

# Use of a Single-Quadrupole Mass Spectrometer for Collision-Induced Dissociation Studies of Multiply Charged Peptide Ions Produced by Electrospray Ionization

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**The feasibility of obtaining the collision-induced dissociation (CID) spectra of multiply charged peptide ions produced by electrospray ionization in a simple and inexpensive single-quadrupole mass spectrometer is demonstrated. Collisional activation was carried out in the high-pressure region between the capillary exit and the skimmer entrance to the mass analyzer. The CID of multiply charged peptide ions is very efficient, and the observed fragment ion intensities are typically 1-5% of the parent ion intensity prior to CID. About 70 pmol of the peptide is consumed in obtaining each CID spectrum. Spectra obtained by CID of multiply charged ions from bradykinin, angiotensin II, two peptides with features similar to tryptic peptides, and a synthetic analogue of a component of TGF- $\alpha$  containing two disulfide bonds are shown. The influence of the primary structure of the peptide on the observed fragmentation pathways is discussed. Although the present single-quadrupole configuration is simple and effective, the inability to choose a particular parent ion for collisional activation makes it less powerful than the triple-quadrupole configuration for mixtures of peptides and peptide samples that yield more than one charge state in the normal mass spectrum. However, it has the potential for inexpensively obtaining sequence information of proteins at high sensitivity by analyzing the pure tryptic peptides obtained by on-line or off-line chromatographic separation of tryptic digests.**

## INTRODUCTION

Electrospray ionization is a soft ionization process that produces multiply charged molecule ions with high efficiency from large polar biomolecules (1-3). With this ionization method, peptides frequently yield multiply protonated molecule ions of the form  $(M + nH)^{n+}$ , where  $n$  is the number of protons attached to the intact molecule  $M$ . In the present paper we will refer to these  $(M + nH)^{n+}$  ions by the abbreviation  $n+$  ions. Among other factors, the extent of protonation depends on the number of basic sites present on the peptide molecule.

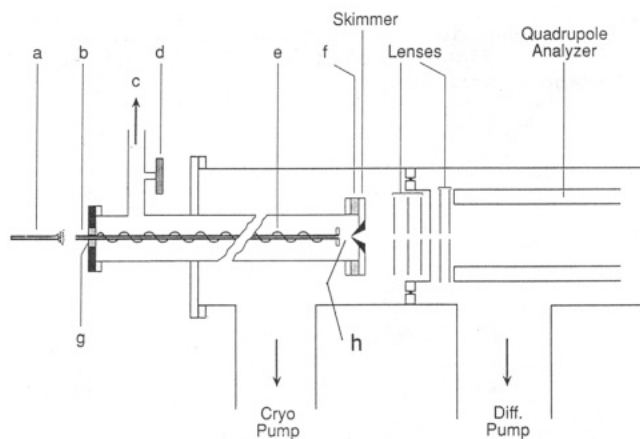
Although tandem mass spectrometry has been extensively used to obtain amino acid sequences of peptides by collision-induced dissociation (CID) of singly charged ions generated by fast atom or ion bombardment (4), relatively few such studies have been made on multiply charged ions. Hunt et al. (5) and Barber et al. (6) showed that sequence information can also be obtained from low-energy CID of  $2+$  peptide ions generated by fast atom bombardment. Smith and co-workers (7-9) demonstrated that multiply protonated protein and peptide ions generated by electrospray ionization fragment readily when subjected to multiple low-energy collisions. This latter group of workers carried out collisional activation in two regions of a triple-quadrupole mass spectrometer: (a) in the region between the sampling orifice and the skimmer and (b) in the collision cell located in the central

RF-only quadrupole. Fragment ions yielding information regarding the sequence of amino acids in the peptide melittin (molecular mass (MM) = 2846) were observed in the CID spectra of the parent  $3+$ ,  $4+$ ,  $5+$ , and  $6+$  molecule ions, respectively (9). These workers also carried out studies of the collisional activation in the nozzle-skimmer interface of a double-quadrupole mass spectrometer and obtained useful information from the peptide melittin (8, 10). Meng et al. have also observed collision-induced fragmentation to occur in the high-pressure region of their electrospray ion source coupled to a magnetic sector mass analyzer (11). Covey et al. (12) showed that tryptic peptides frequently produce  $2+$  molecule ions with ion-spray ionization (a technique closely related to electrospray ionization) because tryptic peptides usually contain a terminal amino group and a basic arginine or lysine residue at the carboxyl terminus. Collisional activation of these  $2+$  ions in the collision cell of a triple-quadrupole mass spectrometer yielded singly charged  $b$  and  $y$  series fragment ions (the nomenclature followed for the fragment ions is a modified version (13) of the original notation proposed by Roepstorff and Fohlman (14)).

Recently, we have designed and constructed a novel electrospray ionization source and coupled it to a single-quadrupole mass analyzer (15). With this instrument, we have obtained electrospray ionization mass spectra of a number of peptides and proteins (15), tryptic digests (16), and transition-metal complexes (17). In the present paper, we demonstrate the capability of this simple mass spectrometric configuration for producing both normal electrospray spectra (exhibiting exclusively intact molecule ions) as well as CID spectra (exhibiting singly or multiply charged fragment ions formed by the collisional activation of the molecule ions). The CID spectra obtained from a number of peptides are found to be comparable in quality and information content to those obtained with more elaborate triple-quadrupole instruments. The sensitivity of this simple instrument is relatively high, requiring the consumption of approximately 70 pmol of peptide for each CID spectrum. An investigation is made of the influence of peptide sequence on the observed fragmentation of multiply protonated peptide ions.

## EXPERIMENTAL SECTION

A schematic representation of the electrospray ionization mass spectrometer used in the present investigation is shown in Figure 1. The instrument has been described in detail previously (15). Briefly, the analyte solution is electrosprayed from a syringe through a stainless steel needle (a) that is maintained at 4-6 kV relative to a stainless steel capillary tube (b). Electrospray produces small, highly charged droplets that attempt to follow the electric field lines and migrate toward the capillary tube. The charged droplets that enter the capillary tube (200 mm in length, 0.5 mm i.d., 1.58 mm o.d.) are focused by the gas (ambient air) flow through the tube toward the central axis and are transported into the first vacuum stage of the mass spectrometer. The tube is heated to 85-90 °C to assist in the evaporation of solvent from the droplets. Ionic species exiting the capillary tube, normally still somewhat solvated, are subjected to an electrostatic field defined by the potential difference  $\Delta V$  between the exit of the



**Figure 1.** Schematic diagram of the electrospray ionization mass spectrometer: (a) 150  $\mu\text{m}$  i.d. syringe needle at 4–6 kV; (b) 0.5 mm i.d., 200 mm long stainless steel capillary tube at 80–350 V; (c) outlet to rotary pump; (d) pirani gauge; (e) heating tape to maintain the capillary at 85–90  $^{\circ}\text{C}$ ; (f) Teflon insulating ring; (g) Teflon swagelok fitting; (h) the capillary exit-skimmer region where collisional activation takes place. The 0.5-mm orifice skimmer, lens elements, and extended mass range ( $m/z$  2000) quadrupole analyzer are all from a Vestec Model 201 thermospray mass spectrometer.

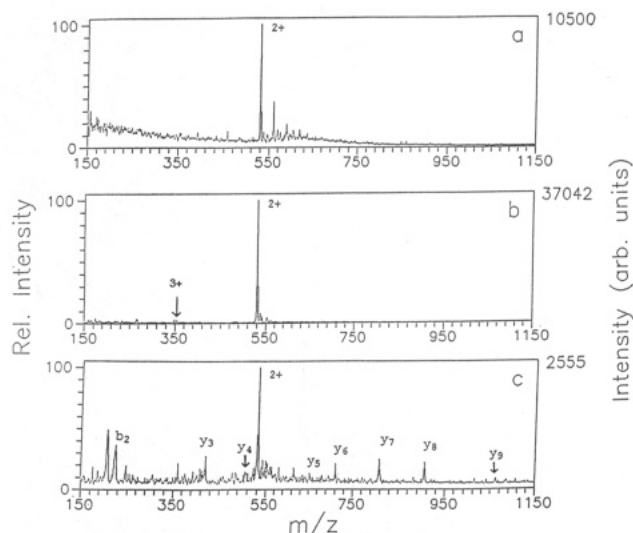
capillary tube ( $V_c = 50\text{--}380$  V) and a coaxial skimmer ( $V_s = 20$  V), spaced 3.3 mm apart. The pressure in the space (h) between the capillary exit and the skimmer is not accurately known, but from the pressure measured by the pirani gauge (d) shown in Figure 1, it is estimated to be in the range of 1–10 Torr. Because of the imposed electric field and the high pressure in this region, the ionic species undergo many energetic collisions and are collisionally activated (8, 10, 15, 17). The electrostatic field can be readily adjusted and provides a fine control over the level of collisional activation. At relatively low levels of activation, complete desolvation of the multiply charged molecule ions can be effected without causing any fragmentation. At higher activation levels, these multiply charged ions can be made to undergo fragmentation. In either case, the molecule ions and/or the fragment ions enter the second-stage pumping region through an orifice in the skimmer. The skimmer potential  $V_s$  defines the energy of the ions entering the mass analyzer. A set of electrostatic lenses focuses the ions emerging from the skimmer into a differentially pumped single-quadrupole mass analyzer. The lenses and the extended  $m/z$  range (2000) quadrupole analyzer are part of a Vestec Model 201 mass spectrometer (Vestec Corp., Houston, TX).

The mass spectra were obtained by using a commercial data system (Vector-1, Teknivent, St. Louis, MO) on an IBM-PC-AT compatible computer. Scan times were typically 0.5–2 min, and multiple scans were added together and averaged to obtain higher signal to noise ratios. This data system records ion abundances at integer  $m/z$  values only. For measurement of accurate  $m/z$  values, the centroids of peaks of interest were determined in the so-called "calibration mode", by scanning a narrow range of  $m/z$  values (typically 5–20  $m/z$  units) (15).

The peptide samples were obtained from the Sigma Chemical Co. (St. Louis, MO) and are listed here together with their sequences and catalog numbers: bradykinin (RPPGFSPFR; B-3259); angiotensin II (DRVYIHPF; A-9525); (Glu-1)fibrinopeptide (EGVNDNEEGFFSAR; F-3261); synthetic renin inhibitor (PHPFHFFVYK; P-2402). The synthetic analogue of a component of transforming growth factor  $\alpha$  (TGF- $\alpha$ ) was provided by Drs. C. R. Wu and J. P. Tam of Rockefeller University. The peptide samples were used without further purification and were dissolved in a mixture of water and methanol (1:1) containing 3–6% acetic acid. The resulting peptide solutions (concentration 9–25 pmol/ $\mu\text{L}$ ) were electrosprayed at a rate of 0.5–1.0  $\mu\text{L}/\text{min}$ .

## RESULTS AND DISCUSSION

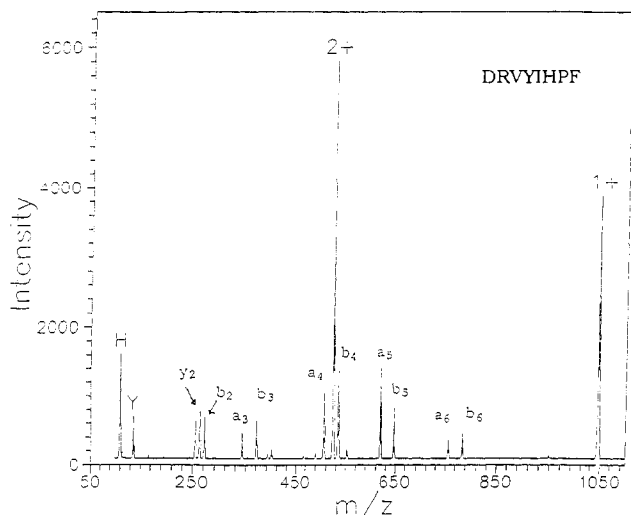
The present electrospray ionization source provides a fine control over the degree of collisional activation of the peptide ions. This collisional activation is carried out in the high-pressure region between the capillary exit and the skimmer



**Figure 2.** Demonstration of the fine control available in desolvating and collisionally activating the 2+ ion of bradykinin. (a) At  $\Delta V = 80$  V, the 2+ ion and associated solvent cluster ions are seen. (b) At  $\Delta V = 160$  V, the 2+ ion is completely desolvated and its intensity increases by a factor of 4. (c) At  $\Delta V = 260$  V, the 2+ ion is highly collisionally activated, reducing its intensity by 94% and yielding a series of  $y$  fragment ions.

(Figure 1) (15, 17). The high degree of control is illustrated in the mass spectra shown in Figure 2 for the nine amino acid residue peptide bradykinin, with the sequence RPPGFSPFR (MM = 1060). The spectra shown in Figure 2 were obtained at three different values of  $\Delta V$ , the potential difference between the exit of the capillary and the skimmer (Figure 1). These three values of  $\Delta V$  yield three different levels of collisional activation. When  $\Delta V = 80$  V, the collisions in this region between the capillary exit and the skimmer are not energetic enough to completely desolvate the 2+ ion of bradykinin (Figure 2a). Although the 2+ ion is dominant, the presence of a large number of solvated 2+ ions is also observed. These solvated ions are mainly of the form  $[\text{M} + 2\text{H} + x\text{H}_2\text{O}]^{2+}$ , with  $x$  ranging from 1 to  $>10$ . As  $\Delta V$  is increased to 160 V, the collisions are more energetic and effective in desolvating the 2+ ion. As more and more 2+ ions are desolvated, the 2+ ion intensity increases and reaches a maximum value upon complete desolvation (Figure 2b). The intensity of the 2+ ion in Figure 2b is nearly 4-fold higher than the 2+ ion intensity in Figure 2a. At  $\Delta V = 160$  V, a low-intensity peak from the 3+ ion of bradykinin is also observed. No trace of the singly protonated molecule is seen. A further increase in  $\Delta V$  results in higher collisional activation of the 2+ and 3+ ions, and these protonated peptide molecules begin to fragment. The spectrum shown in Figure 2c was obtained at  $\Delta V = 260$  V. The 2+ ion intensity has decreased by a factor of 15 relative to its maximum value (Figure 2b), and a number of bradykinin fragment ion peaks appear in the mass spectrum. These fragments are found to correspond primarily to singly charged  $y$  series ions ( $y_3\text{--}y_9$ , inclusive). The sum of the intensities of the fragment ions and the residual parent ion in Figure 2c is approximately 15% of the maximum intensity of the 2+ ion shown in Figure 2b. This high efficiency for observing the fragment ions can be attributed to two factors: (i) the high efficiency of multiple low-energy collisions in causing the dissociation of the doubly charged 2+ ions and (ii) the efficient transmission of fragment ions produced in the region between the capillary exit and the skimmer into the mass analyzer.

It has been shown that the primary structure of a peptide strongly influences the fragmentation pathways observed upon collisional activation (4). Such fragmentation studies have primarily been made on 1+ peptide ions produced by fast



**Figure 3.** CID spectrum of angiotensin II (sequence: DRVYIHPF) obtained at  $\Delta V = 280$  V. A 10 pmol/ $\mu$ L peptide solution is electrosprayed at a rate of 0.6  $\mu$ L/min, and five scans of 130-s duration each are averaged. Total amount consumed is 66 pmol. Peaks from singly charged *a* and *b* series ions and 1+ ion of angiotensin II are seen in the spectrum.

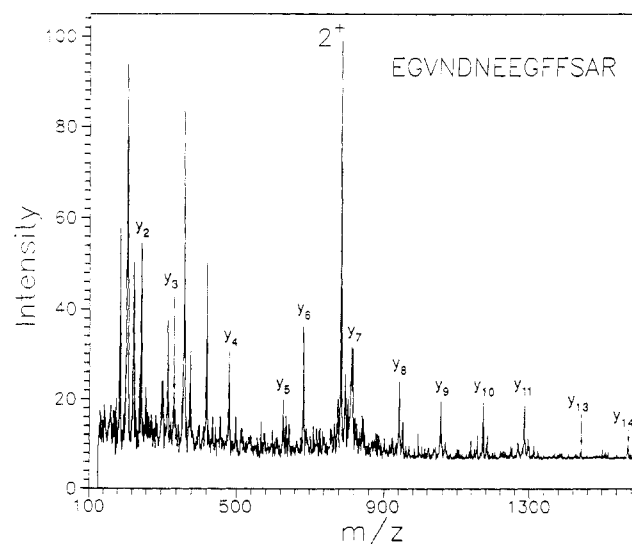
atom bombardment ionization. In the present study, we investigated the influence of primary structure on the fragmentation of 2+ and 3+ peptide ions produced by electrospray ionization. In the octapeptide angiotensin II with the sequence DRVYIHPF (MM = 1046), there are three possible sites of protonation: the N-terminal amino group and the side chains of the arginine and the histidine residues. Because the peptide is electrosprayed from an acidic solution, angiotensin II may therefore be expected to produce molecule ions with a maximum attachment of three protons. The normal electrospray ionization mass spectrum of angiotensin II obtained at  $\Delta V = 160$  V is dominated by the 2+ ion ( $m/z$  524). At this level of activation, the intensities of the 1+ ion ( $m/z$  1047) and 3+ ion ( $m/z$  350) relative to the 2+ ion were respectively 4.6% and less than 0.03%. At a higher level of collisional activation,  $\Delta V = 255$  V, the 2+ ion peak is observed to be attenuated by 90% of its peak value and intense fragment ion peaks appear in the mass spectrum (Figure 3). Two sequences of fragment ion peaks stand out in the CID spectrum corresponding to singly charged *a* and *b* series ions ( $a_3$ - $a_6$  and  $b_2$ - $b_6$ , inclusive). Table I gives a comparison of the measured  $m/z$  values of the most intense fragment ions with the calculated values. The mass accuracy was found to be better than  $\pm 0.2$  unit. The ions at  $m/z$  110 and 136 likely arise from multiple bond cleavages to give histidine and tyrosine immonium ions (4). The ion at  $m/z$  263 probably arises from facile cleavage of the peptide bond between the histidine and the proline residues to give  $y_2$  fragment ion. The ion at  $m/z$  255 may arise from the loss of ammonia from the  $b_2$  fragment ion. The observation of dominant *a* and *b* series ions indicates that after fragmentation the charge is retained primarily on the side chain of the arginine residue at position 2 and/or on the N-terminal amino group. Conversely, the observed low abundance of *y* series ions indicates that the charge is not retained on the histidine residue at position 6. The peak at  $m/z$  1047, corresponding to the 1+ ion is more intense than that observed in the normal spectrum. The increase in intensity may result from a proton-stripping reaction of the 2+ ion caused by collisional activation (7).

Most tryptic peptides have a basic arginine or lysine residue at the C-terminus and a free N-terminal group. Some tryptic peptides may contain one or more internal histidines. Tryptic peptides with no internal histidines generally show intense 2+ ion peaks in the normal electrospray mass spectra (12, 16).

**Table I.** Comparison of the Observed and Calculated  $m/z$  Values for the Intense Peaks Observed in the CID Spectrum of Angiotensin II<sup>a</sup>

obsd $m/z$	assgnt	calcd <sup>b</sup> $m/z$	$\Delta^c$
110.10	His	110.07	+0.03
136.15	Tyr	136.07	+0.08
263.10	$y_2$	263.14	-0.04
272.35	$b_2$	272.14	+0.21
343.35	$a_3$	343.21	+0.14
371.10	$b_3$	371.20	-0.10
506.40	$a_4$	506.27	+0.13
524.10	2+	524.10 <sup>d</sup>	0.00
534.35	$b_4$	534.27	+0.08
619.55	$a_5$	619.36	+0.19
647.30	$b_5$	647.35	-0.05
756.50	$a_6$	756.42	+0.08
784.50	$b_6$	784.41	+0.09
1046.52	1+	1046.54	-0.02

<sup>a</sup>The assignments His and Tyr indicate the immonium ions from these amino acid residues. <sup>b</sup>Calculated  $m/z$  values given are for the monoisotopic peak  $A + 0$ . <sup>c</sup>Difference between the observed and the calculated  $m/z$  values. <sup>d</sup>The calculated  $m/z$  value given for the 2+ ion is the average of the isotopic components.



**Figure 4.** CID spectrum of the 2+ ion of (Glu-1) fibrinopeptide (sequence: EGVNDNEEGFFSAR) obtained at  $\Delta V = 280$  V. A 20 pmol/ $\mu$ L peptide solution is electrosprayed at a rate of 0.5  $\mu$ L/min, and five scans of 81-s duration each are averaged. Total amount consumed is 67 pmol. Almost all the singly charged *y* series ions are observed.

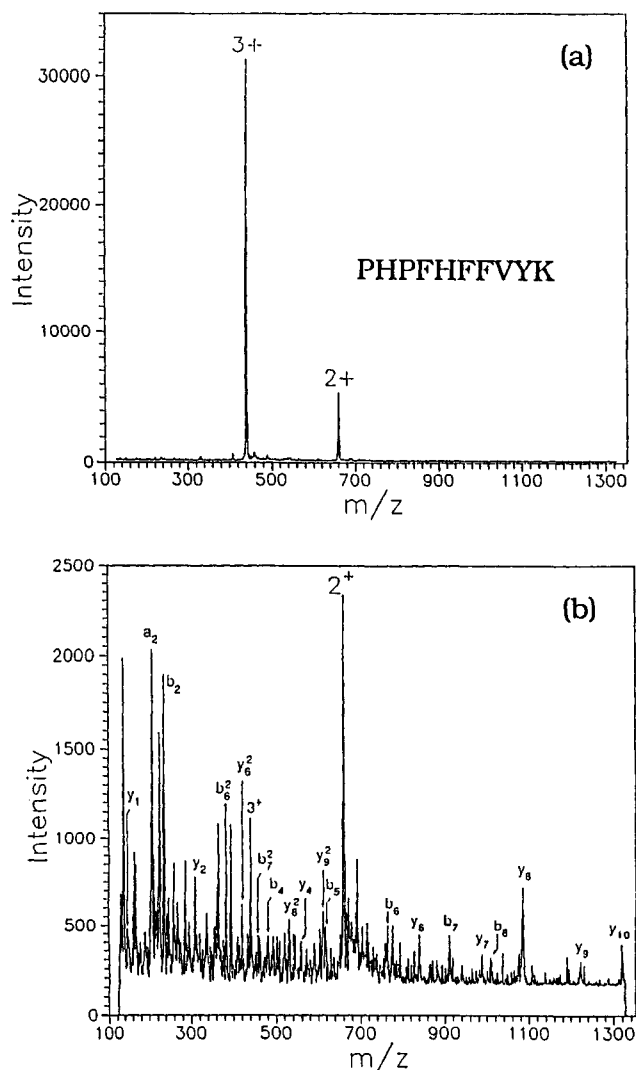
Because of the presence of charge on both ends of the molecule, the cleavage of any CO-NH bond in the peptide by collisional activation may leave both the resulting fragments charged and a complete series of singly charged *y* and *b* series ions may be observed (12), although sometimes only one series (*y* or *b*) dominates the mass spectrum. Thus, the CID of 2+ ions from tryptic peptides is a powerful tool for the analysis of the primary structure of proteins. Tryptic peptides with internal histidines often give a 3+ ion in addition to the 2+ ion (16). The resulting CID spectra of such peptides, obtained with the present configuration, are more complex because both singly and doubly charged fragment ions may be observed (see below).

Figure 4 shows the CID spectrum of a 14-residue peptide, (Glu-1)fibrinopeptide with a sequence EGVNDNEEGFFSAR (MM = 1570.6), obtained at  $\Delta V = 280$  V. This peptide has an arginine at the C-terminus and a free N-terminus, features common to tryptic peptides. At lower levels of collisional activation, the spectrum was dominated by the 2+ ion (data not shown). No 1+ ion was observed. At a higher level of collisional activation (Figure 4) an intense series of singly

charged  $y$  ions was observed. The series was complete except for  $y_1$  and  $y_{12}$ . Interestingly, no complimentary  $b$  series ions were observed. It appears that, after CID, the highly basic arginine residue at the C-terminus retains the proton attached to it, while the less basic N-terminal amino group loses its attached proton. Several intense peaks were observed below  $m/z$  450 (Figure 4) that originate from collisional activation of an unidentified impurity that produces an ion at  $m/z$  538 in the normal spectrum. This impurity is observed at varying levels in a number of our peptide and protein preparations. From collisional activation studies it is found to be polymeric in nature with a 59-unit repeating unit. Because of the single-quadrupole configuration, we cannot select a single parent ion for carrying out the collisional activation. The CID spectrum thus reflects the presence of fragment ions formed from any impurities (peptides or non-peptides) that produce intense peaks with electrospray ionization.

Synthetic renin inhibitor PHPFHFFVYK (MM = 1318.5) has a lysine at the carboxyl terminus and a free amino terminus, features common to tryptic peptides. In addition, the chain contains two internal histidine residues providing the peptide with a total of four basic sites. The normal mass spectrum, obtained at  $\Delta V = 160$  V, exhibits a dominant 3+ ion and a less intense 2+ ion (Figure 5a). The single-quadrupole configuration unfortunately does not allow a single parent ion to be selected for collisional activation. The resulting CID spectrum is expected to be considerably more complex than those shown previously because both singly and doubly charged fragment ions can arise from both the 3+ and 2+ parents. As the collisional energy was raised ( $\Delta V = 260$  V), the 3+ ion intensity decreased by 95%, while the decrease in 2+ ion intensity was only 70% (Figure 5b). This differential decrease is expected because ions with three charges will experience 50% higher collisional activation energy than those with two charges, for a given value of the potential difference  $\Delta V$ . In addition, the 3+ ion is expected to be less stable than the 2+ ion because it experiences a higher level of coulombic repulsion. Even though the spectrum is complex, the assignment of peaks with  $m/z$  values greater than 660 ( $m/z$  value of the 2+ ion) is straightforward because these ions are singly charged and the spectrum is relatively uncongested in this region ( $b_6$ - $b_8$  and  $y_6$ - $y_{10}$  ions were observed). In the region below  $m/z$  660, the fragment assignment becomes more difficult, as these peaks can arise from fragment ions with charge states ranging from 1 to 3. However, several singly charged  $b$  and  $y$  series ions ( $b_2$ ,  $b_4$ ,  $b_5$  and  $y_1$ ,  $y_2$ ,  $y_4$ ) and some of the doubly charged fragment ion peaks ( $y_9^2$ ,  $y_8^2$ ,  $y_6^2$ ,  $b_7^2$ , and  $b_8^2$ , where the superscript indicates the charge state) can be identified. Thus limited sequence information is available from the CID spectrum even in this complex example. It should be emphasized, however, that the assignment of fragment ions below  $m/z$  660 would be extremely difficult if the peptide was of an unknown sequence.

The presence of disulfide bonds in a peptide effects the fragmentation pathways observed upon collisional activation because multiple-bond cleavages are needed to give fragment ions informative of the complete peptide sequence. Such multiple-bond cleavages require higher energies and are less probable than fragmentations involving the cleavage of single bonds. The normal and the CID spectra, shown in Figure 6, of a 19-residue synthetic analogue of a component of TGF- $\alpha$  (sequence: FFCHSGCVGARCEHCDLLA, MM = 2064.4) illustrate this point clearly. This peptide has a total of four basic sites and contains two disulfide bonds. The two disulfide bonds in this peptide are interleaved, a situation that is not uncommon in proteins. The normal spectrum (Figure 6a), obtained at  $\Delta V = 160$  V, shows an intense 3+ ion and a relatively weak 2+ ion (less than 2%). Upon collisional ac-

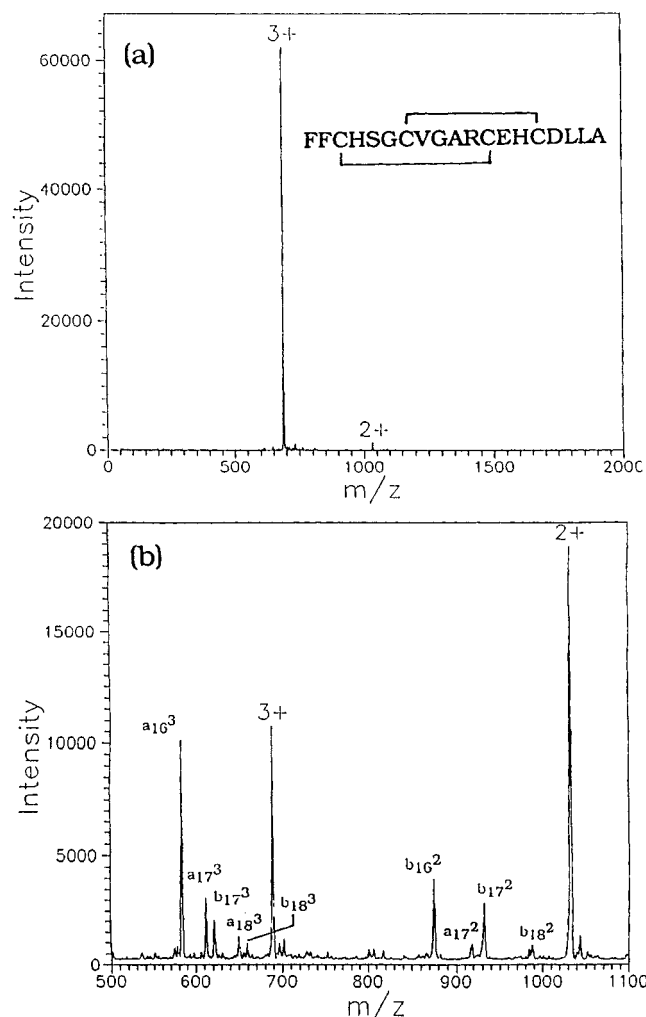


**Figure 5.** (a) Normal electrospray ionization spectrum of a peptide (sequence: PHPFHFFVYK) with four basic sites. At  $\Delta V = 160$  V, both the 3+ and 2+ ions of the peptide are seen. (b) CID spectrum obtained at higher collisional activation ( $\Delta V = 260$  V). Both singly and doubly charged  $b$  and  $y$  series ion peaks are observed. Amount of peptide consumed in obtaining the CID spectrum is about 66 pmol.

tivation in the capillary exit-skimmer region ( $\Delta V = 240$  V), the 3+ ion intensity is observed to be attenuated by 90%. In the region below  $m/z$  1030 intense fragment ion peaks were observed (Figure 6b). Because no peaks were observed beyond  $m/z$  1100 (the fragment ions would have to be singly charged to appear in this region), the CID spectrum is only shown between  $m/z$  500 and 1100. The fragment ion peaks seen in Figure 6b are attributable to the triply charged ( $a_{16}^3$ ,  $a_{17}^3$ ,  $a_{18}^3$ ,  $b_{17}^3$ ,  $b_{18}^3$ ) and doubly charged ( $a_{17}^2$ ,  $b_{16}^2$ ,  $b_{17}^2$ ,  $b_{18}^2$ ) fragment ions. All these fragment ions retain the core of the peptide that contains the disulfide bonds. In the region below  $m/z$  500 two peaks from singly charged fragment ions ( $y_1$  and  $y_2$ ) were observed. Thus the CID spectrum only provides information about the last three C-terminal amino acid residues and the two N-terminal amino acid residues. This finding is consistent with the observation of Loo et al. (18) that the low-energy collisions of multiply charged molecule ions generated from proteins with disulfide bonds yielded fragment ions from regions primarily near the ends of the molecules.

## CONCLUSION

The feasibility of obtaining CID spectra of multiply charged ions produced by electrospray ionization in a simple and inexpensive single-quadrupole mass spectrometer is demon-



**Figure 6.** (a) Normal electrospray ionization mass spectrum of 19-residue synthetic analogue of a component of TGF- $\alpha$ , containing two disulfide bonds and four basic sites. At  $\Delta V = 160$  V, an intense 3+ ion peak and a weak 2+ ion peak are observed. (b) The CID spectrum obtained at  $\Delta V = 240$  V. The spectrum is shown between  $m/z$  values of 500 and 1100 for reasons of clarity (see text). The amount of peptide consumed during the time the CID spectrum was obtained is 21 pmol.

strated. CID was carried out in the high-pressure region between the capillary exit and the skimmer entrance to the mass analyzer. The present instrumental configuration provides a fine control over the level of collisional activation, and this enables one to obtain either the normal electrospray ionization mass spectra or the CID spectra. Collision-induced dissociation of multiply charged ions is very efficient, as evident from the observed fragment ion intensities, which are found to be typically in the range 1–5% of the parent ion intensity prior to collisional activation. About 70 pmol of the peptide is consumed in obtaining each CID spectrum.

The instrument is especially suited for obtaining structural information from tryptic peptides, because they have basic

sites at both ends of the molecule and often produce intense 2+ ions. Collisional activation of 2+ ions of these peptides produced singly charged *b* and/or *y* series fragment ions giving sequence information, as has been observed previously in double- and triple-quadrupole instruments.

Although the single-quadrupole configuration is simple and effective, it has a disadvantage for CID investigations compared with more complex triple-quadrupole configurations. A single parent ion cannot be selected, and the presence of any impurities in the sample (e.g. (Glu-1) fibrinopeptide described above) or the presence of molecule ions with different charge states (e.g. synthetic renin inhibitor described above) can complicate the interpretation of the CID spectra. However, this simple instrumental configuration has the potential for obtaining structural information from *pure* peptides obtained by an on-line or off-line separation of peptide mixtures, such as tryptic digests of proteins.

**Registry No.** B-3259, 58-82-2; A-9525, 4474-91-3; F-3261, 103213-49-6; P-2402, 75645-19-1; TGF- $\alpha$  synthetic analogue, 130408-47-8.

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