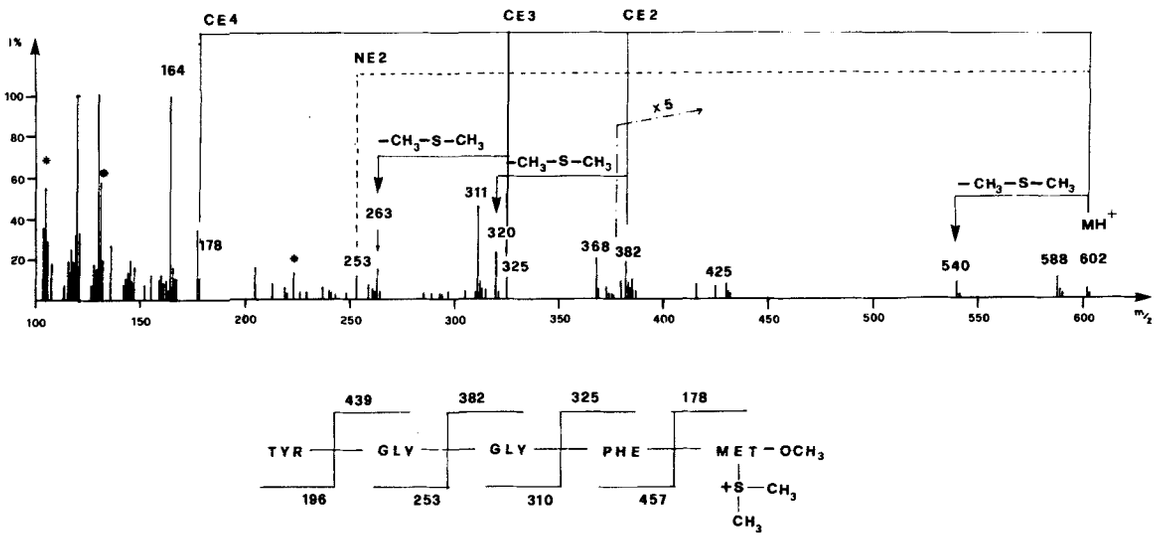


Figure 4. FAB mass spectrum of the mixture from partial methanolysis of peptide **3**.

methylcysteine residue may occur. When comparing the FAB spectrum of glutathione (Table 2) with that of the methanolytic mixture (Fig. 2), it is evident, however, that the amount of sequence information contained in the spectrum of the methanolysed peptide is considerably increased.

Methionine-containing peptides are methylated on sulphur to the extent of about 50% during methanolysis, with formation of an *S*-methylsulphonium ion. Under FAB conditions, this preformed ion is desorbed from the matrix and undergoes a gas-phase elimination of methyl sulphide, with formation of an intense daughter ion. Thus, three series of sequence-determining peaks are observed for methanolytic fragments containing methionine: those containing the unmodified methionine residue, those containing *S*-methylated methionine residue and those corresponding to the elimination of methyl sulphide from the *S*-methylmethionine residue. Although the occurrence of chemical modifications and gas-phase fragmentations results in the production of more complicated spectra, appropriate interpretation permits an insight into the amino acid sequence.

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