Applied Biosystems

High-Energy, Collision Induced Dissociation of Peptides with the Applied Biosystems 4700 Proteomics Analyzer

Purpose

Obtain more information with confidence using the Applied Biosystems 4700 Proteomics Analyzer with TOF/TOF™ Optics—high-energy, collision induced dissociation (CID) produces data-rich product ion spectra that allow unambiguous peptide sequence assignments.

Overview

The Applied Biosystems 4700 Proteomics Analyzer with TOF/TOF Optics is uniquely designed to allow the user control of fragmentation conditions and simultaneously provides both high-energy, collision induced dissociation spectra and low-energy unimolecular dissociation spectra. High-energy, collision induced dissociation of peptides is carried out by activation of selected peptide precursor ions after collisions with a gas. This process takes place in a collision cell and the ions enter the cell with energies typically in the 1-2 keV range.

These high-energy collisions result in the production of low mass and internal fragment ions, ions from amino acid side chain fragmentations, and ions specific to particular amino acids. These additional fragmentation patterns of peptides are generally different from those observed in low-energy fragmentation spectra typically seen in triple quadrupole, ion trap, and orthogonal quadrupole time-of-flight instruments. Although low-energy fragmentation produces relatively simple, easy-to-interpret spectra, it does not generate sufficient collisional velocity to effectively generate immonium ion fragments, which provide valuable amino acid composition information; nor can it cleave the side chains necessary to distinguish between the isobaric amino acids leucine and isoleucine.

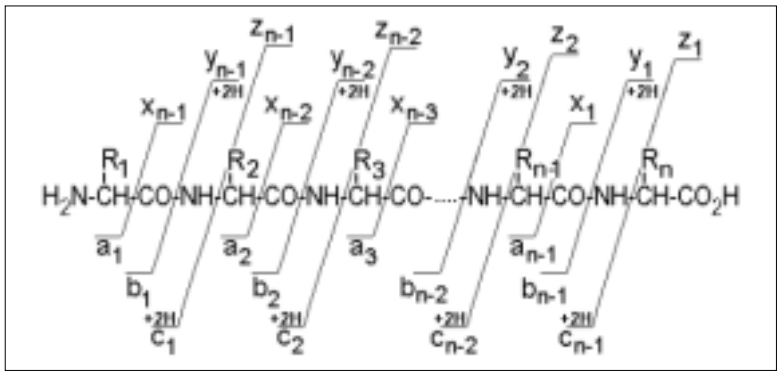

Figure 1. Nomenclature of fragment ions of peptides. In general, these fragment ions are observed in the high-energy CID fragmentation mass spectra of peptides, although not necessarily all in the same spectrum. In low-energy fragmentation spectra, only some of these fragment ion types are present.[1]

The presence of complementary fragment ion series, abundant low mass and internal fragment ions, ions from amino acid side chain fragmentations, and ions specific to particular amino acids are extremely advantageous and allow you to more confidently interpret peptide fragment ion mass spectra. The fragmentation patterns and fragment ions predominant or unique to high-energy CID are briefly discussed below.

Key Features

- Patented TOF/TOF Optics easily generate reproducible high-energy CID fragment ion series and unimolecular disassociation, delivering definitive detailed structural information for accurate protein identification and enhanced characterization
- Complementary fragment ion series, abundant low mass immonium ions, internal fragment ions, and ions specific to particular amino acids provide confidence in your results
- High-energy CID provides side chain fragmentation that allows differentiation between leucine and isoleucine residues
- GPS Explorer™ Software quickly and accurately identifies proteins from uninterpreted high-energy CID fragment spectra

Experimental Conditions

The observations discussed below were made by the close examination of a collection of peptide fragment ion mass spectra, which were acquired with the Applied Biosystems 4700 Proteomics Analyzer. Peptides were generated by enzymatic digestion of proteins, using trypsin, endopeptidase Glu-C, or thermolysin. The MALDI matrix used was ∝-cyano-4-hydroxy-cinnamic acid. Collision energies

were 1 keV and air was used as the collision gas. The acquired MS and MS/MS data were then submitted to a database for protein identification using GPS Explorer™ Software with the integrated Mascot™ search engine software.

Results and Discussion

The CID mass spectra of peptides generated with the Applied Biosystems 4700 Proteomics Analyzer contain sequence fragment ions resulting not only from cleavages along the peptide bonds (Figure 1) but also from side chain fragmentations (Figure 2).

This is consistent with similar observations made with respect to high-energy CID mass spectra of peptides obtained with magnetic deflection four-sector mass spectrometers in the late 1980s.[2,3] The side chain-specific fragments can be used to confirm the assignment of a particular amino acid to the peptide sequence. This is because most amino acids have unbranched, symmetrical side chains, so in fragment ion mass spectra a single high-energy *d* or *w* fragment ion is produced from loss of the portion of the side chain attached at the ß-carbon atom. Two amino acids, isoleucine and threonine, have two different groups (methyl-ethyl and methyl-hydroxyl, respectively) attached to the ß-carbon, and so two different *d* or *w* fragments may be produced. In the case of isoleucine, this feature allows its differentiation from the isomeric leucine. Valine also has a branched side chain but the two γ-substituents are the same (methyl groups), so effectively only one *d* or *w* ion is produced from this amino acid. Loss of the entire side chain (cleavage between the ∝- and ß-carbon atoms) results in *v*-type fragment ions.

Figure 3 shows the complete series of *y*-type fragment ions that allows for the unambiguous determination of the sequence of this peptide de novo. The presence of other C-terminal, as well as a number of N-terminal fragment ions allows for straightforward confirmation of all amino acid assignments. Also shown are the side chain fragments present for a number of the amino acids and, specifically, for Leu-2 (w_7) and Ile-7 (w_{2a} and w_{2b}), which allow for the differentiation of these two isomeric amino acids. These high-energy fragments are completely absent in low-energy peptide fragmentation spectra.[1]

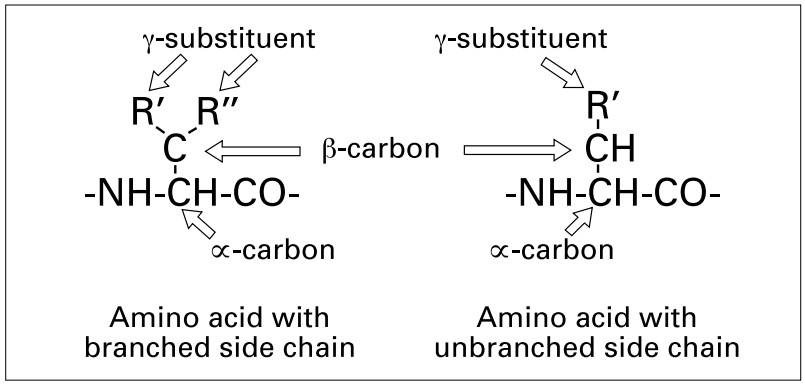

Figure 2. Amino acid side chain substituents.[1]

Another feature of high-energy CID mass spectra of peptides is the presence, at low mass, of ions characteristic of the amino acids present in the sequence. These ions are either immonium ions of the individual amino acids or fragments thereof. While the absence of an immonium ion does not necessarily preclude the presence of the associated amino acid, the presence of certain immonium ions or other low mass fragments is a good indication that the corresponding amino acids are present in the sequence. A list of frequently observed immonium and other low mass ions for the common protein amino acids appears in Table 1. All fragment ion m/z values shown in Figures 3, 4, and 5 are for mono-isotopic (^{12}C isotope peak) and are generally within 0.02 u from the calculated exact mass values.

The presence of basic amino acids at or near a peptide N- or C-terminus results in producing N-terminal (*a,b,c,* and *d*) or C-terminal (*x,y,z,v,* and *w*) fragment ions respectively.

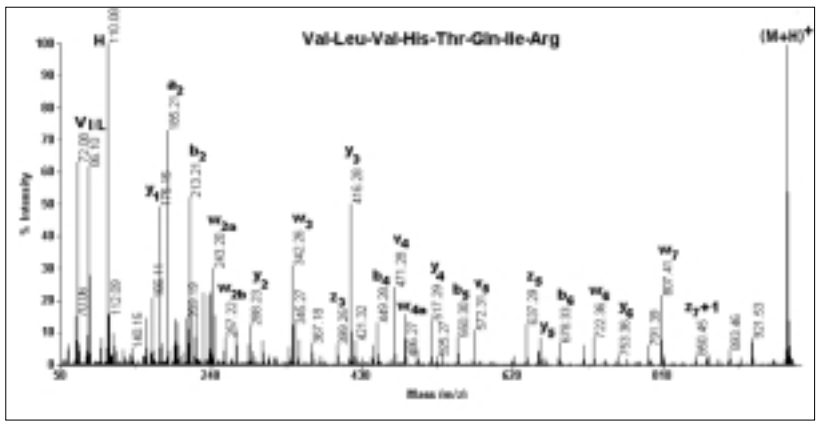

Figure 3. High-energy CID fragment ion mass spectrum of the octapeptide VLVHTQIR [(M+H)⁺ m/z 965.5], generated by digestion with trypsin of yeast proteins, isolated in a proteomics experiment. For clarity, not all the ions that can be assigned to the sequence have been labeled—in this figure as well as in Figures 4 and 5.

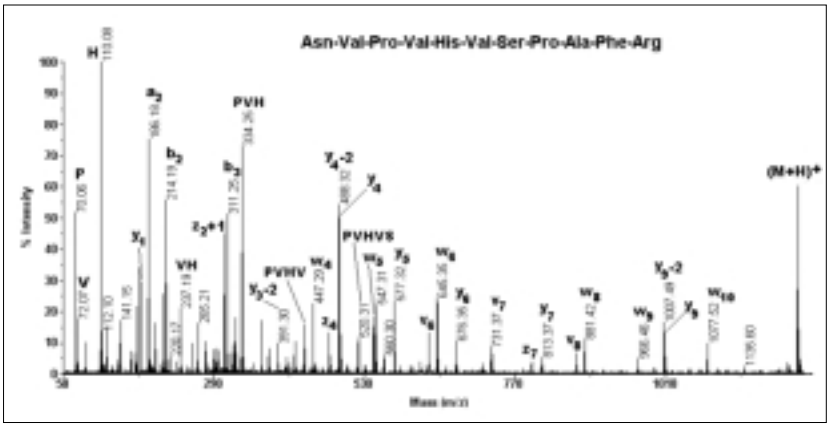

Figure 4. High-energy CID fragment ion mass spectrum of the undecapeptide NVPVHVSPAFR, [(M+H)⁺ m/z 1222.7] generated by digestion with trypsin of yeast proteins, isolated in a proteomics experiment.

The peptide example in Figure 4 has a basic Arginine residue at the peptide C-terminus that results in the production of predominantly C-terminal fragments. Also worth noting is the presence of the abundant *y-2* (Y_i) fragments confirming the Proline residues (y_4-*2* and y_9-*2*). In addition, a number of internal fragment ions resulting from cleavage at Pro are present, as well as immonium ions indicative of the amino acids present in the peptide.

If no basic amino acid is present near the N-terminus then both N-terminal and C-terminal fragments may be produced, often *b*- and *y*-type ions as shown in Figure 5. This is not different from what has been previously described for peptide fragment ion mass spectra generated with either high- or low-energy CID. However, the information content of the fragment ion mass spectra generated with the Applied Biosystems 4700 Proteomics Analyzer is greater than in spectra generated with other instruments. For example, complementary ion series (e.g. *a, b,* and *d*) are frequently present in these spectra (Figures 3 and 4). Although it can be argued that such fragment ion mass spectra are more complex because they contain more fragment ions, this complexity is moderated by the absence of multiply charged fragments, as the precursor ions in MALDI are almost always singly charged. In fact, such complementary information allows for greater confidence in the interpretation of such data de novo.[4] Furthermore, uninterpreted high-energy CID tandem mass spectra of peptides generated with the Applied Biosystems 4700 Proteomics Analyzer can be used successfully for protein identification via database searching, using the Applied Biosystems GPS Explorer™ Software, which operates in concert with Mascot™ database search software.

Conclusion

The high-energy peptide CID mass spectra obtained with the Applied Biosystems 4700 Proteomics Analyzer contain a wealth of information in the form of often-complementary fragment ion series, which can be very advantageous in the interpretation of such data. Amino acid side chain fragment ions unique to high-energy CID allow for the differentiation of leucine and isoleucine; abundant immonium ions provide information related to the amino acid composition of a particular peptide; and internal and other high-energy fragments increase the confidence in the interpretation of these spectra.

Since high-energy fragmentation pathways of peptides were elucidated in great detail with magnetic deflection sector instruments, the Applied Biosystems 4700 Proteomics Analyzer with GPS Explorer software builds on that already extensive knowledge. Also, the unique abilities to control fragmentation conditions and produce valuable structural information with high-energy CID—all with great sample throughput—are key innovations that the Applied Biosystems

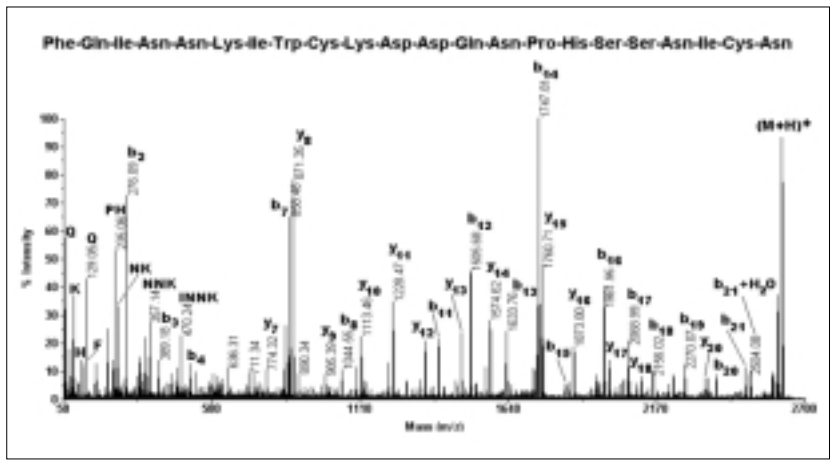

Figure 5. High-energy CID fragment ion mass spectrum of the 22-amino acid peptide FQINNKIWCKD-DQNPHSSNICN [(M+H)⁺ m/z 2618.1], generated by digestion with chymotrypsin of ∝-lactalbumin (cysteines are reduced but not alkylated). No basic amino acid is present at either the N- or the C-terminus of the peptide, and as a result both *b*- and *y*-type fragment ions are produced that can be used to confirm the amino acid assignments. The very abundant y_8 and b_{14} fragments are indicative of the particular lability of the Asn-Pro bond.

Amino acid	Residue mass[b]	Immonium ion m/z[b]	Other low mass ions m/z[b]
Gly (G)	57.0215	30.0344	
Ala (A)	71.0371	44.0500	
Ser (S)	87.0371	60.0449	
Pro (P)	97.0528	70.0657	
Val (V)	99.0684	72.0813	41.0391, 55.0548, 69.0704
Thr (T)	101.0477	74.0606	
Cys (C)	103.0092	76.0221	
Ile (I)	113.0841	86.0970	44.0500, 72.0449
Leu (L)	113.0841	86.0970	44.0500, 72.0449
Asn (N)	114.0429	87.0558	70.0293
Asp (D)	115.0269	88.0399	70.0293
Gln (Q)	128.0586	101.0715	56.0500, 84.0449, 129.1028
Lys (K)	128.0950	101.1079	56.0500, 84.0813
Glu (E)	129.0426	102.0555	84.0449
Met (M)	131.0405	104.0534	
His (H)	137.0589	110.0718	
Phe (F)	147.0684	120.0813	91.0548
Arg (R)	156.1011	129.1140	70.0657, 100.0875, 112.0875
CaC[c] (C)	160.0306	133.0436	
CmC[d] (C)	161.0147	134.0276	
Tyr (Y)	163.0633	136.0762	91.0548, 107.0497
Trp (W)	186.0793	159.0922	77.0391, 117.0578, 130.0657, 132.0813
PeC[e] (C)	208.0670	181.0799	106.0657

Table 1. Immonium and other low mass ions observed in high-energy CID fragment ion mass spectra of peptides.[a]

[a] Data in this table was taken from Reference 1.
[b] All mass values are monoisotopic
[c] Carbamidomethyl Cys
[d] Carboxymethyl Cys
[e] Pyridylethyl Cys

4700 Proteomics Analyzer brings to MALDI mass spectrometry instrumentation.

In addition, the Applied Biosystems 4700 Proteomics Analyzer can be fully automated to provide comprehensive peptide and protein analysis consistently, day after day. Supporting multiple workflows including 2-D gels, MDLC, and proprietary ICAT™ reagent technology, the Applied Biosystems 4700 Proteomics Analyzer is a flexible, high powered, straightforward system for MS and MS/MS that increases your productivity and helps solve your complex biological problems.

References

[1] Papayannopoulos, I.A. 1995. *Mass Spectrom. Rev.* 14:49–73.

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[4] Yergey, A.L., et al. 2002. *J. Am. Soc. Mass Spectrom.* 13:784–791.

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