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International Journal of Mass Spectrometry 190/191 (1999) 313–320

International Journal of Mass Spectrometry

Collision-induced dissociation of singly charged peptide ions in a matrix-assisted laser desorption ionization ion trap mass spectrometer

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Received 30 December 1998; accepted 29 March 1999

Abstract

We document the systematics of collision-induced dissociation of singly charged peptide ions in a matrix-assisted laser desorption/ionization (MALDI) ion trap mass spectrometer. We show that singly charged peptide ions with *m/z* ratios extending to 3500 can be effectively fragmented using a newly devised excitation scheme, termed red shifted off-resonance large amplitude excitation (RSORLAE), and classify the dominant features of the resulting collision-induced dissociation spectra as follows. (1) Peptides ions with a *m/z* value of <1500 can be effectively fragmented independent of their amino acid composition. (2) Peptides ions with $m/z < 3000$ that contain lysine but not arginine can be extensively fragmented. (3) Arginine-containing peptide ions undergo highly preferential fragmentation at the C-termini of aspartic/glutamic acid residues. (4) Peptide ions containing a C-terminal lysine residue as well as one or more arginine residues readily rearrange to lose the terminal lysine. (5) Proline-containing peptide ions fragment preferentially at the N-termini of the proline residues. These systematics are helpful for the verification of peptide primary structures and for formulating efficient strategies for protein identification using tandem MALDI ion trap mass spectrometric data. (Int J Mass Spectrom 190/191 (1999) 313–320) © 1999 Elsevier Science B.V.

Keywords: MS/MS; Tandem mass spectrometry; Peptide sequence; Protein identification; MALDI; Ion trap mass spectrometer; Collision-induced dissociation

1. Introduction

Recent developments in protein identification incorporating database searching with mass spectrometric (MS) data have established the high value of data obtained from collision-induced-dissociation (CID) spectra of individual peptide ions [1–4]. Database searching with mass spectral CID data frequently yields reliable, unambiguous identifications. Currently, the majority of such CID data is derived from doubly (and sometimes triply charged) peptide ions produced by electrospray ionization (ESI) from trypsin digests of proteins. The wide use of electrospray ionization (ESI) for this purpose derives from the fact that tryptic peptides yield mostly doubly charged ions by ESI and that doubly charged peptide ions undergo extensive and readily interpretable fragmentation upon collisional excitation. ESI is also conveniently

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Dedicated to J.F.J. Todd and R.E. March in recognition of their original contributions to quadrupole ion trap mass spectrometry.

PII S1387-3806(99)00064-0

carried out from peptide solutions that are directly infused into the mass spectrometer or are subjected to on-line liquid chromatographic separation just prior to MS analysis. Finally, there are several highly evolved instruments that prove to be well suited for acquiring CID spectra of peptide ions from the continuous ion current ESI source. These instruments include the triple-stage quadrupole mass spectrometer [5], the ion trap mass spectrometer [4] and more recently the orthogonal injection quadrupole-time-of-flight mass spectrometer [6–8].

Pulsed ion sources, especially those incorporating matrix-assisted laser desorption/ionization (MALDI), can also be used for obtaining tandem MS data from peptides. Instruments adapted for this purpose include the axial-geometry time-of-flight (TOF) analyzer fitted with an electrostatic mirror [9–11], the orthogonal geometry quadrupole-TOF instrument [12], and the ion trap mass spectrometer [13,14]. A potential advantage of MALDI is its ability to produce information-rich spectra from complex mixtures of peptides without the requirement for chromatographic separation of the peptides or removal of biochemical additives such as buffers and salts. This ability to acquire CID spectra from unfractionated peptide mixtures is especially attractive for protein identification involving protein/DNA database searches, where the amount of available sample is often severely limited.

To take advantage of these good properties of MALDI, we recently constructed a MALDI ion trap mass spectrometer and demonstrated the utility of the instrument for protein identification and analysis of post-translational modifications [15,16]. We devised an excitation scheme [termed red shifted off-resonance large amplitude excitation (RSORLAE)] that enables the deposition of sufficiently large amounts of energy to allow the efficient decomposition of singly charged peptide ions with *m/z* up to 3500 [17]. In the present article, we describe the results of an investigation of the CID fragmentation of more than 200 different singly protonated peptides. The observed fragmentation systematics prove helpful for the verification of peptide primary structures and for formulating efficient strategies for protein identification using tandem MALDI ion trap MS data.

2. Experimental

Peptides were purchased from the Sigma Chemical Co. (St. Louis, MO). *Escherichia coli* RecA protein was provided by Dr. Kenji Adzuma (The Rockefeller University) and rabbit phosphatase inhibitor-I was provided by Dr. Andrew Czernik (The Rockefeller University). Modified bovine trypsin and endoproteinase Lys-C were sequencing grade from Boehringer Mannheim (Indianapolis, IN). Digestions of proteins were carried out according to protocols suggested by the manufacturer. All experiments were performed using our custom-built MALDI ion trap mass spectrometer. A detailed description of the instrument can be found in [14]. RSORLAE [17] was used to obtain the MS/MS spectra. The amplitude and period of the excitation was respectively 21 $V_{p\text{-}p}$ and 30 ms, except for the MS/MS spectrum of N-acetyl-β-endorphin, which was excited for 120 ms. Excitation frequencies were in the range 59–102 kHz, chosen to be ~5% to the red of the resonant frequency of the ion to be dissociated [17]. The matrix solution used for the present measurements was 2,5-dihydroxybenzoic acid (DHB) solution in water/acetonitrile 1:1 (v/v) (2× dilution of a saturated solution of DHB.) Samples were prepared for mass spectrometry by thoroughly mixing 1 μL of the sample solution with 1 μL of the matrix solution directly on the sample probe and allowing the solution to dry at room temperature.

3. Results and discussion

We obtained MALDI ion trap CID mass spectra of more than 200 different peptides and found that the features observed in these spectra depend on the peptide molecular mass, amino acid composition, and sequence. Our results lead to a relatively simple classification scheme for the fragmentation of singly protonated peptides, which we summarize by the following five general rules.

3.1. Peptide ions with m/z value <1500 can be effectively fragmented independent of their amino acid composition

The RSORLAE scheme [17] (which supplies higher amounts of energy than conventional resonant

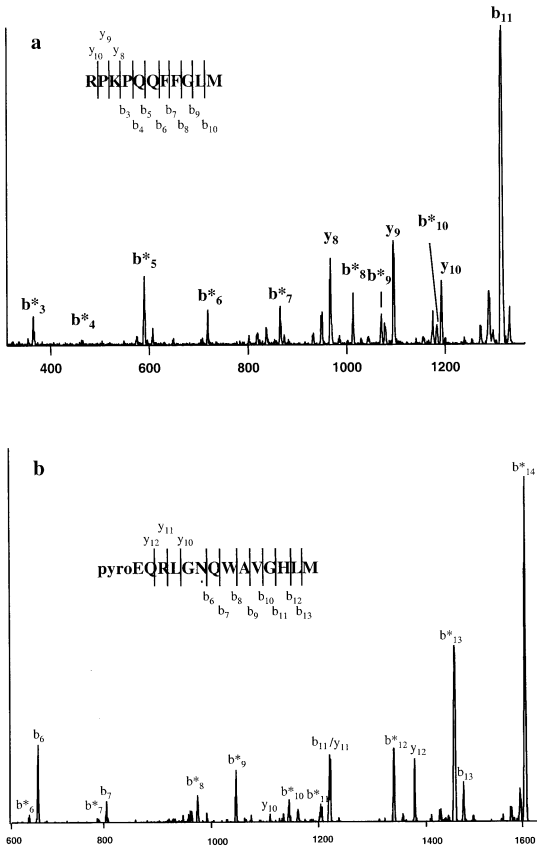

Fig. 1. MS/MS spectra of (a) substance P and (b) bombesin. A description of the nomenclature used for the fragment ions can be found in [18]. Ions resulting from the loss of H_2O and/or NH_3 from *b* and *y* ions, are here denoted *b** and *y**.

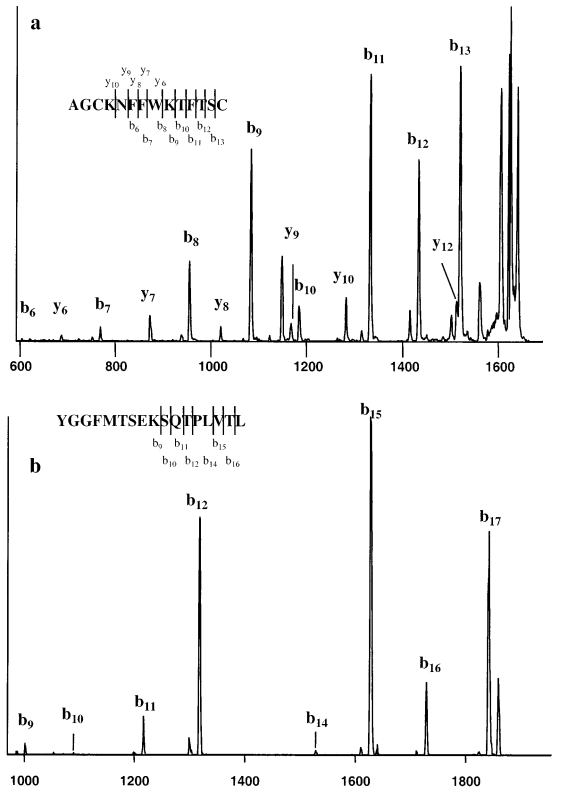

Fig. 2. MS/MS spectra of (a) reduced somatostatin and (b) β-endorphin.

excitation) allows effective fragmentation of singly protonated peptide ions with *m/z* values <1500, relatively independent of the amino acid composition and sequence. Fig. 1 shows MS/MS spectra of substance P and bombesin, two peptides that are commonly used as benchmarks for evaluating CID. The spectra were taken with a 100 fmol loading on the mass spectrometer sample probe and with a mass isolation resolution of ~4 Da. CID using RSORLAE is seen to produce extensive sequencing information, generating predominately *b* and *y* type ions [18] (as well as ions arising through additional loss of H_2O/NH_3 from the *b* and *y* ion species, which are here denoted *b** and *y**).

3.2. Peptide ions that contain lysine but not arginine produce extensive fragmentation

Singly protonated peptides ions with *m/z* < 3000 that contain lysine but not arginine can be fragmented extensively, although usually not at every peptide bond. Examples of MS/MS spectra of peptides in this category (reduced somatostatin, β-endorphin, amyloid-β-protein 12–28, and N-acetyl-β-endorphin 1–27) are shown in Figs. 2–4, using 1 pmol of each peptide loaded onto the sample probe. In this category, peptide ions with *m/z* values as high as 3000 undergo fragmentation as readily as do those with *m/z* = 1000—a surprising deviation from the general trend of reduced efficacy of dissociation as a function of increasing molecular mass.

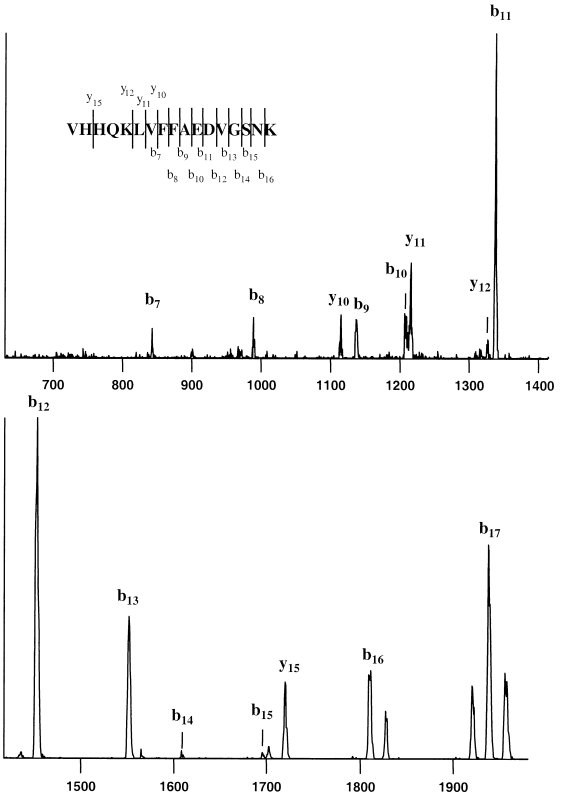

Fig. 3. The MS/MS spectrum of the amyloid β protein 12–28.

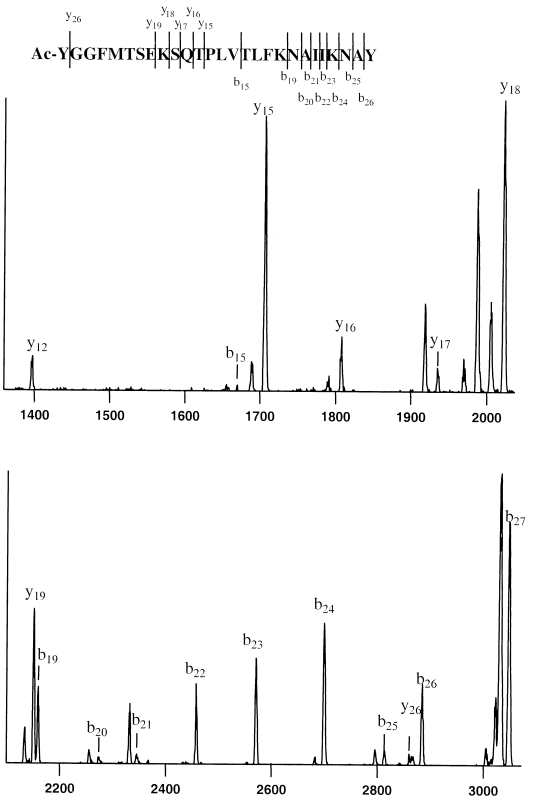

Fig. 4. The MS/MS spectrum of N-acetyl-β-endorphin.

3.3. Arginine-containing peptide ions undergo preferential fragmentation at the C-termini of Asp/Glu residues

We have reported previously that singly protonated, arginine-containing peptide ions undergo preferential fragmentation at the C-termini of Asp/Glu residues [19]. This fragmentation gives rise to intense *y* type ions if the Arg residue is located C-terminal to the acidic residues, or *b* type ions if the Arg residue is located N-terminal to the acidic residues. Peptides in this category can be readily fragmented for *m/z* values as large as 3700. Lys-containing peptides that do not contain Arg sometimes exhibit similar selective fragmentation at Asp, but the preference is not as dramatic as for Arg-containing peptides. The fragmentation of peptides containing both Arg and Lys as well as acidic residues is less readily characterized and the preference for fragmentation at acidic residues can sometimes be lost completely for peptides containing multiple Arg and Lys residues. The CID of Arg-containing peptides that do not contain acidic amino acids also proves difficult to characterize although the fragmentation is found to be strongly dependent on the number and relative position of Arg residues in the sequence. Thus, peptide ions with multiple Arg residues resist fragmentation when the Arg residues are positioned towards the middle of the sequence, but fragment relatively easily when they contain <3 Arg residues positioned close to the termini.

3.4. Peptide ions containing a C-terminal lysine residue as well as one or more arginine residues readily rearrange to lose the terminal lysine

During an investigation of the CID spectra of peptides generated by digestion of proteins by endoproteinase Lys-C (i.e. peptides that terminate in a

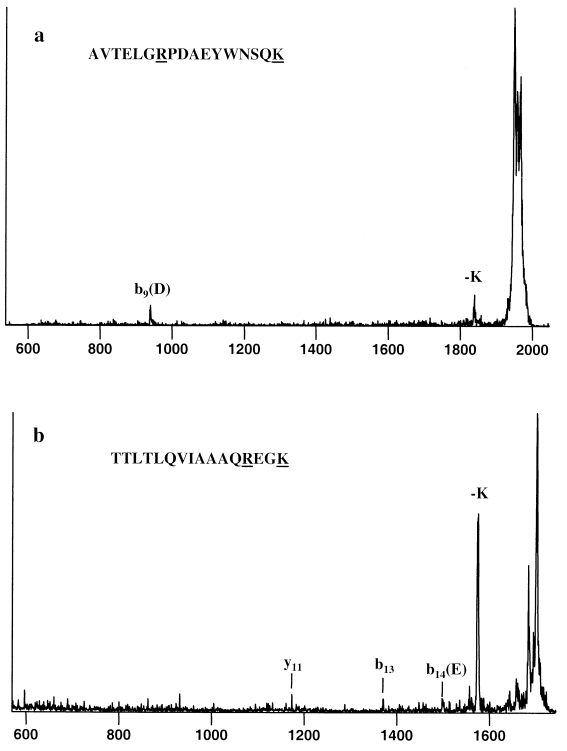

Fig. 5. MS/MS spectra of Lys-C peptides with the sequence (a) AVTELGRPDAEYWNSQK and (b) TTLTLQVIAAAQREGK.

C-terminal Lys), we frequently observed a relatively intense fragment ion with a *m/z* ~ 129 less than the precursor ion. This preferential fragmentation was especially evident when the peptides contained one or more Arg residues in addition to the C-terminal Lys. In some cases (see, e.g. Fig. 5), the intensity of this fragment ion was higher than or comparable with fragments generated by the previously discussed preferential cleavage at Asp/Glu residues. This intense fragment ion arises through the loss of the C-terminal Lys by a rearrangement reaction that formally yields a c_{n-1} ion fragment [18]. In cases where the sequence of the peptide is unknown, it proves difficult to differentiate this type of rearrangement from preferred cleavage of an N-terminal Glu residue because the latter also yields an intense fragment ion with a mass 129 Da less than that of the protonated precursor species.

3.5. Proline-containing peptide ions undergo preferential fragmentation at the N-termini of the proline residues

Proline-containing singly protonated peptides undergo moderately preferential fragmentation at the N-termini of the Pro residues. This preferred fragmentation has also been observed in other types of MS measurement, e.g. ESI triple quadruple MS [20] and ESI-ion trap MS [21]. In the present investigation, we observed relatively intense fragment ions arising from the cleavage at the N-terminal of Pro and fragment ions arising from the cleavage at the C-terminal were usually absent or very weak. Examples of this phenomenon are evident in Fig. 1(a) (strong b_3^* and weak b_4^*), Fig. 2(b) (strong b_{12} and missing b_{13}), and Fig. 4 (strong y_{15} and missing y_{14}), generating a gap in a series of sequencing ions. For Arg-containing peptides, the selective fragmentation at Pro residues is not as dominant as that observed at Asp/Glu residues. Exceptions are sometimes observed to the weak cleavage at the C-terminal of Pro [see, e.g. y_8 in Fig. 1(a)]. The moderate cleavage selectivity at Pro for singly charged peptide ions in the MALDI ion trap mass spectrometer should be contrasted with the more dominant cleavage selectivity at Pro observed for doubly charged peptide ions in a ESI ion trap mass spectrometer (where fragmentation at Pro is even more pronounced than that at Asp [21]).

3.6. Tandem ion trap mass spectrometry of peptide ions from unfractionated enzymatic digestions of proteins

We have also evaluated the feasibility of performing MS/MS experiments on unfractionated peptides produced by enzymatic digestions of proteins. More than 50 different proteins were included in this study. CID spectra obtained from individual peptides in the proteolytic mixtures exhibited the same general features as those outlined above. Peptide ions of interest were isolated with a mass resolution of 4–7 Da in the ion trap using a procedure that we have described previously [22]. Although this mass isolation resolution is modest, it is usually sufficient to allow analysis of quite complex peptide mixtures without prior

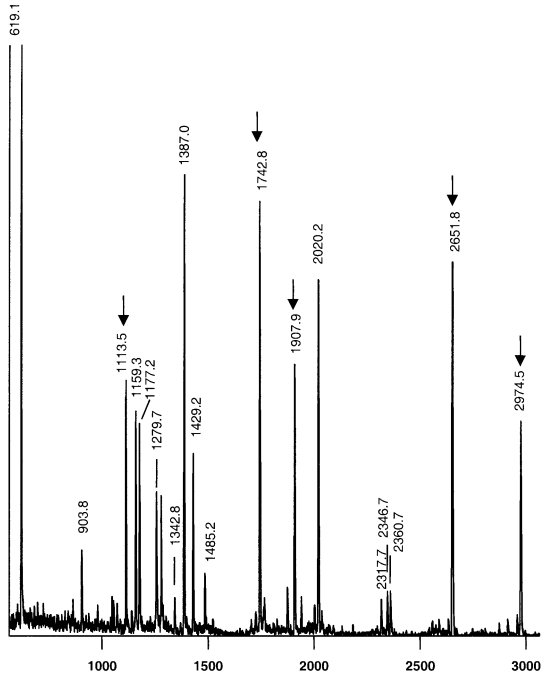

Fig. 6. The MALDI/ion trap mass spectrum of products of trypsin digestion of the *E. Coli* RecA protein.

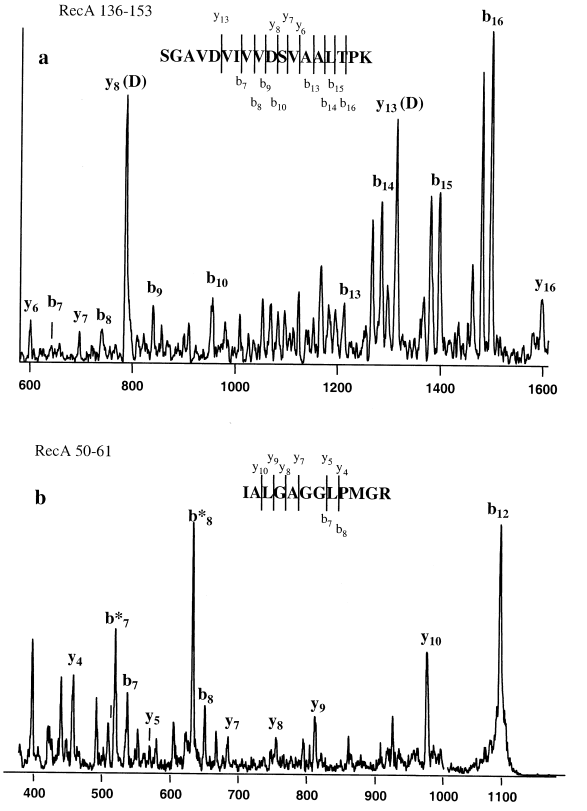

Fig. 7. MS/MS spectra of (a) RecA 136–153 and (b) RecA 50–61.

chromatographic separation. We illustrate the procedure in Figs. 6–8. Fig. 6 displays a mass spectrum of a trypsin digest of the 38 kDa *E. Coli* RecA protein. Figs. 7 and 8 show MS/MS spectra of the five individual components that are marked with arrows in Fig. 6. The patterns of fragmentation of these peptides belong to the categories described above. The amount of sample loaded onto the probe for the spectra displayed in Figs. 6–8 was ~0.5 pmole per peptide, and we did not observe any sign of sample depletion at the end of the experiment. This relatively low consumption of sample is one of the advantages of MALDI, allowing a small quantity of digest to be interrogated by a very large number of laser shots in order to obtain maximal information. Indeed, CID spectra can be obtained for all the peptides detected in Fig. 6 from one such loading.

The signal-to-noise ratios (S/N) of the CID spectra in Fig. 7 obtained from the complex mixture were observed to be lower than those obtained from the CID of single peptides, (Figs. 1–4), even when the sample loading per peptide was the same. We believe that this reduced S/N is related to the limited charge capacity of the ion trap. If the trap is filled with ions having many different *m/z* values, then the number of ions at any particular *m/z* will be limited, and relatively fewer ions will be available for dissociation after mass isolation of the peptide species of interest.

The S/N ratios are improved for CID spectra in which relatively few dissociation channels are accessible to the precursor peptide ions. This is apparent by comparing Fig. 7 (Lys-containing peptides that yield extensive fragmentation) with Fig. 8 (Arg- and Asp/Glu-containing peptides that undergo highly selective fragmentation). The number of dissociation channels is restricted to the number of acidic residues present in the peptide, leading to an improved S/N ratio. Although the content of sequence information is reduced in such cases, substantial sensitivity gains are observed. This sensitivity proves crucial for the identification of small amounts of protein isolated by gel electrophoresis [15].

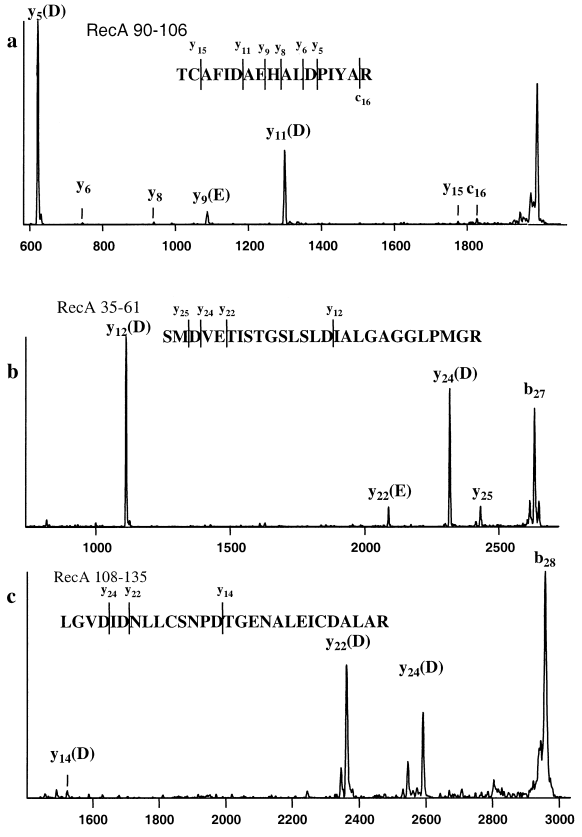

Fig. 8. MS/MS spectra of (a) RecA 90–106, (b) RecA 35–61, and (c) RecA 108–135.

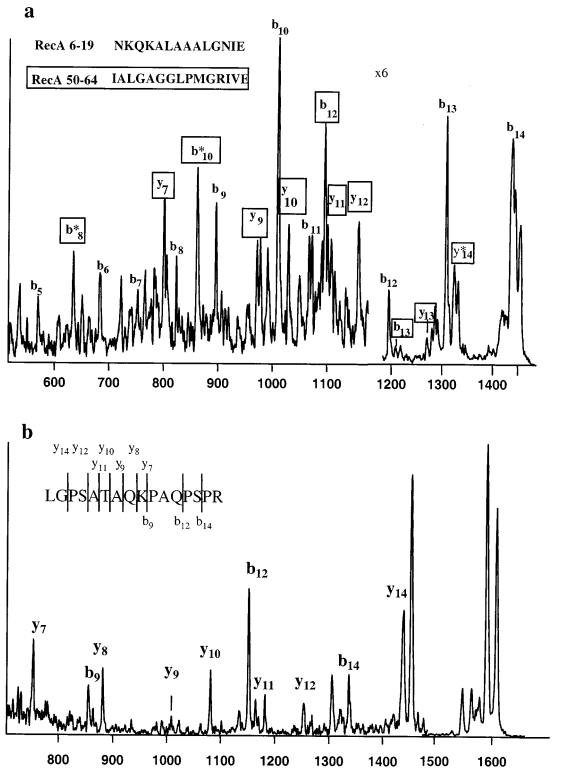

Fig. 9. MS/MS spectra of (a) RecA 6–19 and RecA 50–64 and (b) the phosphatase inhibitor-I 127–142.

We have found MALDI ion trap MS/MS to be of great value for confirming peptide sequences. A particularly challenging situation occurs in MS peptide mapping when two peptides in a protein have sufficiently similar *m/z* to preclude a definitive assignment. Such ambiguities can be resolved by MS/MS. Fig. 9 shows an example of this application of tandem MS. In a V8 protease peptide mapping of the RecA protein, two potential V8 fragments, RecA 6–19 (*m/z* 1455) and RecA 50–64 (*m/z* 1454), could not be readily differentiated in the MS spectrum of the digest. The resolution and mass accuracy of the MALDI ion trap instrument were insufficient to permit unambiguous assignment of the species present in the single unresolved peak. Indeed, even with isotopically resolved peaks, such assignments can be problematic because of overlapping isotopic components. The present ambiguity was resolved with a MS/MS experiment in which the CID spectrum clearly revealed the coexistence of both candidate peptides [Fig. 9(a)]. Notwithstanding the low intensities of the fragment ions, the measured masses of fragment ions were sufficiently accurate to ensure the unambiguous assignments indicated in the figure. Fig. 9(b) illustrates a second example of ambiguity in which we observed a peak in a tryptic digest of the protein phosphatase inhibitor-I. The measured mass could be assigned either to inhibitor-I 127–142 (*m/z* 1606) or inhibitor-I 70–82 (*m/z* 1607). It was important to distinguish these two possibilities because we were interested in phosphorylation of phosphatase inhibitor I by MAP and CDC2 kinase, and each candidate peptide contained a consensus site for phosphorylation by these proline-directed kinases. The MS/MS spectrum [Fig. 9(b)] reveals the presence of inhibitor-I 127–142 with no hint of inhibitor-I

70–82. Note again the preferred cleavage at the N-terminal of Pro (see y_{14}, b_9, b_{12}, and b_{14}).

4. Conclusions

Tandem MALDI ion trap mass spectrometry is not well suited for *de novo* peptide sequencing because preferential fragmentation at Asp/Glu residues precludes definition of a continuous series of sequence ions. By contrast, the technique is well suited for applications in which proteins are identified by correlation of the MS and MS/MS information obtained from a digest of the protein with information obtained from protein/DNA databases. Search protocols that use individual peptide masses together with limited MS/MS data from the peptides can be very rapid because they require neither the observation of an uninterrupted series of sequencing ions nor exhaustive interpretation of the CID spectra [3]. Using such procedures, we have demonstrated previously that the MALDI ion trap mass spectrometer is a powerful tool for the identification of proteins [15]. The preferential fragmentation at the C-terminal of Asp/Glu of Arg-containing peptides is of special value because the limited number of dissociation channels reduces signal dilution effects and at the same time provides useful constraints for searching the protein/DNA databases [3]. The MALDI ion trap mass spectrometer is expected to be particularly useful for searching expressed sequence tag (EST) databases [23]. Because these EST databases contain only short fragments of gene sequences, it is often necessary to obtain MS/MS data from a large number of peptides from a given protein digest before one of them is identified in the EST database. The present procedure allows continued interrogation of the peptides in a digest until either an EST is identified or until there are no further peptides to investigate.

We have documented the systematics of collision-induced dissociation of singly protonated peptide ions produced by MALDI in an ion trap mass spectrometer, and provide a simple classification scheme to describe the resulting fragmentation. Singly protonated peptide ions with $m/z < 3500$ can be effectively fragmented, although the fragmentation exhibits different degrees of selectivity depending on the mass and amino acid composition/sequence of the peptide. We have also demonstrated that MALDI ion trap tandem mass spectrometry can be routinely performed with unfractionated complex peptide mixtures.

Acknowledgements

The authors wish to acknowledge the NIH (grant no. RR00862) for supporting this work and Dr. Kenji Adzuma and Dr. Andrew Czernik for kindly supplying the protein samples.

References

[1] J.R. Yates, Electrophoresis 19 (1998) 893.
[2] M. Mann, M. Wilm, Anal. Chem. 66 (1994) 4390.
[3] D. Fenyo, J. Qin, B.T. Chait, Electrophoresis 19 (1998) 998.
[4] D. Arnott, W.J. Henzel, J.T. Stults, Electrophoresis 19 (1998) 968.
[5] D.F. Hunt, R.A. Henderson, J. Shabanowitz, K. Sakaguchi, H. Michel, N. Sevilir, A.L. Cox, E. Appella, V.H. Engelhard, Science 255 (1992) 1261.
[6] A.N. Verentchikov, W. Ens, K.G. Standing, Anal. Chem. 66 (1994) 126.
[7] A. Shevchenko, I. Chernushevich, W. Ens, K.G. Standing, B. Thomson, M. Wilm, M. Mann, Rapid Commun. Mass Spectrom. 11 (1997) 1015.
[8] H.R. Morris, T. Paxton, A. Dell, J. Langhorne, M. Berg, R.S. Bordoli, J. Hoyes, R.H. Bateman, Rapid Commun. Mass Spectrom. 10 (1996) 889.
[9] X. Tang, W. Ens, K.G. Standing, J.B. Westmore, Anal. Chem. 60 (1988) 1791.
[10] T.J. Cornish, R.J. Cotter, Anal. Chem. 65 (1993) 1043.
[11] R. Kaufmann, B. Spengler, F. Lutzenkirchen, Rapid Commun. Mass Spectrom. 7 (1993) 902.
[12] A.N. Krutchinsky, A.V. Loboda, V.L. Spicer, R. Dworschak, W. Ens, K.G. Standing, Rapid Commun. Mass Spectrom. 12 (1998) 508.
[13] V.M. Doroshenko, R.J. Cotter, Rapid Commun. Mass Spectrom. 10 (1996) 65.
[14] J. Qin, R.J.J.M. Steenvoorden, B.T. Chait, Anal. Chem. 68 (1996) 1784.
[15] J. Qin, D. Fenyo, Y. Zhao, W.W. Hall, D.M. Chao, C.J. Wilson, R.A. Young, B.T. Chait, Anal. Chem. 69 (1997) 3995.
[16] J. Qin, B.T. Chait, Anal. Chem. 69 (1997) 4002.
[17] J. Qin, B.T. Chait, Anal. Chem. 68 (1996) 2108.
[18] K. Biemann, Biomed. Environ. Mass Spectrom. 16 (1988) 99.
[19] J. Qin, B.T. Chait, J. Am. Chem. Soc. 117 (1995) 5411.
[20] J.A. Loo, C.G. Edmonds, R.D. Smith, Anal. Chem. 65 (1993) 425.
[21] J. Qin, B.T. Chait, unpublished.
[22] J. Qin, B.T. Chait, Anal. Chem. 68 (1996) 2102.
[23] M.S. Boguski, T.M. Lowe, C.M. Tolstoshev, Nat. Genet. 4 (1993) 332.