~~Protein sequencing by tandem mass spectrometry~~

(collision-activated dissociation/liquid secondary-ion mass spectrometry/apolipoprotein B)

DONALD F. HUNT, JOHN R. YATES III, JEFFREY SHABANOWITZ, SCOTT WINSTON, AND CHARLES R. HAUER

Department of Chemistry, University of Virginia, Charlottesville, VA 22901

ABSTRACT   Methodology for determining amino acid sequences of proteins by tandem mass spectrometry is described. The approach involves enzymatic and/or chemical degradation of the protein to a collection of peptides which are then fractionated by high-performance liquid chromatography. Each fraction, containing as many as 10–15 peptides, is then analyzed directly, without further purification, by a combination of liquid secondary-ion/collision-activated dissociation mass spectrometry on a multianalyzer instrument. Interpretation of collision-activated dissociation mass spectra is described, and results are presented from a study of soluble peptides produced by treatment of apolipoprotein B with cyanogen bromide and trypsin.

Current strategy for sequencing proteins in our laboratory by tandem mass spectrometry (1) involves digestion of the protein with site-specific reagents such as cyanogen bromide or trypsin followed by fractionation of the resulting mixture by high-performance liquid chromatography (HPLC). Peptides in each fraction are then ionized by liquid secondary-ion mass spectrometry (LSIMS) (2) on a triple-quadrupole mass spectrometer. Sample is dissolved in a viscous matrix such as glycerol or monothioglycerol and then exposed to a beam of 6- to 8-keV ($1 \text{ eV} = 1.602 \times 10^{-19}$ J) Cs$^+$ ions in the ion source of the mass spectrometer. Peptides are sputtered into the gas phase under these conditions, and the resulting mass spectrum consists largely of (M+H)$^+$ ions characteristic of the molecular weight of each peptide in the sample (3). In a second experiment, the first quadrupole of the instrument is used to select a single (M+H)$^+$ ion from the mixture and to transmit it to quadrupole 2, a collision chamber, where the peptide undergoes collisions with argon atoms and suffers fragmentation primarily at the various amide linkages in the molecule. The resulting fragment ions are then transferred to the third quadrupole, which separates them according to mass. The end result is a mass spectrum containing ions characteristic of the sequence of amino acids in the selected peptide. Repetition of this process under computer control provides sequence information on each peptide in the mixture. Presently, the above approach is limited by the 1800-Da mass range of our triple-quadrupole instrument. Maximum sequence information is obtained only when the protein under investigation is cleaved into peptides of molecular mass under this ceiling. New instrument developments should remedy this situation shortly (4).

The major strength of the tandem mass spectrometry method is that it provides extensive sequence information over the whole length of a protein chain in a single series of experiments that involve minimal effort directed toward separation and purification of oligopeptide fragments. Total time for biochemical manipulation, HPLC fractionation, and instrumental analysis of samples from a single protein digest seldom exceeds 4 or 5 days. Even with the limited mass range

of our existing instrumentation, it is usually possible to obtain sequence information at the 10 nmol level covering 25–60% of a 50-kDa protein from this single experiment.

Another important feature of the tandem mass spectrometry method is that it does not require that the sample be purified to a high degree of homogeneity. The approach works well as long as the target protein is a major component of the protein mixture (5). In this situation the initial enzyme digest and analysis will produce peptide sequences from each protein in the mixture. Subsequent digests with enzymes of different specificity generate sequence information that allows the original set of peptides to be overlapped and assigned to a specific protein. This approach has proved to be of particular value in situations where the goal of the analysis is to locate peptide sequences containing 4 or more adjacent residues of the 11 amino acids that allow construction of unique oligonucleotide probes for the corresponding mRNA coding for the protein in question. Use of tandem mass spectrometry frequently allows this experiment to be performed at a much earlier stage of protein purification than would otherwise be possible. Model studies on both small linear (6) and cyclic (7) peptides indicate that the tandem mass spectrometry approach will also facilitate sequence analysis of cyclic peptides of unknown structure.

Here we present additional information on the tandem mass spectrometry sequencing method and illustrate its use for obtaining structural data on the relatively small number of soluble tryptic peptides produced by limited proteolysis of insoluble cyanogen bromide fragments from human apolipoprotein B (apoB). Sequence information on about 60% of this protein has recently become available from studies on the corresponding cDNA (8–11). Partial sequence information from the related rat apoB has also been published (12, 13).

MATERIALS AND METHODS

Mass Spectrometry. All spectra were recorded on a triple-quadrupole mass spectrometer assembled with components from Finnigan-MAT (San Jose, CA). The design and construction of this instrument have been described in detail (14). Briefly, the triple quadrupole consists of a conventional model 4500 ion source followed by three quadrupole filters (Q1, Q2, and Q3) and a conversion dynode electron multiplier detector.

Samples for mass analysis were prepared by adding 1 μl of a 5% acetic acid solution containing the peptide or mixture of peptides at the 0.1–1.0 nmol/μl level to 1 μl of thioglycerol on a stainless steel or gold-plated stainless steel probe tip, 2 mm in diameter. The resulting mixture was then subjected to bombardment by a beam of 6- to 8-keV Cs$^+$ ions generated from a Cs$^+$ ion gun (2) (Antek, Palo Alto, CA) mounted directly on the Finnigan model 4500 ion source. Peptides sputtered into the gas phase as (M+H)$^+$ ions by this process

---

Abbreviations: LSIMS, liquid secondary-ion mass spectrometry; CAD, collision-activated dissociation; apoB, apolipoprotein B; Lxx, Leu or Ile.

were subjected to mass analysis by placing rf potential on Q1 and Q2 and a combination of rf and dc potential on Q3. In this mode of operation Q1 and Q2 function as ion-focusing devices and transmit all ions of all masses up to m/z 1800. Q3 functions as a mass filter and separates the ions according to mass. The result is a main-beam mass spectrum containing $(M+H)^+$ ions characteristic of the molecular weight of each peptide in the mixture.

Sequence analysis of the peptides in the mixture was performed on a second 1-μl aliquot of the above solution. For this experiment, the collision cell, Q2, was filled with argon to a pressure of 5 millitorr (1 torr = 133 Pa) and operated in the rf-only mode with a dc offset of −10 V. The solids probe was maintained at a potential of 0–10 V, and Q1 and Q3 were operated in the rf/dc mode with offset potentials of −4 and −30 V, respectively. Collision energies varied, therefore, between −10 and −20 V. Those at the lower end were employed for peptides of molecular mass below 600 Da, and those at the higher end were used for peptides of molecular mass above 1400 Da. To maximize ion transmission through the system, Q1 was usually tuned so as to pass a window of masses 3–9 units wide. Approximately 5 scans for each of 3 or 4 peptide $(M+H)^+$ ions in the mixture were usually acquired under data-system control before depletion of the matrix occurred as a result of continuous bombardment by Cs^+ ions from the Antek gun. Ion currents from these scans were summed and displayed as a single collision-activated dissociation (CAD) mass spectrum. When more than 3 or 4 peptides occurred in a particular sample, additional aliquots of the sample solution were used to complete the analysis.

Isolation and Proteolytic Digestion of ApoB. Low density lipoprotein of density <1.05 g/ml, isolated from human plasma obtained from healthy normolipidemic adults who had fasted overnight (15), was delipidated (15) and then cleaved with cyanogen bromide (16). The resulting fragments (25 mg, 50 nmol) were suspended in 50 mM ammonium bicarbonate and treated with 100 μl of 1 mM HCl containing trypsin at 1 μg/μl. Digestion was terminated by lyophilizing the solution after 24 hr. The residue was placed in 800 μl of a 9:1 solution of 5% acetic acid/1-propanol, and the resulting suspension was then sonicated for 5 min and centrifuged. Peptides in the supernatant were fractionated by HPLC on a μBondapak C_{18} preparative column, using gradient elution from 10:90 (vol/vol) to 40:60 (vol/vol) 1-propanol/5% acetic acid at a flow rate of 1 ml/min for 45 min. After lyophilization of the 1-ml fractions, each tube was treated with 50 μl of 5% acetic acid. A 0.5- to 1.0-μl aliquot was employed for analysis of the fractions by mass spectrometry.

Methyl Ester Formation. A standard solution of 2 N HCl in methanol was prepared by adding 1.6 ml of acetyl chloride dropwise with stirring to 10 ml of methanol. After the solution had stood at room temperature for 5 min, 200-μl aliquots of the reagent were added to each of the lyophilized HPLC fractions. The reaction was allowed to proceed for 2 hr at room temperature, and the solvent was then removed by lyophilization. Samples were dissolved in 50 μl of 5% acetic acid, and a 0.5- to 1.0-μl aliquot was used for recording mass spectra.

Edman Degradation (17). Aliquots of each HPLC fraction estimated to contain 5–10 nmol of each peptide were lyophilized in polypropylene test tubes and then treated with 15 μl of H_2O/pyridine (1:1) and 15 μl of a 5% solution of phenyl isothiocyanate in pyridine. The resulting solution was allowed to stand at 37°C for 10–15 min and was then extracted once with 60 μl of heptane/ethyl acetate (2:1). Both layers were taken up in a polypropylene Pipetman tip (Rainin Instruments, Woburn, MA), and the aqueous layer was then ejected back into the reaction vessel and lyophilized. The resulting residue was treated with 20 μl of anhydrous trifluoroacetic acid for 10 min at 37°C. After the acid had been removed by lyophilization, the residue was dissolved in 30 μl of water and

the resulting solution was extracted once with 100 μl of *n*-butyl acetate. Following lyophilization of the aqueous layer, the residue was dissolved in 10 μl of 5% acetic acid. Mass spectra were recorded on 1-μl aliquots of this solution.

RESULTS AND DISCUSSION

Results of CAD experiments on soluble peptides in the HPLC fractions from a limited trypsin digest of cyanogen bromide-treated apoB are presented in Table 1. Complete sequence information was obtained on 34 peptides containing about 5% of the total residues in apoB, if a molecular mass of 500 kDa

Table 1. Soluble peptides obtained by treating suspended cyanogen bromide fragments of apoB with trypsin

| Fr.[a] | $(M+H)^+$ m/z | | cDNA[d] | Amino acid sequence |
	Under.[b]	Der.[c]		
16	375	—	280–282[e]	Asp-Lxx-Lys
	389	—	279–281[f]	Lxx-Thr-Arg
	654	—	839–844[f]	Gly-Thr-Lxx-Ala-His-Arg
	695	709	866–871[f]	Ala-His-Lxx-Asn-Lxx-Lys
	851	865		Lxx-His-Val-Ala-Gly-Asn-Lxx-Lys
	895	937		Val-His-Glu-Lxx-Lxx-Glu-Arg
17	—	421	53–55[f]	Phe-Lxx-Lys
	444	458	84–87[g]	Ala-Lxx-Lxx-Lys
	522	536	291–294[e]	Ala-Tyr-Lxx-Arg
	—	580	102–105[g]	Tyr-Glu-Lxx-Lys
	572	586		Val-Asn-Lxx-Val-Lys
	659	673	1178–1183[f]	Ala-Lxx-Gln-Ser-Lxx-Lys
	758	786		Gln-Ala-Glu-Ala-Val-Lxx-Lys
	987	1001	252–260[g]	Val-Ala-Gln-Val-Thr-Gln-Thr-Lxx--Lys
18	502	530	1163–1166[f]	Glu-Lxx-Lxx-Lys
	599	627		Lxx-Glu-Pro-Lxx-Lys
	—	999	16–23[e]	Glu-Gln-Lxx-Thr-Pro-Glu-Lxx--Lys
	973	1029	1014–1021[f]	Gln-Lxx-Asp-Asp-Lxx-Asp-Val--Arg
19	742	756	42–48[e]	Ala-Ala-Lxx-Gln-Ala-Lxx-Arg
	961	989	582–590[f]	Lxx-Asp-Val-Thr-Thr-Ser-Lxx--Gly-Arg
20	550	578	656–659[f]	Lxx-Asp-Phe-Arg
21	666	694		Phe-Ser-Lxx-Asp-Gly-Lys
	721	749		Ala-Lxx-Phe-Gly-Glu-Gly-Lys
	759	787	544–550[e]	Ala-Lxx-Val-Asp-Thr-Lxx-Lys
	844	872		Lxx-Ala-Val-Asp-Lxx-Thr-Gly--Arg
	912	954		Lxx-Asp-Asp-Lxx-Pro-Lxx-Ala--Arg
	1028	1042		Val-Asn-Ser-Lxx-Lxx-Gln-Gln--Val-Lys
	1486	1514		His-Lxx-Gln-Asn-Lxx-Asp-Lxx--Gln-His-Lxx-Ala-Gly-Lys
22	547	561	24–28[e]	Ser-Ser-Lxx-Lxx-Lys
32	861	889		Lxx-Thr-Lxx-Pro-Asp-Phe-Arg
	1034	1076	1161–1169[f]	Asp-Asn-Val-Phe-Asp-Gly-Lxx--Val-Arg
35	—	789	622–628[f]	Phe-Lxx-Thr-Pro-Gly-Lxx-Lys
	1070	1098		Lxx-Glu-Lxx-Pro-Lxx-Pro-Phe--Asn-Lys
44	1039	1067	1446–1455[f]	Lxx-Ala-Pro-Gly-Glu-Lxx-Thr--Lxx-Lxx-Lxx

Lxx = Leu or Ile.
[a]HPLC fraction.
[b]Underivatized peptide.
[c]Peptide methyl ester.
[d]Residue numbers refer to peptides within protein fragments sequenced by conceptual translation of cDNA [refs. 8 ([f]), 10 ([g]), and 11 ([e])] and not to actual residue numbers in the intact protein.

Chemistry: Hunt *et al.*

Proc. Natl. Acad. Sci. USA 83 (1986)   6235

is assumed for the intact protein. The amino acids leucine and isoleucine have the same mass and are therefore not differentiated by the existing methodology. Positions containing one or the other of these residues are designated Lxx.

Shown in Fig. 1a is the main-beam mass spectrum recorded

for an aliquot of HPLC fraction 21. Signals for peptide $(M+H)^+$ ions appear at m/z 666, 721, 759, 844, 912, 1028, and 1486. Sequences determined for these peptides appear in Table 1. CAD mass spectra of the peptide $(M+H)^+$ ions at m/z 666, 1486, 912, and 759 are shown in Fig. 1 b–e, respectively. Major fragmentation pathways observed for oligopeptide $(M+H)^+$ ions are shown in Schemes I and II. Ion types are labeled according to the nomenclature proposal of Roepstorff and Fohlman (18).
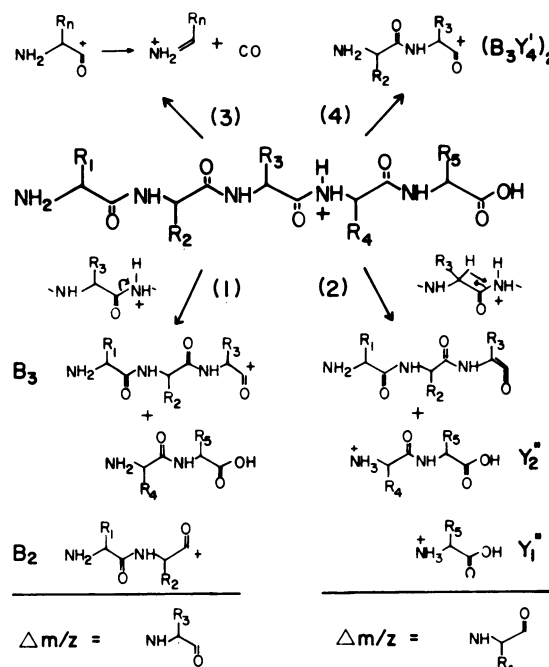
Scheme I
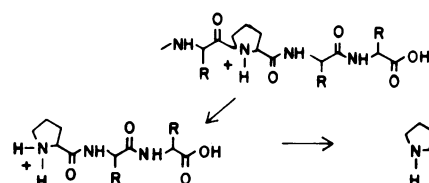
Scheme II

In the acidic matrix employed for LSIMS experiments, glycerol or thioglycerol containing 5% acetic acid, peptides exist as cations with a proton located on the amino terminus or on the side chains of such basic residues as arginine and lysine. Solvation with matrix molecules delocalizes the charge and stabilizes protonated species. In the gas phase, solvent molecules are absent and charge delocalization can only occur by internal solvation, a folding process that undoubtedly brings one or more of the amide linkages in close proximity to the protonated amino terminus. Since the proton affinity of a single peptide amide linkage ($\approx$217 kcal/mol; ref. 19) is comparable to that of a three-carbon primary amine in the gas phase ($\approx$218 kcal/mol; ref. 20), internal solvation provides a convenient pathway for proton-transfer reactions that distribute the charge onto the various amide linkages of the peptide backbone. Intramolecular hydrogen bond formation involving other amide linkages in the peptide liberates at least an additional 7 kcal/mol (19) and provides an additional driving force for preferential charge localization on the various amide bonds in the gas phase. Since hydrogen bond strength is greatest when the bond angle separating the attached atoms approaches 180°, maximal hydrogen bond stabilization is probably achieved by intramolecular solva-
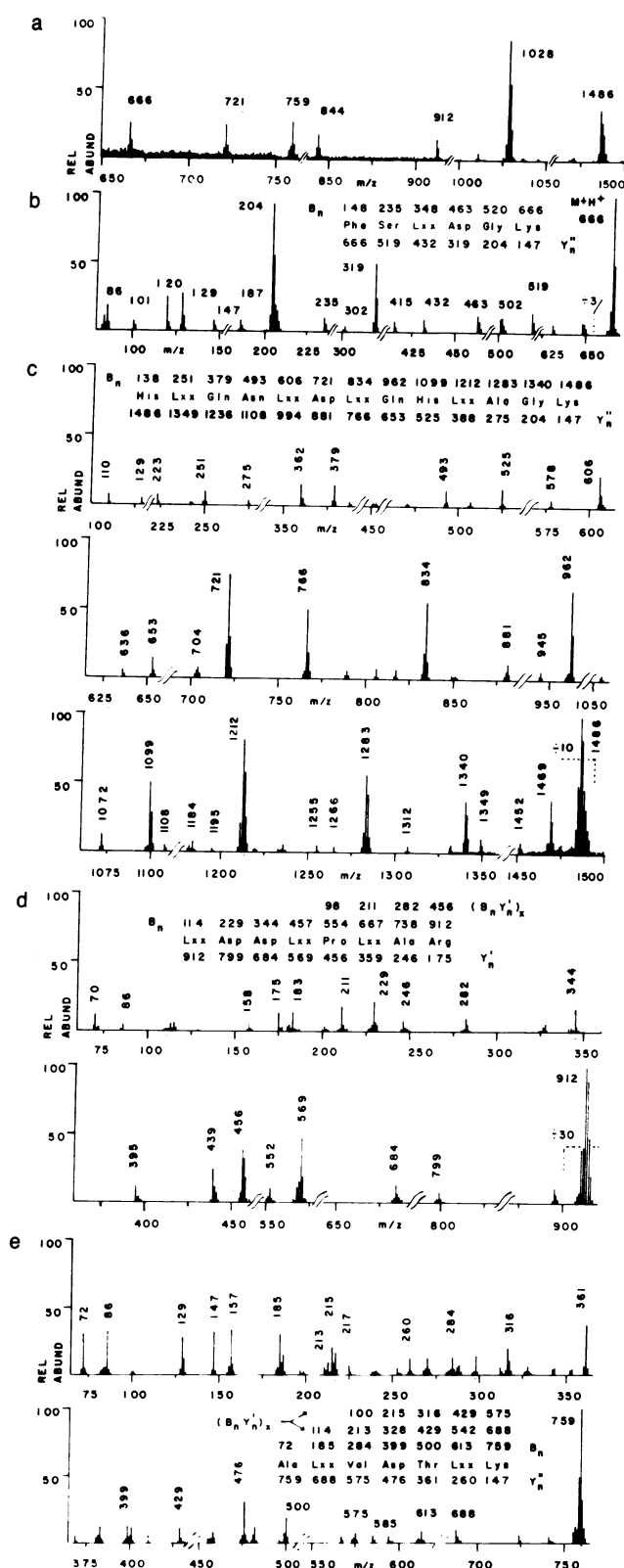

FIG. 1. Main-beam mass spectrum of peptides in HPLC fraction 21 (a) and CAD mass spectra of m/z 666 (b), m/z 1486 (c), m/z 912 (d), and m/z 759 (e). Abscissae, m/z; ordinates, relative abundance.

tion involving amide linkages on nonadjacent residues in the peptide chain.

When the collection of oligopeptide molecules protonated on the various amide linkages undergo low-energy (10–30 eV) collisions with argon atoms in the collision cell, kinetic energy is converted to vibrational energy (21) and many of the ions suffer fragmentation at one of the amide bonds. Cleavage of these bonds can occur by pathways 1 or 2 in Scheme I. Ions of type B, containing the amino terminus, and type Y″ (18), containing the carboxyl terminus, are produced in these two reactions. If the energy transferred in the collision process is relatively high, fragmentation pathway 1, involving cleavage of a single bond, is favored (22). Ions of type B are formed preferentially. If the amount of kinetic energy converted to potential energy during collision is relatively small, pathway 2, which involves simultaneous bond formation and cleavage and therefore a lower activation energy (22), should dominate the observed fragmentation. Ions of type Y″, produced by hydrogen transfer to the amide nitrogen and elimination of closed-shell ketene or cyclic species, should form preferentially.

Distribution of charge between the B- and Y″-type fragments discussed above is also dependent on the number and distribution of residues in the peptide chain that have high gas-phase basicity: lysine, arginine, histidine, tryptophan, and proline. In the low-energy collision regime (10–30 eV) employed in the present study, neutral and charged fragments produced from a particular peptide molecule do not necessarily separate immediately following the bond-cleavage step. Instead, the two partners frequently spiral about each other and suffer one or more encounters before the kinetic energy released in the fragmentation overcomes the charge–dipole force of attraction between the two particles (23). Proton transfer to the fragment of highest basicity during these additional collisions provides a possible explanation for the frequently observed preferential distribution of charge on B or Y″ type ions containing basic amino acid residues.

Fig. 1*b* shows an example of a CAD spectrum dominated by ions of type Y″. In this particular case, the peptide is relatively small and the basic lysine residue at the carboxyl terminus has a significant effect on the nature of ions observed in the spectrum. Numbers below the amino acid sequence in Fig. 1*b* refer to m/z values for expected ions of this type. Values for possible ions of type Y″ containing the carboxyl-terminal amino acid are provided in column 5 of Table 2. As shown in Scheme I, the mass difference between homologous Y″-type ions corresponds to the elements -NHCHRCO-. Mass values for the possible compositions of this type are provided in column 4 of Table 2. Predicted m/z values for fragments of type Y_1'' derived from the peptide Phe-Ser-Lxx-Asp-Gly-Lys are obtained by adding sequentially the incremental masses in column 4 for Gly, Asp, Lxx, Ser, and Phe to m/z 147, the value of Y″ for Lys found in column 5 of Table 2.

Numbers located above the amino acid sequence in Fig. 1*b* refer to m/z values expected for fragments of type B produced by pathway 1 in Scheme I. Column 3 of Table 2 contains values for the possible ions of type B. Note that the mass difference between homologous B-type ions (Scheme I) also corresponds to the elements -NHCHRCO-. Predicted m/z values for fragments of types B_2–B_5 derived from the peptide Phe-Ser-Lxx-Asp-Gly-Lys are obtained by adding sequentially the incremental masses in column 4 for Ser, Lxx, Asp, and Gly to that for B_1 at m/z 148. The m/z value for the $(M+H)^+$ ion is calculated by adding the incremental mass for Lys plus an additional 18 units, the mass corresponding to -OH and H^+, to that of B_5. Fig. 1*c* shows an example of a peptide CAD spectrum dominated by ions of type B. Spectra of this type are almost always observed when the basic residue histidine is present at or close to the amino terminus of the peptide sample.

Two other major ion types found in most CAD spectra result from cleavage of at least two bonds internal to the peptide chain. The first of these ion types is shown as arising from pathway 3 in Scheme I, appears at the low-mass end of the spectrum, and has the general formula $NH_2 = CHR^+$ or NH_2CHRCO^+. Possible masses for these ions are listed in columns 2 and 3 of Table 2. Although ions of this type are

Table 2. Mass values used in the interpretation of peptide CAD mass spectra

Amino acid	$NH_2 = CHR^+$ m/z	Type B, NH_2CHRCO^+ m/z	Incremental mass, -NHCHRCO-	Type Y″, $^+NH_3CHRCOOH$ m/z	
Gly	30	58	57	76	(90)
Ala	44	72	71	90	(104)
Ser	60 [140]	88 [168]	87 [167]	106 [186]	(120)
Pro	70	98	97	116	(130)
Val	72	100	99	118	(132)
Thr	74 [155]	102 [184]	101 [183]	120 [200]	(134)
Cys	76	104	103	122	(136)
Leu	86	114	113	132	(146)
Ile	86	114	113	132	(146)
Asn[a]	87	115	114	133	(147)
Asp	88 (102)	116 (130)	115 (129)	134	(162)
Gln[b]	101	129	128	147	(161)
Lys[b]	101	129	128	147	(161)
Glu	102 (116)	130 (144)	129 (143)	148	(176)
Met	104	132	131	150	(164)
His	110	138	137	156	(170)
Phe	120	148	147	166	(180)
Arg[c]	129	157	156	175	(189)
CM-Cys	134	162	161	180	(208)
Tyr	136	164	163	182	(196)
Trp[d]	159	187	186	215	(229)

Values in parentheses refer to the corresponding methyl esters. Values in brackets (for Ser and Thr) are for the corresponding phosphorylated derivatives.
[a–d]Values also correspond to those for the dipeptide Gly-Gly ([a]), Gly-Ala ([b]), or Gly-Val ([c]) or for the dipeptides Gly-Glu, Ala-Asp, and Val-Ser ([d]).

seldom observed for all residues in a peptide, those that do appear provide partial information about the amino acid composition of the sample. In Fig. 1*b*, ions at m/z 86, 120, and 101 plus 129 indicate the presence of Lxx, Phe, and Lys, respectively.

Pathway 4 in Scheme I shows the second fragment ion species that results from multipoint cleavage of the peptide backbone. These ions have the same formula as B ions and are designated as being of type $(B_xY_y')_z$. The letters B_x and Y_y indicate the points of cleavage to produce the carboxyl terminus and amino terminus of the fragment, respectively, and the number (z) outside the parentheses describes the number of amino acid residues present in the ion. Fortunately, ions of this type are usually found in low abundance, seldom contain more than two or three amino acid residues, and therefore occur only at the low-mass end of the spectrum. Peptides containing proline are an exception to this generalization. Because the imino group in proline is part of a five-membered ring, amide linkages involving proline are devoid of hydrogen and exhibit a gas-phase proton affinity that is several kcal/mol higher than other amide bonds in the peptide backbone. Protonation and cleavage of the proline amide bond to produce a Y''-type ion is a highly favored process and often dominates the CAD spectra of peptides containing this residue. Intramolecular transfer of the proton to the other amide linkages in the ion and subsequent cleavage by pathway 1 results in a series of ions of type $(B_xY_y')_z$, as shown in Scheme II.

Fig. 1*d* displays the CAD spectrum of a peptide containing proline and illustrates the importance of the above fragmentation pathway. Ions corresponding to type $(B_xY_y')_z$ are observed at m/z 70 (98 − CO), 211, and 282 and terminate at m/z 456, which corresponds to Y_4''. The remainder of the sequence is read from additional Y''-type ions at m/z 569, 684, and 799. Type-B ions are observed in low abundance at m/z 229, 344, 457, and 554 and provide support for the assigned sequence.

All of the ion types discussed above can undergo loss of water if the particular fragment contains serine, homoserine, or threonine. Two signals separated by 18 mass units result from this process. Loss of ammonia to produce a pair of signals separated by 17 mass units is also a common occurrence for all fragments containing arginine, lysine, glutamine, and asparagine. Elimination of phosphoric acid from phosphoserine and phosphothreonine and of methyl mercaptan from methionine are also observed. Loss of carbon monoxide from B-type ions is a general phenomenon.

The spectrum shown in Fig. 1*e* is considerably more complex than those discussed above because it contains ions corresponding to all of the various ion types in approximately the same abundance. Comparison of this CAD spectrum with that recorded for the corresponding methyl ester greatly simplifies the task of interpretation because all signals containing the carboxyl terminus and aspartic, glutamic, or S-carboxymethylcysteine residues shift by increments of 14 mass units in the spectrum of the methyl ester. Fragments derived from the amino terminus remain at the same mass in both spectra unless they contain one of the above three acidic residues. CAD spectra of the corresponding methyl esters were used to check all sequences in Table 1.

Note that glutamine and lysine residues have identical mass. Differentiation of lysine and glutamine is accomplished by on-probe acetylation (24) or by treatment of the sample with phenyl isothiocyanate (16, 25). Values given for lysine in Table 2 shift by 42 mass units following acetylation and by

135 mass units following treatment with Edman reagents. No shift in mass is observed for fragments containing glutamine following treatment of the sample under either of the above conditions. Use of phenyl isothiocyanate is the preferred method because the identity of the amino-terminal residue can then be confirmed by performing one cycle of Edman degradation on the mixture of peptides and by using LSIMS to obtain the molecular mass of each peptide shortened by one amino acid (16, 25). All peptides in Table 1 were checked in this manner.

1. Hunt, D. F., Bone, W. M., Shabanowitz, J., Rhodes, J. & Ballard, J. M. (1981) *Anal. Chem.* **53**, 1704–1706.
2. Aberth, W., Straub, K. M. & Burlingame, A. L. (1982) *Anal. Chem.* **54**, 2029–2034.
3. Barber, M., Bordoli, R. S., Sedgwick, R. D. & Tyler, A. N. (1981) *J. Chem. Soc. Chem. Commun.*, 325–327.
4. Cody, R. B., Jr., Amster, I. J. & McLafferty, F. W. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 6367–6370.
5. Fitton, J. E., Hunt, D. F., Marasco, J., Shabanowitz, J., Winston, S. & Dell, A. (1984) *FEBS Lett.* **169**, 25–29.
6. Lippstreu-Fisher, D. L. & Gross, M. L. (1985) *Anal. Chem.* **57**, 1174–1180.
7. Eckart, K., Schwarz, H., Tomer, K. B. & Gross, M. L. (1985) *J. Am. Chem. Soc.* **107**, 6765–6769.
8. Knott, T. J., Rall, S. C., Jr., Innerarity, T. L., Jacobson, S. F., Urdea, M. S., Levy-Wilson, B., Powell, L. M., Pease, R. J., Eddy, R., Nakai, H., Byers, M., Priestley, L. M., Robertson, E., Rall, L. B., Betsholtz, C., Shows, T. B., Mahley, R. W. & Scott, J. (1985) *Science* **230**, 37–43.
9. Deeb, S. S., Motulsky, A. G. & Albers, J. J. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 4983–4986.
10. Protter, A. A., Hardman, D. A., Schilling, J. W., Miller, J., Appleby, V., Chen, G. C., Kirsher, S. W., McEnroe, G. & Kane, J. P. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 1467–1471.
11. Law, S. W., Lackner, K. J., Hospattankar, A. V., Anchors, J. M., Sakaguchi, A. Y., Naylor, S. L. & Brewer, H. B., Jr. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 8340–8344.
12. LeBoeuf, R. C., Miller, C., Shively, J. E., Schumaker, V. N., Balla, M. A. & Lusis, A. J. (1984) *FEBS Lett.* **170**, 105–108.
13. Lusis, A. J., West, R., Mehrabian, M., Reuben, M. A., LeBoeuf, R. C., Kaptein, J. S., Johnson, D. F., Shumaker, V. N., Yuhasz, M. P., Schotz, M. C. & Elovson, J. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 4597–4601.
14. Hunt, D. F., Giordani, A. B., Rhodes, J. & Herold, D. A. (1982) *Clin. Chem. (Winston-Salem, N.C.)* **28**, 2387–2392.
15. Socorro, L. & Camejo, G. (1979) *J. Lipid Res.* **20**, 631–638.
16. Bradley, W. A., Rhode, M. F., Gotto, A. M., Jr., & Jackson, R. L. (1978) *Biophys. Biochem. Res. Commun.* **81**, 928–935.
17. Bradley, C. V., Williams, D. H. & Hanley, M. R. (1982) *Biochem. Biophys. Res. Commun.* **104**, 1223–1230.
18. Roepstorff, P. & Fohlman, J. (1984) *Biomed. Mass Spectrom.* **11**, 601.
19. Meot-Ner, M. (1984) *J. Am. Chem. Soc.* **106**, 278–283.
20. Aue, D. H. & Bowers, M. T. (1979) in *Gas Phase Ion Chemistry*, ed. Bowers, M. T. (Academic, New York), pp. 1–51.
21. McLafferty, F. W. (1983) *Tandem Mass Spectrometry* (Wiley-Interscience, New York).
22. Williams, D. H. & Howe, I. (1972) *Principles of Organic Mass Spectrometry* (McGraw-Hill, New York).
23. Lias, S. G. & Ausloos, P. (1975) *Ion Molecule Reactions* (American Chemical Society, Washington, DC), pp. 64–89.
24. Morris, H. R., Panico, M. & Taylor, G. W. (1983) *Biochem. Biophys. Res. Commun.* **117**, 299–305.
25. Gibson, B. W. & Biemann, K. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 1956–1960.