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Fragmentation reactions of protonated peptides containing phenylalanine: a linear free energy correlation in the fragmentation of H–Gly–Xxx–Phe–OH

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Abstract

The fragmentation reactions of a variety of protonated tri- and tetra-peptides containing phenylalanine have been examined using metastable ion studies and energy-resolved collision-induced dissociation studies. For peptides with the sequence H–Gly–Xxx–Phe–OH (Xxx = Gly, Ala, Val, Leu, Phe) the major primary fragmentation of MH^+ involves cleavage of the C-terminus amide bond to form either the b_2 ion or the y_1'' ion. For metastable ion fragmentation it is found that $\log([b_2]/[y_1''])$ increases linearly with the increase in the gas phase basicity of H–Xxx–OH. This linear free energy correlation is in contrast to the lack of such a correlation in the fragmentation of protonated H–Gly–Xxx–Gly–OH [J. Mass Spectrom. 30 (1995) 290]. When Phe is the central residue in tripeptides, the major primary fragmentation reaction involves formation of the b_2 ion which fragments further to the a_2 ion; at higher internal energies the a_2 ion fragments to give the phenylalanine immonium ion which becomes the dominant fragment. When Phe is in the N-terminus position, as in Phe–Gly–Gly–OH, the phenylalanine immonium ion is the dominant fragment and is formed, in part, directly by fragmentation of MH^+. The fragmentation of the tetrapeptides H–Gly–Gly–Phe–Leu–OH, H–Phe–Gly–Gly–Phe–OH and H–Val–Ala–Ala–Phe–OH are more complex but show a substantial directional effect of the phenylalanine residue(s). (Int J Mass Spectrom 217 (2002) 185–193) © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Fragmentation reactions; Protonated peptides; Phenylalanine residues

1. Introduction

Collision-induced dissociation (CID) of protonated peptides continues to be an important method of obtaining the amino acid sequence of the peptide [1–4]. As a result of many such studies, the main features of the fragmentation of protonated peptides have been elucidated, at least in a phenomenological sense, as illustrated in Scheme 1 [5,6]. However, the factors which control the relative abundances of the various fragment ions are less clearly understood, largely because the various approaches to elucidating fragmentation pathways and mechanisms have only occasionally been applied in a systematic way to the fragmentation of peptides.

One approach which shows some promise is to probe for a correlation of fragment ion intensities with the thermochemical properties of the constituent amino acids. Isa et al. [7] have carried out a systematic study of the high-energy CID mass spectra of several series of protonated dipeptides H–Xxx–Gly–OH, H–Gly–Xxx–OH, H–Xxx–Leu–OH and

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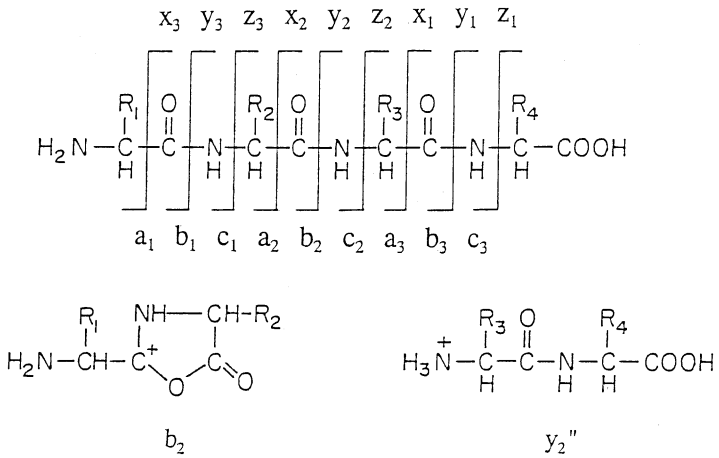


Scheme 1.

H–Leu–Xxx–OH. They focused on formation of the y_1'' ion and showed that the proton affinity of the C-terminal amino acid must be greater than that of the N-terminal amino acid for the y_1'' ion to be observed. A more quantitative study was carried out by Morgan and Bursey [8] on protonated tripeptides H–Gly–Gly–Xxx–OH where it was observed that $\log([y_1'']/[b_2])$ showed a linear increase with increasing proton affinity of H–Xxx–OH, a linear free energy correlation [9]. When the variable amino acid was in the N-terminal position, as in H–Xxx–Gly–Gly–OH, $\log([y_2'']/\Sigma$ fragment ions) was found to decrease in a linear fashion with an increase in the proton affinity of H–Xxx–OH [10]. By contrast for protonated peptides, H–Gly–Xxx–Gly–OH, no correlation of fragment ion intensities with the proton affinity of H–Xxx–OH could be discerned [10]. Vaisar and Urban [11] have shown that, in the low-energy CID of protonated acyl–Ala–Pro–NH_2 compounds, $\log([y_1'']/\Sigma$ fragment ions) increased linearly with the proton affinity of the acyl group, the latter being taken as a measure of the nucleophilicity of the acyl group. The results were interpreted as supporting the formation of neutral oxazolones [12–14] during fragmentation to form the y_1'' ion.

Recent work in this laboratory [15] has shown that, in the fragmentation of protonated H–Val–Xxx–OH peptides, $\log([y_1'']/[a_1])$ increased linearly with PA(H–Xxx–OH). However, for the protonated dipeptides H–Xxx–Phe–OH, $\log([a_1]/[y_1''])$ gave a poor correlation with proton affinity or gas-phase basicity of H–Xxx–OH. When H–Xxx–OH was an aliphatic amino acid a good correlation of $\log([a_1]/[y_1''])$ with the Taft–Topsom σ_α for the alkyl group [16] was observed (σ_α is a measure of ion/induced dipole stabilization of charge sites by the alkyl group). The results for the protonated dipeptides were interpreted in terms of initial formation of a proton-bound complex of an aziridinone and an amino acid which may fragment to form either a protonated amino acid (y_1'') or a N-protonated aziridinone with the corresponding neutrals being an aziridinone and an amino acid. Ab initio calculations showed that the N-protonated aziridinone is unstable and eliminates CO to form the a_1 immonium ion.

In the present work, a detailed study has been made of the fragmentation of protonated peptides containing phenylalanine. In the course of this study it was found that, in the fragmentation of protonated peptides H–Gly–Xxx–Phe–OH, $\log([b_2]/[y_1''])$ was a linear function of the gas-phase basicity of H–Xxx–OH (GB(H–Xxx–OH)). This is in contrast to the results of Morgan and Bursey [10] who found no linear free

energy correlation in the fragmentation of protonated H–Gly–Xxx–Gly–OH. The reasons for this difference will be discussed as will the significance of the correlation observed. The fragmentation reactions of a variety of protonated peptides containing phenylalanine will be discussed to illustrate the influence of the phenylalanine residue on the fragmentation reactions observed.

2. Experimental

Metastable ion studies were carried out by B/E linked scans [17,18] on a VG Analytical (Manchester, UK) 70-250S EB double-focusing mass spectrometer controlled by an Opus data system. Ionization was by fast atom bombardment (FAB) using a Xe atom beam of 8 keV energy with the sample dissolved in glycerol or thioglycerol.

Collision-induced dissociation (CID) studies were performed using an electrospray ionization/quadrupole mass spectrometer (VG Platform, Micromass, Manchester, UK). It is well known [19,20] that CID can be achieved in the interface region between the atmospheric pressure source and the quadrupole mass analyzer, so-called cone voltage CID. It has been established [21–23] that the average energy imparted to the decomposing ions increases as the field in this interface region increases and recent work [24–26] has shown that, by varying this field in steps, energy-resolved mass spectra [27–29] comparable to those obtained in variable, low-energy CID in quadrupole cells are obtained. The results are presented in the following as breakdown graphs expressing the percent of total ion signal as a function of the cone voltage, a measure of the field in the interface region. Ionization was by electrospray with the sample, at micromolar concentration in 1:1 CH_3CN/H_2O, being introduced into the source at a flow rate of 30 μL min^{-1}. The electrospray capillary was held at 2.5–3.0 kV and N_2 was used as both nebulizing and drying gas.

All peptide samples were obtained from BACHEM Biosciences (King of Prussia, PA).

Table 1
Metastable ion fragmentation of protonated H–Gly–Xxx–Phe–OH

Xxx	Ion signal (%)		
	b_3	b_2	y_1''
Gly[a]	16.8	1.9	73.3
Ala	13.1	9.9	77.0
Val	7.6	46.8	45.7
Leu	10.0	52.9	37.1
Phe	11.2	65.5	23.3

[a] y_2'' (7.9%) also observed.

3. Results and discussion

3.1. Fragmentation of protonated H–Gly–Xxx–Phe–OH

Table 1 presents the metastable ion mass spectra of five protonated tripeptides of structure H–Gly–Xxx–Phe–OH where Xxx is varied; the breakdown graphs for the five protonated species are presented in Figs. 1–5. On the metastable ion time frame

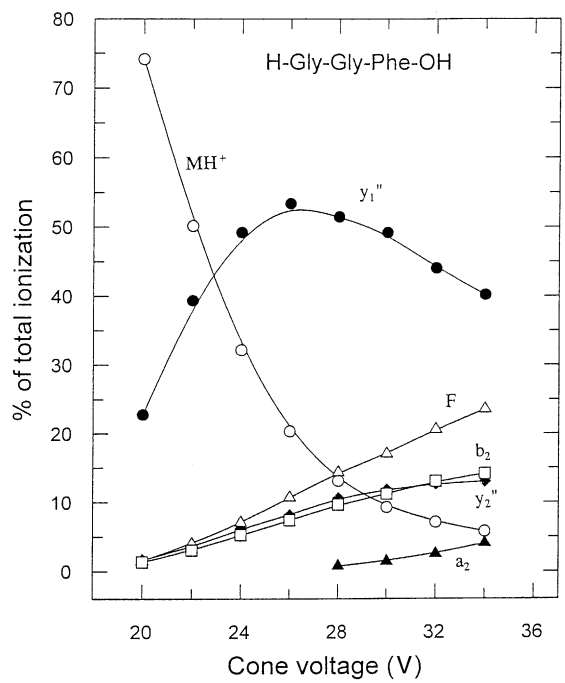


Fig. 1. Breakdown graph for protonated H–Gly–Gly–Phe–OH.

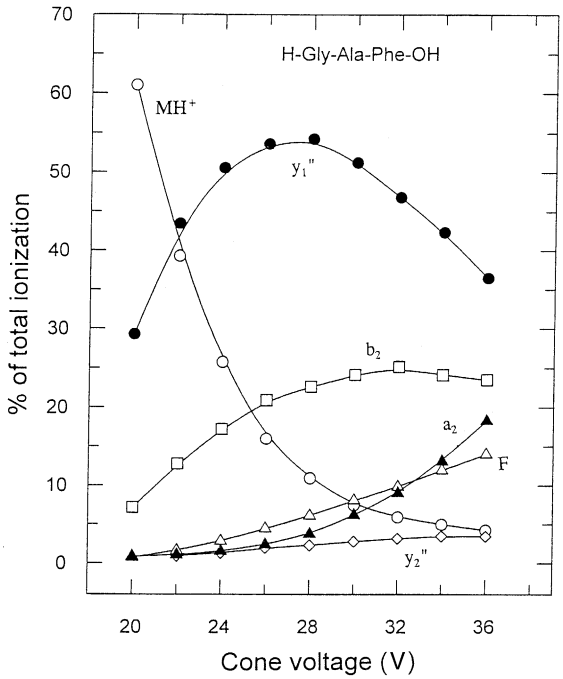


Fig. 2. Breakdown graph for protonated H–Gly–Ala–Phe–OH.

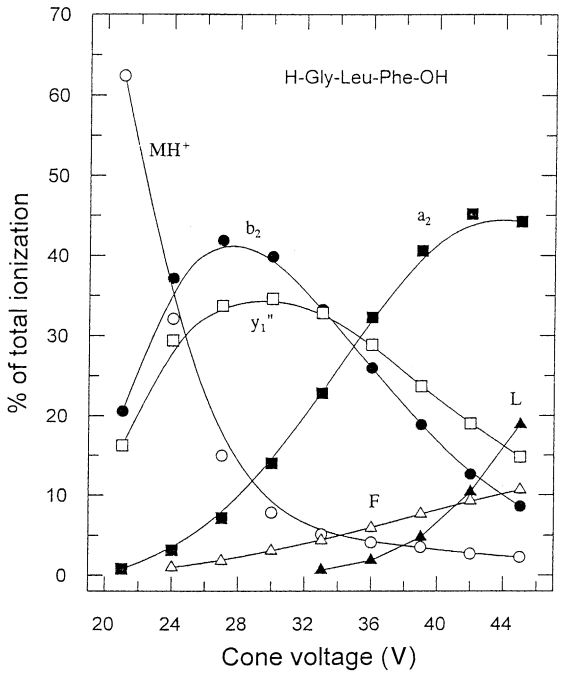


Fig. 4. Breakdown graph for protonated H–Gly–Leu–Phe–OH.

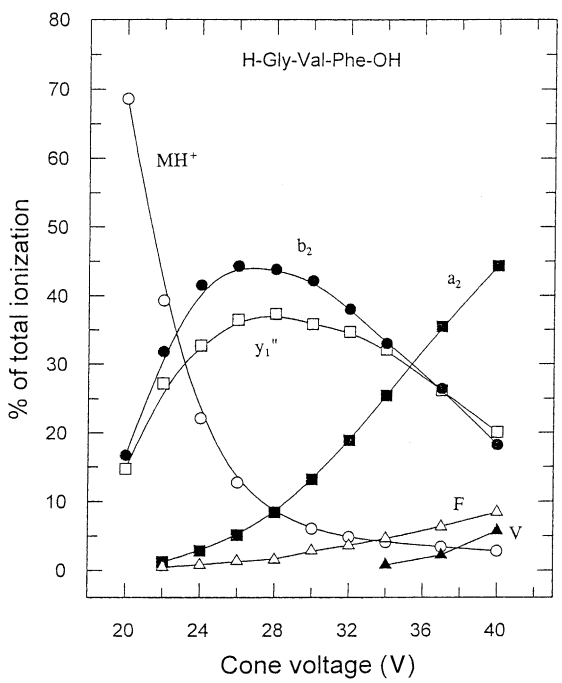


Fig. 3. Breakdown graph for protonated H–Gly–Val–Phe–OH.

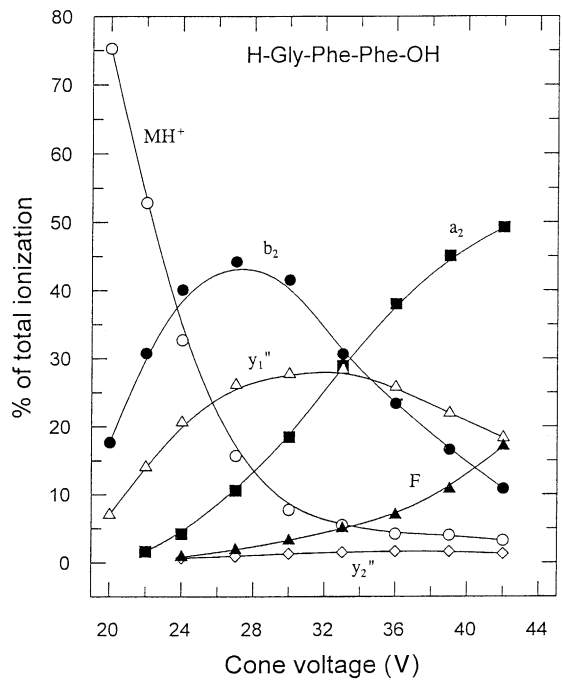


Fig. 5. Breakdown graph for protonated H–Gly–Phe–Phe–OH.

there is minor elimination of H_2O from the MH^+ ions to form the b_3 ion; however, the major fragmentation route involves cleavage of the C-terminus amide bond to form either the b_2 ion or the y_1'' ion, with the former increasing substantially in importance as the variable central residue is changed from Gly to Phe. Under CID conditions (Figs. 1–5) formation of the b_3 ion is of negligible importance, the major primary fragmentation products being the b_2 and the y_1'' ions. With increasing collision energy (increasing cone voltage) the y_1'' ion shows fragmentation to the phenylalanine immonium ion (F) while the b_2 ion fragments further to the a_2 ion. At the highest collision energies the a_2 ions show further fragmentation to give the immonium ion derived from the central amino acid as has been reported earlier in several examples [30,31].

Fig. 6 shows a plot of $\log([b_2]/[y_1''])$ vs. the gas-phase basicity (GB) [32] of the central amino acid H–Xxx–OH for the metastable ion data of Table 1. A satisfactory linear correlation ($r = 0.985$) is obtained. (A similar linear correlation is observed if $\log([b_2]/[y_1''])$ is plotted vs. the proton affinity of H–Xxx–OH.) The linear free energy correlation observed in the present system is in contrast to the lack of any linear correlation reported by Morgan and Bursey [10] for fragmentation of protonated H–Gly–Xxx–Gly–OH. Examination of their experimental data shows that the b_2 ion was the dominant

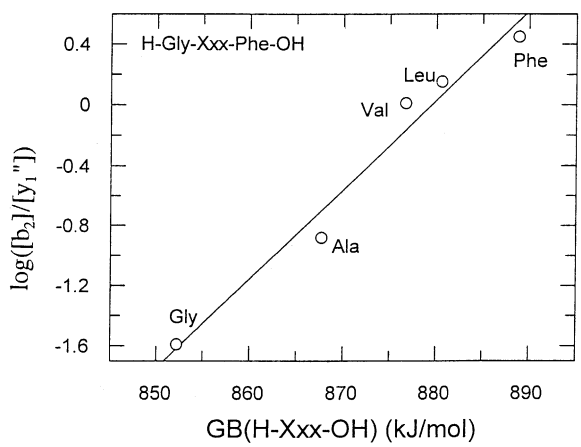


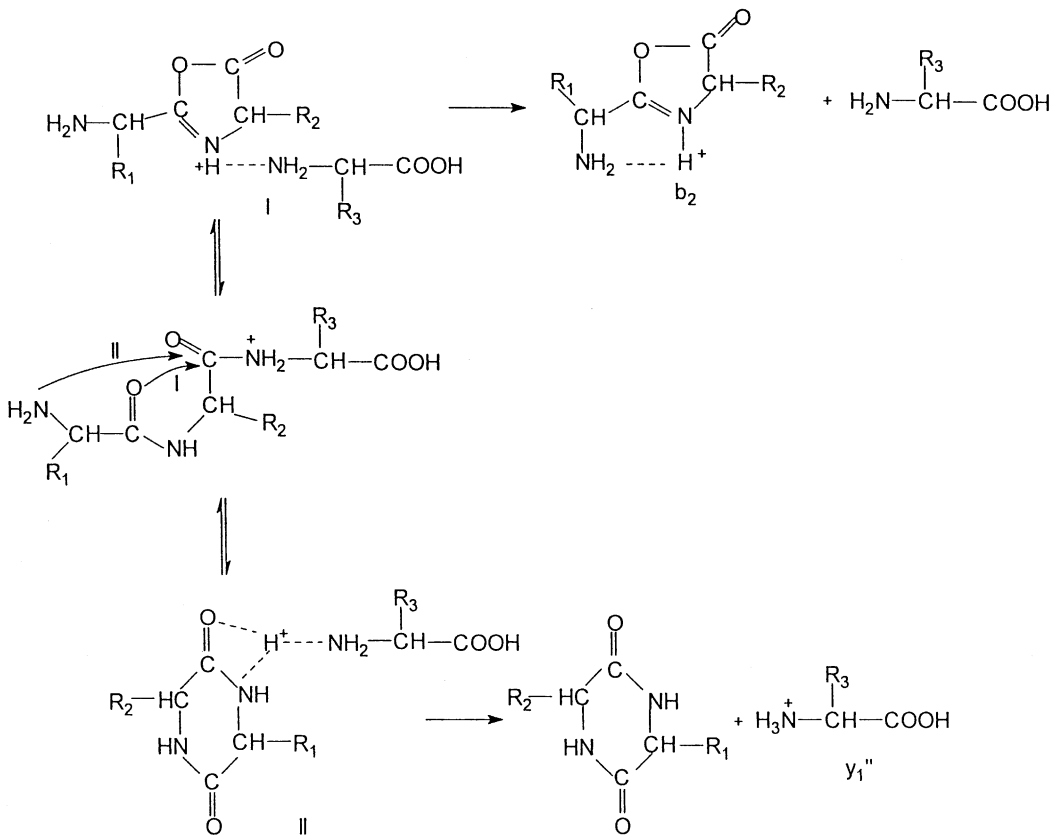
Fig. 6. $\log([b_2]/[y_1''])$ as a function of GB(H–Xxx–OH).

fragment ion in all cases with the y_1'' ion observed only for H–Gly–Gly–Gly–OH. In effect, the C-terminus glycine residue has a low gas-phase basicity and cannot compete for charge retention upon cleavage of the amide bond. In the present system, the greater basicity of the C-terminus phenylalanine results in competition between the N-terminus fragment and the C-terminus fragment for retention of the charge on cleavage of the amide bond.

The available evidence [12–14,33] indicates that, in the fragmentation of protonated tripeptides, the b_2 ion has the structure of a protonated oxazolone. However, when the y_1'' ion is formed the accompanying neutral is not an oxazolone but rather a diketopiperazine. This is illustrated in Scheme 2. The linear free energy correlation observed in the present study shows that the R_2 substituent is influencing the energy required to reach the ion–neutral complex I in the same fashion as it influences the basicity and proton affinity of $H_2NCH(R_2)COOH$. The R_2 side-chain is remote from the site of action in forming the complex II and should have little effect on the energy requirements to form this complex. There is an alternative rationalization possible, however. Wysocki et al. [34] have suggested that b_2 ion formation may occur from the *O*-protonated species rather than the *N*-protonated species shown in Scheme 2. Thus, one cannot discount the possibility that y_1'' ion formation occurs from the *N*-protonated species, as shown, but b_2 ion formation occurs from the *O*-protonated species and the effect of the R_2 substituent is to change the fraction of MH^+ ions which are *O*-protonated.

3.2. Other peptides containing phenylalanine

The metastable ion mass spectra and the energy-resolved CID mass spectra of a number of protonated tri- and tetra-peptides containing phenylalanine were also studied. Fig. 7 shows the breakdown graph for protonated H–Gly–Phe–Gly–OH. Not unexpectedly, the b_2 ion is the dominant low energy fragmentation product. This also is true in the metastable ion mass spectra where minor formation of the b_3 ion (2.6%) and major formation of the b_2 ion (97.4%)



Scheme 2.

was observed. The breakdown graph shows further fragmentation of the b_2 ion to the a_2 ion which, at higher internal energies, fragments to form the phenylalanine immonium ion (F); this sequential fragmentation of the Gly–Phe b_2 ion has been observed previously [30]. Protonated H–Gly–Phe–Ala–OH and H–Ala–Phe–Gly–OH (data not shown) exhibited a similar behavior in that the b_2 ion was the major primary fragment with formation of a_2 at higher cone voltages and formation of the phenylalanine immonium ion at even higher cone voltages.

The breakdown graph for protonated H–Phe–Gly–Gly–OH (Fig. 8) shows formation of the y_2'', b_2 and a_1 fragment ions, with the latter dominating at higher cone voltages. In metastable ion fragmentation of MH^+ formation of b_3 (9.3%), b_2 (68.8%), y_2'' (2.8%) and a_1 (19.1%) ions was observed. In an earlier study [30] of metastable ion fragmentation of MH^+ in the quadrupole cell of a BEqQ mass spectrometer formation of b_3 (0.8%), b_2 (76.9%) and a_1 (22.3%) was reported. It seems clear that the a_1 ion is originating, at least in part, directly from fragmentation of the protonated tripeptide. The a_1 ion also originates, in part, by fragmentation of the b_2 ion [30]. A similar domination of the breakdown graph by the a_1 ion is shown in the fragmentation of protonated H–Leu–Gly–Phe–OH (Fig. 9) where the a_1 ion (leucine immonium ion) is the dominant ion at higher cone voltages. The metastable ion mass spectra of MH^+ showed formation of b_3 (10.4%), y_2'' (26.2%), b_2 (25.8%), y_1'' (32.3%) and a_1 (5.2%). Thus, it appears in this case as well, that the a_1 ion originates, at

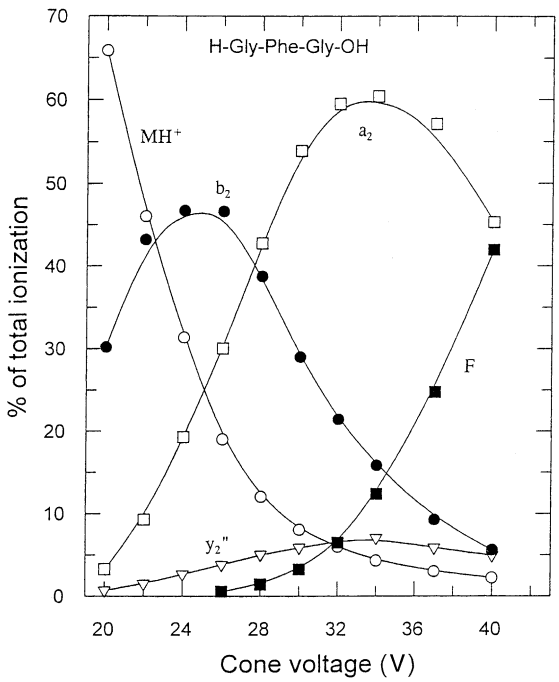


Fig. 7. Breakdown graph for protonated H–Gly–Phe–Gly–OH.

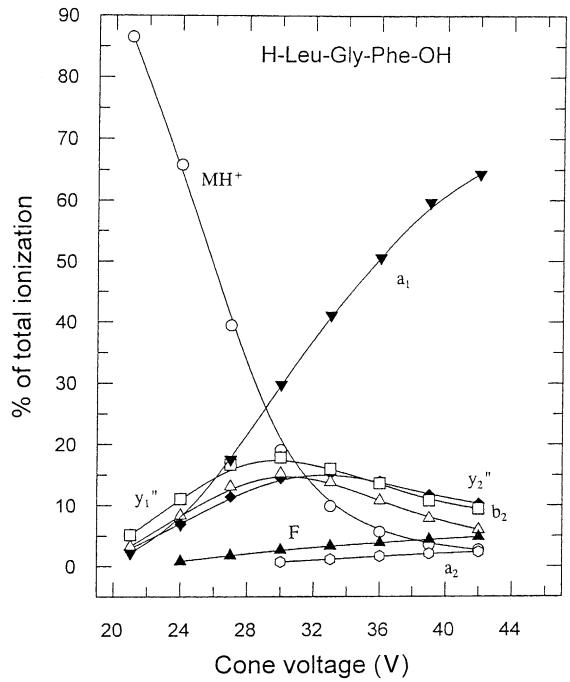


Fig. 9. Breakdown graph for protonated H–Leu–Gly–Phe–OH.

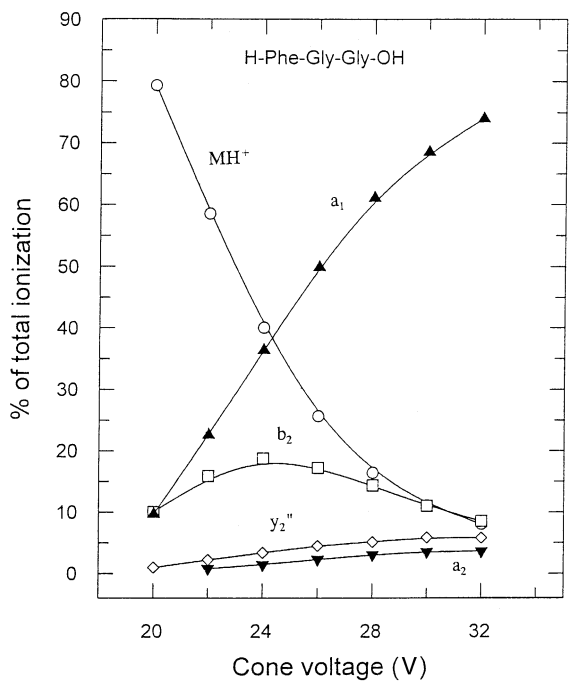


Fig. 8. Breakdown graph for protonated H–Phe–Gly–Gly–OH.

least in part, directly by fragmentation of the protonated tripeptide. The a_1 ion also is formed by further fragmentation of the b_2 ion [13,31].

Three tetrapeptides, H–Gly–Gly–Phe–Leu–OH (GGFL), H–Phe–Gly–Gly–Phe–OH (FGGF) and H–Val–Ala–Ala–Phe–OH (VAAF) were studied. The metastable ion mass spectra and the CID mass spectra at 36 V cone voltage are summarized in Table 2. The fragmentation reactions observed are more varied and complex than those observed for the tripeptides. In metastable ion fragmentation elimination of H_2O to give, nominally, the b_4 ion is a significant fragmentation route; however, this pathway is of only minor importance under CID conditions. For both GGFL and FGGF formation of the phenylalanine immonium ion (F) becomes important upon collisional activation. For GGFL metastable ion fragmentation of the b_3 ion observed in the FAB mass spectrum resulted in formation of the a_3 ion (67.3%) and the phenylalanine immnonium ion (F) (32.7%). An earlier metastable ion study [30] of the fragmentation of protonated

Table 2
Metastable ion and CID fragmentation of protonated tetrapeptides

Ion	Base peak (%)					
	GGFL		FGGF		VAAF	
	m^*	CID	m^*	CID	m^*	CID
b_4	45.2	7.6	45.2	2.8	22.0	
a_4		3.5	18.4	4.2		
y_3''		2.4	65.3	37.8	22.0	10.8
b_3	100	79.6	77.9	45.4	77.3	48.9
a_3		11.5				
y_2''	44.7	49.7	100	100	100	100
b_2		2.8	31.6	52.7	12.4	66.9
a_2				10.5		13.7
y_1''		9.9		22.0		14.5
F		100		61.2		1.3
L		15.3				
V						8.2

H–Phe–Leu–OH (the y_2'' ion from GGFL) showed major fragmentation to form the phenylalanine immonium ion, indicating a second pathway to this product. For FGGF, the phenylalanine immonium ion, F, presumably originates primarily by further fragmentation of the b_3 and b_2 ions although formation directly from MH^+ under CID conditions cannot be excluded. An earlier study [30] has shown that the Phe–Gly b_2 ion does fragment under CID conditions to give both the a_2 and a_1 ions with the letter predominating. It is interesting that the y_2'' ion is formed in primary fragmentation rather than the y_1'' ion. This presumably reflects the fact that the proton affinities of dipeptides generally are greater than that of either constituent amino acid [35]. The y_1'' ion also is a secondary product for protonated VAAF originating, at least in part, by further fragmentation of the y_2'' as shown earlier [15].

4. Conclusions

The most striking observation in the present work is that fragmentation of protonated H–Gly–Xxx–Phe–OH involves primarily cleavage of the C-terminus amide bond to produce b_2 or y_1'' ions and that $\log(b_2/y_1'')$ increases in a linear fashion with the increase in the gas-phase basicity of H–Xxx–OH. This linear free energy correlation contrasts with the lack of such a correlation in the fragmentation of protonated H–Gly–Xxx–Gly–OH [10]. In the latter case the gas-phase basicity of H–Gly–OH is sufficiently low that, in cleavage of the amide bond, formation of the b_2 ion is overwhelmingly favored in all cases studied. In the present system the gas-phase basicity of H–Phe–OH is sufficiently high that formation of the y_1'' competes effectively on cleavage of the amide bond. Accepting the reaction pathways outlined in Scheme 2, it is apparent that the substituent on Xxx affects the activation barrier to reach the protonated oxazolone in essentially the same fashion as it affects the gas-phase basicity or proton affinity of H–Xxx–OH.

In simple tripeptides, such as H–Gly–Phe–Gly–OH, H–Gly–Phe–Ala–Oh, H–Ala–Phe–Gly–OH and H–Phe–Gly–Gly–OH, the phenylalanine residue plays a dominant role in determining the primary fragmentation reaction(s); in all cases the phenylalanine immonium ion $C_6H_5CH_2CH{=}NH_2^+$ becomes the dominant fragment ion at high internal energies. For tetrapeptides containing Phe the fragmentation reactions of MH^+ are more varied and complex. Even when Phe is the C-terminus residue, as in H–Phe–Gly–Gly–Phe–OH and H–Val–Gly–Gly–Phe–OH, formation of the y_2'' ion as a primary fragment is favored over formation of y_1'', protonated phenylalanine; indeed, the y_1'' ion is a secondary product arising from fragmentation of the y_2'' ion.

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