Field Desorption Mass Spectra of Gastrine Peptides and Glutathione Derivatives

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Oligopeptides comprising the sequence of the C-terminal tetrapeptide of gastrine, Trp-Met-Asp-Phe-NH2, and several derivatives of glutathione, γ-Glu-Cys(SR)-Gly, were characterized by field desorption mass spectrometry. The field desorption mass spectra obtained at various field ion emitter temperatures reveal abundant molecular ions and fragmentation reactions that yield partial sequence information. In the series of glutathione derivatives investigated, characteristic ions formed by cleavage of the γ-Glu-Cys peptide bond determine the substituent at the Cys residue and can therefore be used to identify corresponding conjugation products of drug metabolites with glutathione.

Introduction

A variety of mass spectrometric methods for the structure analysis of oligopeptides has been developed and tested in the last years [1–3]. Particularly, derivatization reactions to enhance the notoriously low volatility of peptides for mass spectral analysis have been studied extensively [4]. The most advanced approach developed by Biemann et al. [5] involves multiple derivatization steps that enable the analysis of complex mixtures of di- to tetra/penta-peptides by gas chromatography–mass spectrometry (g.c.–m.s.) using electron impact (e.i.) ionization. The value of this approach to the sequence elucidation of polypeptides containing up to 50 amino acid residues has been demonstrated [5, 6]. However, major limitations of derivatization procedures for the mass spectral analysis of peptides are that they require relatively large amounts of sample and present difficulties with certain polar amino acid residues. Moreover, information about chain-terminal or side chain substituents that frequently determine the biological activity of peptides will be lost during the necessary hydrolysis steps. More recently, studies by McLafferty et al. have shown that useful spectra and partial sequence information of larger oligopeptides can be obtained by chemical ionization (c.i.) mass spectrometry [7], but still some degree of (N-, C-terminal) derivatization is required for attaining vaporization of the peptide.

Since its introduction by Beckey and Schulten several years ago, field desorption (f.d.) mass spectrometry has become an established tool for the molecular weight determination of large, polar and/or thermally unstable molecules [8, 9]. Abundant molecular ions were obtained in f.d. mass spectra of underivatized oligopeptides with up to nine amino acid residues [10, 11]. However, little systematic work has yet been done to investigate the question whether the fragmentation observed in f.d. spectra of oligopeptides can be used to obtain, beyond the molecular ion of the peptide, additional structural information that might be complementary to other ionization techniques. A previously published paper on oligopeptides [11] and f.d. mass spectral work on tripeptides in our laboratory [12] indicated the presence of some sequence-specific fragment ions upon f.d. ionization, at appropriate field ion emitter temperatures. Such additional information would be especially useful in the case of...
substituted or “blocked” physiological peptides (such as many peptide hormones) that can not be isolated in amounts sufficient for derivatization and alternate mass spectral techniques. As part of a systematic study of biological peptides by f.d. mass spectrometry, we have investigated the f.d. mass spectra of two such examples: a) the tetrapeptide-amide, Trp-Met-Asp-Phe-NH₂, which represents the biologically essential C-terminal sequence of human gastrine I [13], using di- and tripeptides of this sequence as models to evaluate possible fragment ions under f.d. ionization; b) several derivatives (conjugates) of glutathione (γ-Glu-Cys-Gly, GSH), an important cellular “trapping” agent in many metabolic detoxication pathways of drugs. The potential utility of f.d. mass spectrometry for the direct structural characterization of GSH derivatives was of particular interest, since selective hydrolysis reactions for the separate analysis of the substrate metabolite are usually not possible for this type of conjugate. C.i. mass spectrometry of the GSH derivatives investigated in this study did not result in their positive structural characterization [14]. F.d. mass spectra of some GSH conjugates with reactive metabolites were already briefly reported [14].

**Results**

**Gastrine peptides**

Abundant molecular ions of the C-terminal tetrapeptide of gastrine I, Trp-Met-Asp-Phe-NH₂, were obtained in several f.d. spectra at emitter anode currents of 10 mA or greater. The spectrum at 14 mA (Fig. 1) that contained the most useful fragmentation pattern still shows the M⁺-ion (m/e 596) as well as the MH⁺-ion (m/e 597) with relative intensities of more than 30%. The appearance of “cluster” molecular ions (mainly MH⁺) is believed to arise from field emitter surface reactions and has been consistently observed in f.d. mass spectrometry [8]. As a consequence, f.d. fragment ions frequently also occur as cluster ions, with formal abstraction and addition of hydrogen. With this consideration, a series of fragmentation products leading to partly overlapping sequence information can be identified from the spectrum of Trp-Met-Asp-Phe-NH₂. If ions arising by direct cleavage of peptide bonds from the molecular ion are assigned C₁, C₂ etc., (C-terminus) and N₁, N₂ etc. (N-terminus), all possible C-terminal “sequence” ions (m/e 164, 165, C₁; m/e 279, 280, C₂; m/e 411, C₃) are found. Of the three possible N-terminal ions, N₃ (m/e 432, 433) and N₂ (m/e 317, 318) are observed, while a fragment corresponding to N₁ (m/e 187) is missing and was not observed in repetitive scans at emitter heating currents from 12 to about 18 mA. While the C-terminal ions can be rationalized with amine endgroups, both acylium (as known from e.i. fragmentation pathways) and keten are possible endgroups for the N-terminal ions but can not be determined exactly due to the uncertainty in the mass assignment of f.d. fragment ions. However, a fairly good consistency in the formation of these ions was found.

**Experimental**

The synthesis of the gastrine peptides has been described previously [15]. The dipeptide Met–Asp–OH was a commercial product (Serva, Heidelberg, W.-Germany). It was homogenous on thin-layer chromatography in methanol/water/acetic acid, 4/4/1. Glutathione was purchased from Merck, Darmstadt, W.-Germany. The GSH derivatives were isolated as conjugation products with reactive metabolites of the drugs acetylhydrazine, isopropylhydrazine and other compounds after incubation with rat liver microsomes as described previously [16]. Details of the isolation procedures, characterization and biological properties of these compounds have been reported [17].

The f.d. mass spectra were recorded with a Varian MAT CH 711 double focusing spectrometer equipped with an e.i./f.i./f.d. combination ion source. Activated 10 µm tungsten field ion emitters were used once for each peptide. The peptides were dissolved in DMSO at concentrations of 50–100 µg/20 µl and loaded on the emitter by the dipping technique [8]. The emitter was then introduced in the ion source by a direct insertion lock and heated gradually (2–3 min) from ambient to a maximum heating current of approximately 20 mA. During this time repetitive spectra were recorded on a magnetic tape of a computer (Varian SS 100). The emitter heating current at which the highest relative abundance of molecular ions occurred is referred to as “best anode temperature” (BAT) [8]. Both spectra recorded at BAT and further spectra at greater emitter temperatures were evaluated for fragment ions. Instrumental conditions were: 1000 (10%) resolution, 11 kV potential between field emitter anode and cathode, 220 °C source filament temperature, 2.8 kV voltage of the electron multiplier used for ion detection. E.i. spectra of high boiling perfluorokercsine (Merck, Darmstadt, W.-Germany) were used for mass calibration.
Table I. Fragment ions formed by direct cleavage of peptide bonds in the f.d. mass spectra of Trp-Met-Asp-Phe-NH$_2$ (I), Met-Asp-Phe-NH$_2$ (II), Asp-Phe-NH$_2$ (III) and Met-Asp-OH (IV).

<table>
<thead>
<tr>
<th>m/e</th>
<th>Partial sequence</th>
<th>N, C-terminal sequence ion$^a$ (% relative intensity)$^b$</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>433</td>
<td>Trp-Met-Asp</td>
<td>N$_2$ (8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>411</td>
<td>Met-Asp-Phe-NH$_2$</td>
<td>C$_3$ (6)</td>
<td></td>
<td>MH$^+$ (100)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>318</td>
<td>Trp-Met</td>
<td>N$_2$ (35)</td>
<td></td>
<td>C$_3$ (13)</td>
<td>MH$^+$ (100)</td>
<td></td>
</tr>
<tr>
<td>280</td>
<td>Asp-Phe-NH$_2$</td>
<td>C$_2$ (6)</td>
<td></td>
<td>C$_2$ (11)</td>
<td></td>
<td>MH$^+$ (100)$^c$</td>
</tr>
<tr>
<td>247</td>
<td>Met-Asp</td>
<td>N$_2$ (11)</td>
<td></td>
<td>C$_2$ (13)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>187</td>
<td>Trp</td>
<td>N$_1$ (—)</td>
<td></td>
<td>C$_1$ (15)</td>
<td>C$_1$ (16)</td>
<td></td>
</tr>
<tr>
<td>164</td>
<td>Phe-NH$_2$</td>
<td>C$_1$ (20)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>132</td>
<td>Met</td>
<td>N$_1$ (—)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>117</td>
<td>Asp</td>
<td>N$_1$ (20)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^a$ Partial sequences starting from the N-terminus are assigned N$_1$, N$_2$, . . ., sequences starting from the C-terminus C$_1$, C$_2$ etc.

$^b$ See Table II for emitter heating currents at which spectra were obtained.

$^c$ MH$^+$-ion, m/e 265 of Met-Asp-OH.

$^d$ Same m/e-value as C$_1$-ion of Met-Asp-OH.

by comparison with the f.d. mass spectra of the model peptides, Met-Asp-Phe-NH$_2$, Asp-Phe-NH$_2$ (Fig. 2), and Met-Asp-OH (spectrum not shown). Thus, a C$_2$-ion is also observed in the spectrum of Met-Asp-Phe-NH$_2$ (molecular ion of Asp-Phe-NH$_2$), and the C$_1$-ion, Phe-NH$_2$ appears in the spectra of all three homologues peptides. Except for the N$_1$-ion that is missing in the spectrum of Met-Asp-Phe-NH$_2$, N-terminal ions were also observed for the di- and tripeptides (Table I).

Less consistently, other fragmentation products were observed. In the spectra of all peptides, elimination of water and/or NH$_3$, and CO$_2$ or COOH leads to abundant ions at emitter temperatures above BAT, but can not be differentiated clearly within the corresponding cluster ions. These fragments seem to occur unspecifically in f.d. mass spectra of peptides [11, 12]. Among other direct fragmentation reactions of the peptide backbone, several ions originate by N-alkyl cleavage, such as m/e 449 and 263 in the spectrum of Trp-Met-Asp-Phe-NH$_2$ and m/e 262/263 and 149 in the spectrum of Met-Asp-Phe-NH$_2$ (Table II). In the f.d. spectra of Met-Asp-Phe-NH$_2$ and Asp-Phe-NH$_2$, some fragments could also be assigned to direct C-C cleavage reactions, for example ions at m/e 307/308 and m/e 191/192 (cf. Fig. 2). The formation of these ions was quite inconsistent at
Table II. F.d. fragment ions of peptides I, II, III and IV formed by N-Alkyl cleavage or other direct fragmentation of the peptide backbone.

<table>
<thead>
<tr>
<th>Peptide</th>
<th>mA</th>
<th>Fragment ion, m/e (% relative intensity)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>14</td>
<td>449(8), 263(7), 149(8)</td>
</tr>
<tr>
<td>II</td>
<td>10</td>
<td>308(17), 307(15), 263(8), 262(5), 149(10), 148(9)</td>
</tr>
<tr>
<td>III</td>
<td>10</td>
<td>192(15), 191(18), 131(15), 88(19)</td>
</tr>
<tr>
<td>IV</td>
<td>9</td>
<td>160(10), 148(8), 104(15)</td>
</tr>
</tbody>
</table>

a Cf. Table I.
b Emitter heating current at which spectrum was recorded.

different field emitter temperatures, and they may be of little value for obtaining additional information of the peptide sequence. However, with the exception of the fragment m/e 228 in the spectrum of Met-Asp-Phe-NH₂ that might arise by elimination of water from m/e 246, there was a notable lack of more abundant ions corresponding to multiple fragmentation or fragmentation of the peptide side chain.

Derivatives of glutathione

Glutathione (GSH) as well as several of its derivatives isolated as conjugates with various drug
cleavage of the γ-Glu-Cys peptide bond was found, whereas in no case cleavage of the Cys-Gly bond was observed (Fig. 3 and 4). The spectra of all GSH derivatives investigated showed characteristic fragments at m/e 130 and m/e 84 of high abundance, which are likely to explain by the formation of a pyroglutamyl ion. The tendency for a preferential formation of pyroglutamyl has been previously observed in f.d. mass spectra of oligopeptides containing α-Glu at the N-terminus [18]:

Consistent with the formation of the pyroglutamyl ion, the complementary fragment ions of the Cys-Gly residue were found throughout the series of metabolites [17] gave molecular peaks of high intensities in their f.d. spectra, at a range of emitter heating currents from 10 to 15 mA. More consistently than in the case of the gastrine peptides, the MH⁺-ion was the predominant molecular ion species (m/e 308 for GSH, Fig. 3,a). At emitter temperatures which produced fragment ions (about 12 mA heating current), all GSH derivatives showed elimination of H₂O and/or CO₂ (e.g., m/e 290 and 263 for GSH, m/e 332 and 305 for S-Acetylglutathione), presumably by simple pyrolytic processes. However, as major fragmentation pathway, the

Table III. Fragment ions formed by cleavage of γ-Glu-Cys peptide bonds in the f.d. mass spectra of glutathione derivatives.

<table>
<thead>
<tr>
<th>R</th>
<th>m/e (%) relative intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>13 178 (11)</td>
</tr>
<tr>
<td>CH₃</td>
<td>14 192 (22)</td>
</tr>
<tr>
<td>C₂H₅</td>
<td>15 232 (14)</td>
</tr>
<tr>
<td>CO₂CH₃</td>
<td>12 208 (100)</td>
</tr>
<tr>
<td>CH₃COH</td>
<td>16 220 (42)</td>
</tr>
<tr>
<td>CH₃COCH₃</td>
<td>15 220 (25)</td>
</tr>
<tr>
<td>CH₃C₆H₅</td>
<td>14 268 (14)</td>
</tr>
<tr>
<td>1-(2-hydroxy)-estradiol</td>
<td>18 446 (18)⁺</td>
</tr>
</tbody>
</table>

⁺ Emitter heating current at which spectrum was recorded.

Table: Fragment ions (464-H₂O)⁺.
GSH derivatives. Although for GSH itself, the fragment Cys-Gly (m/e 178) was rather weak, the corresponding ions of the S-substituted derivatives appeared partially with high abundances (Table III). Therefore, these characteristic ions provide a possibility for the direct substrate identification of

![Diagram](image1)

**Fig. 4.** F.d. mass spectra of γ-Glu-Cys(CH₃)-Gly at 14 mA (a) and γ-Glu-Cys(C₆H₅)-Gly at 12 mA (b) emitter heating current.

![Diagram](image2)

**Fig. 5.** F.d. mass spectra of γ-Glu-Cys-(1-(2-hydroxyestradiol))-Gly at 14 mA (a) and 18 mA (b) emitter heating current.
corresponding conjugation products of drugs and their metabolites by f.d. mass spectrometry, in addition to the molecular ion. As an example for the utility of f.d. mass spectrometry to identify corresponding metabolite conjugates with GSH, we investigated a GSH conjugation product of 2-hydroxyestradiol which was isolated as a metabolite during studies of the hepatic microsomal metabolism of estradiol [14]. The f.d. spectrum of this compound (Fig. 5) showed at 14 mA emitter heating current almost exclusively the molecular ion MH+ (m/e 594) consistent with a GSH conjugate of hydroxylated estradiol. In the spectrum recorded at 18 mA heating current, no molecular ion but several fragmentation products providing structural information are obtained (Fig. 5, b). Besides the intensive ion of the pyroglutamyl residue (m/e 130), a complementary ion at m/e 464 is not found, but a cluster of peaks centered at m/e 446 instead, which is likely to be formed by cleavage of the Gly-Cys bond and additional elimination of water from the steroid skeleton. In addition, the two abundant fragment ions at m/e 320 and m/e 288 provide direct evidence to identify the conjugation position of the metabolite at the Cys residue (see fragmentation scheme depicted in Fig. 5).

Discussion

Consistent with the previous studies of oligopeptides by f.d. mass spectrometry, abundant molecular ions were readily obtained in the f.d. spectra of the gastrine peptides and glutathione derivatives which, without any derivatization, did not give useful structure information by e.i. or c.i. mass spectrometry [14]. Thus, the e.i. mass spectra of GSH and its derivatives were dominated by a multiplicity of pyrolytic fragments in the low mass region (M. Przybylski, unpublished observation). Derivatization procedures of peptides containing amino acids with multiple functional groups as those studied here would lead to a drastical increase in molecular weight already for smaller oligopeptides, into a mass range that might be difficult to determine even if sufficient volatility for e.i. or c.i. mass spectral analysis is obtained. Thus, the f.d. mass spectrometric molecular weight determination might be a valuable initial step in the problem of elucidating the structure of oligopeptides isolated in small amounts from biological material. Molecular ions have been obtained in f.d. mass spectra of peptides containing amino acids such as Arg [10] and His [18] that are known to present difficulties with other mass spectrometric methods. However, more systematic work is needed to show whether a molecular ion determination of oligopeptides can be obtained with certainty by f.d. mass spectrometry. A critical example has been observed previously in f.d. mass spectra of tripeptides with a-Glu at the N-terminus because of the tendency to form pyroglutamyl [12], yielding abundant (M–H2O)±-ions. In the series of GSH derivatives (N-terminal γ-Glu), pyroglutamyl formation did not affect molecular weight determination but even leads to fragment ions that allow to differentiate specifically the binding site of corresponding drug metabolite-GSH conjugates [14] (c.f. Table III). A problem in the definite molecular weight determination of peptides by f.d. mass spectrometry could be the formation of cluster ions [8, 19, 20]. Besides the addition of a proton (MH+·ion), peaks due to the addition of H2O or other small molecules such as ethanol present in the sample solute have been observed [11], although these ions are generally of lower intensity than the molecular ion. In the case of a completely unknown sample, a certain molecular weight determination might therefore best be obtained in connection with an independent amino acid analysis which, likewise, will be very useful for the interpretation of fragment ions in f.d. mass spectra.

In addition to the molecular ion information available, the present f.d. mass spectral data of peptides seem to indicate that at least some degree of useful structure information can be obtained from fragment ions arising above BAT. In the series of the gastrine peptides, an almost complete sequence of peptide bond cleavage products ("sequence ions" [21]) was found. Although the mechanisms of f.d. fragmentation pathways are largely unknown, fragment ion formation seems to originate mainly from the molecular ion directly which, in the case of peptides, facilitates greatly their assignment to parts of the molecular structure. The observation from this and previous investigations that f.d. fragmentation often yields complementary breakdown products [22] might be a further advantage that facilitates the analysis of sequence ions. The question of differentiating thermal and field-induced fragmentation reactions has recently been investigated in a detailed model study on the f.d. fragmentation of methionine [23].
using techniques such as isotope labelling and high resolution data. For oligopeptides, an important factor might be the drastic changes in volatility and corresponding desorption rates within the molecular size range that seems practicable at present for f.d. analysis (peptides with up to 15 to 20 amino acid residues as suggested by Winkler et al. from a f.d. mass spectrometric study of glucagon [24]). To better understand these key questions, f.d. mass spectrometric studies with model peptides of various sizes are presently subject of further work in our laboratory.