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Letter

Does in-source decay occur independent of the ionization process in matrix-assisted laser desorption?

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Abstract

The influence of the acidic and basic characters of constituent amino acid residues on the peptide fragment ions produced by in-source decay under matrix assisted laser desorption/ionization (MALDI) conditions has been studied using positive- and negative-ion experiments. Whereas the in-source decay spectra of peptides containing basic Arg and/or Lys residues near the N-terminus showed so-called c_n- and a_n-series ions in positive-ion mode, a peptide that has an acidic amino acid cluster near the N-terminus and a basic residue near the C-terminus characteristically formed y_n- and z_n-series ions in the positive-ion in-source decay spectrum. These results indicated that fragment ion series produced by in-source decay depend strongly upon the acidic and basic characters of the constituent amino acid residues and the near N- and C-termini. It was suggested that in-source decay processes occur intrinsically at NH–C^{α} and CO–NH bonds independent of the formation of molecular-related ions, and that the cleavages at the NH–C^{α} and CO–NH bonds occurred independently and were dependent on the matrix used. (Int J Mass Spectrom 181 (1998) L1–L6) © 1998 Elsevier Science B.V.

Keywords: MALDI; In-source decay; Peptides

1. Introduction

In protein studies, it is an important theme to quickly obtain amino acid sequence information from appropriate methods. Although tandem mass spectrometry (MS/MS) is a promising method for sequencing the peptides obtained by fragmentation of a protein, sophisticated skills are often required to operate the machines and to understand the product ion spectra. The applicability of in-source decay (ISD) in matrix-assisted laser desorption (ionization) (MALDI) coupled with a delayed ion extraction time-of-flight mass spectrometry (TOF-MS) to the amino acid sequencing of proteins was first reported as a new method by Brown et al. [1]. Fortunately, the MALDI/TOF-ISD spectrum is obtainable without the fragmentation of proteins and the special skills. A novelty of the ISD in the study of proteins and peptides is the appearance of the peptide fragment

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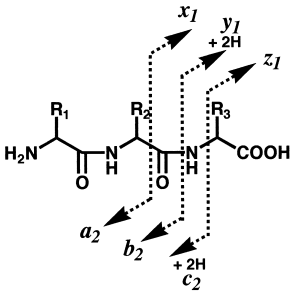


Scheme 1.

ions called c_n-, y_n-, and z_n-series ions followed by the Biemann nomenclature [2] that is shown in Scheme 1. Those are quite different characteristics from the conventional mass spectrometric degradation methods such as collision-induced dissociation (CID) that typically show b_n-, y_n-, and w_n-series ions and post-source decay (PSD) that may show a_n- ($a_n - 17$)-, b_n-, and ($b_n - 17$)-series ions, as pointed out by Reiber et al. [3]. This means that ISD may compensate for the lack of sequence information from CID and PSD methods. The observation of the c_n-series ions is especially convenient for understanding the ISD product ion spectra obtained. For the practical use of ISD, it is important to clarify the relationship(s) between ISD and ionization processes under MALDI conditions. That is, do the ISD fragment ions originate from the molecular-related ions, $[M + H]^+$ and $[M - H]^-$, or not? In this respect, Brown and his co-workers [4] have stated on the basis of the comparison of positive- and negative-ion MALDI/TOF-ISD spectra that the ion activation leading to the ISD of analytes takes place independent of the ionization process. With respect to the characteristic fragment ion series appeared in the ISD, CID, and PSD spectra, we always have to pay adequate attention to the primary sequence, especially the presence of such ionizable amino acid residues as anionic aspartic acid and glutamic acid and as cationic arginine, histidine and lysine, as well as the zwitterionic state of peptides and proteins NH_3^+-(CHR_n)-COO^-. Using a peptide containing an acidic cluster at the N-terminus (pyrrolidone–carboxylic acid-EEEEETAGAPQGLFRG-NH_2), we report here evidence for the independence of the ISD on the ionization process under MALD(I) conditions and the strong dependence of the ISD fragment ions on the primary sequence. With respect to the degradation characteristics in ISD, further, a matrix effect on the production of the fragment ions is noted.

2. Experimental and materials

MALDI mass spectra were obtained with a PerSeptive Voyager-DE STR TOF mass spectrometer (Perkin-Elmer, PerSeptive Biosystems Inc., Framingham, MA) equipped with a model VSL-337ND nitrogen laser (Laser Science, MA; 337 nm, 3 ns pulse length) and a dual microchannel plate detector (Galileo, MA). The ion acceleration voltage and the delay time were 20 kV and 122 ns, respectively, and the linear TOF mode was employed for in-source decay experiments. The sample was dissolved in 0.1% trifluoroacetic acid (TFA) at a concentration of 100 pmol/mL and 1 mL of the sample solution was mixed with a matrix (9 mL). The matrix was a saturated solution of α-cyano-4-hydroxycinnamic acid (CHCA) or 2,5-dihydroxybenzoic acid (DHB) in 0.1% TFA/acetonitrile (3:2 v/v). The solution (0.5 mL) was deposited on a sample plate, and the solvents were removed by drying in air at room temperature. The matrix reagents were purchased from Aldrich (Milwaukee, WI).

Substance P—an undecapeptide RPKPQQFFGLM-NH_2 (Mr 1347), human angiotensin I—a decapeptide DRVYIHPFHL (Mr 1296), and porcine pancreastatin (33–49)—a hexadodecapeptide pyrrolidone–carboxylic acid (Pyr)-EEEEETAGAPQGLFRG-NH_2 (Mr 1830), were obtained from Peptide Institute (Minoh, Osaka, Japan).

3. Results and discussion

The phenomenon of ISD itself is normally observed in a conventional ionization method such as electron ionization, and it is useful for structure information of organic compounds because the ISD

provides a considerable number of fragment ions in their mass spectra. The ISD occurs within the residence time in the ionizing cell through energy conversion from the external energy sources [ie. fast atom bombardment (FAB), electron irradiation, and laser pulse] to the internal energy of analyte molecules. The current soft-ionization methods such as MALDI, FAB, and electrospray ionization (ESI) are mild in character, so that mass spectra obtained tend to show only molecular-related ions such as $[M + H]^+$, $[M + Na]^+$, $[M + nH]^{n+}$, $[M - H]^-$, and $[M - nH]^{n-}$, and often lack informative fragment ions for structure analysis.

As described by Brown et al. [5], the observation of ISD fragment ions and the extent of ISD are significantly influenced by the MALDI matrix employed and laser fluence. An effective matrix for ISD is 2,5-dihydroxybenzoic acid (DHB), which typically provides c_n- and y_n-series ions [1,3]. Although the observation of c_n- and y_n-series ions has been reported in a positive-ion MALDI/TOF-ISD experiment for the substance P with a DHB matrix [1], a similar experiment for the substance P we performed here resulted instead in c_n- and a_n-series ions, as shown in Fig. 1(a). The laser fluence used was a moderate value that was set for 2200 in fluence value, whereas a threshold fluence without fragmentation was 2000 in the setting value. The use of a higher laser fluence of 2500 lowered the signal-to-noise ratio of the c_n- and a_n-series ions and broadened the peak shape of fragment ions. Further, the peak abundance of the a_n- to c_n-series ions increased by increasing laser fluence [Fig. 1(b)]. The negative-ion ISD spectrum of the substance P with DHB showed an intense peak of $[M - H]^-$ ion at m/z 1346 and showed few useful fragment ions for sequence analysis (data not shown). The use of another typical MALDI matrix, CHCA, provided an intense $[M + H]^+$ ion at m/z 1348 of the substance P at a relatively lower fluence of 1500 without fragmentation. The application of a higher laser fluence of 2200 to the substance P with CHCA resulted in the formation of a_n-series ions with lower signal-to-noise ratios, although the c_9 ion was observed, as shown in Fig. 1(c). The lack of c_n-series ions in Fig. 1(c) with CHCA, as compared to Fig. 1(a)

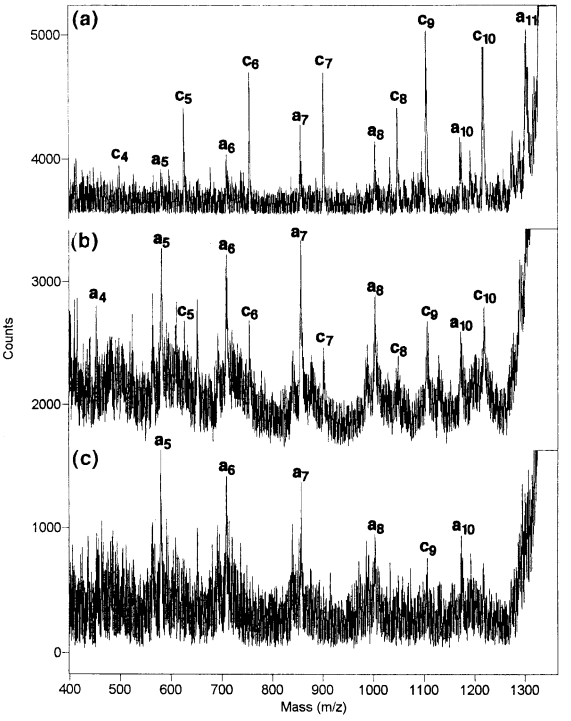
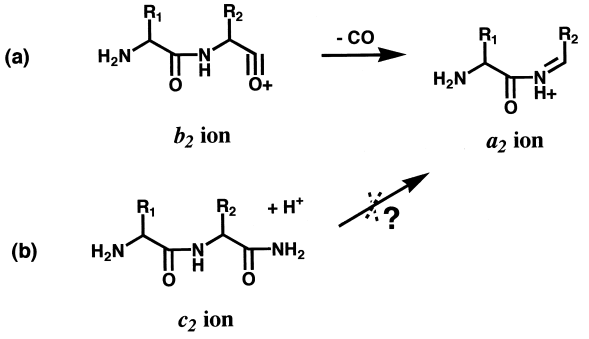


Fig. 1. Positive-ion MALDI/TOF-ISD spectra of the substance P, RPKPQQFFGLM-NH_2 (Mr 1347), obtained with a 2,5-dihydroxybenzoic acid matrix utilizing (a) a moderate laser fluence 2200, (b) a higher laser fluence 2500, and (c) with α-cyano-4-hydroxycinnamic acid matrix utilizing a higher laser fluence 2200.

with DHB, is a significant matrix effect on ISD phenomenon. This matrix effect suggests that DHB effectively cleaves at NH–C^α bonds of the peptide backbone. Although the increase of the peak abundance of a_n- to c_n-series ions in Fig. 1(a) and 1(b) suggests that the fragment ions of c_n proceed to a_n with degradation because of the higher laser fluence, the matrix effect implies that the c_n- and a_n-series ions form independently under MALDI conditions. This is interesting in connection with the mechanism of the ISD on peptide fragmentation.

The a_n-series ions are often observed with d_n-series ions in high-energy CID spectra of peptides [6,7] and are produced by the loss of a neutral CO from b_n-series ions [8]. The a_n-series ions further proceed to d_n-series ions [8]. Since little about the formation and fragmentation of c_n-series ions has been studied, the fragmentation relationships among



Scheme 2.

a_n-, b_n-, and c_n-series ions cannot be described in detail. Considering the matrix effect described above, however, it seems unlikely that the c_n-series ions fragment to a_n-series ions (Scheme 2).

Positive-ion MALDI/TOF-ISD spectra of the angiotensin I with the DHB matrix also showed mainly c_n- and a_n-series ions, and the peak abundance of the a_n- to c_n-series ions increased by increasing laser fluence, as shown in Fig. 2(a) and 2(b). On the other hand, the ISD spectrum with the CHCA matrix was lacking in the c_n-series ions as compared to the DHB matrix [Fig. 2(c)]. The negative-ion ISD spectrum hardly provides information for sequence analysis. In the positive-ion ISD spectra of the angiotensin I, the c_6 fragment ion was not observed because of the presence of Pro-7, and instead the fragment ions of b_6 and a_6 were observed. From the above results of Fig. 1 and Fig. 2, it was confirmed that DHB was an effective matrix for obtaining the c_n-series ions of the peptides compared with CHCA. In other words, DHB was an effective matrix for cleaving the NH–C^{α} bonds compared with CHCA.

The preferential observation of the c_n- and a_n-series ions in the positive-ion ISD spectra described above may be attributed to the presence of basic amino acid residues near the N-terminus, i.e. Arg and Lys for the substance P and Arg for the angiotensin I, as well as the basic nature of both N-termini. The formation of the c_n- and a_n-series ions may be generally explained in the following two ways: (1) the fragment ions are originated from molecular-related ions such as $[M + H]^+$ and $[M - H]^-$ in the residence time after ionization events, and (2) the production of molecular-related ions and fragment ions occurs independently, i.e. neutral fragments as the precursor of c_n-series ions are produced directly from neutral analytes via energy transfer from matrix molecules activated by laser irradiation, and the resulted neutral fragments are ionized according to the acidic and basic characters of the constituent amino acid residues. The former explanation (1) means that the degradation may occur by charge-mediated processes. In comparison, the latter one (2) states that the ISD is an intrinsic and specific process that occurs immediately after the sudden energy impingement by laser pulse in MALDI or particle bombardment in FAB and in plasma desorption (PD), and that the ISD and ionization process occur simultaneously and indepen-

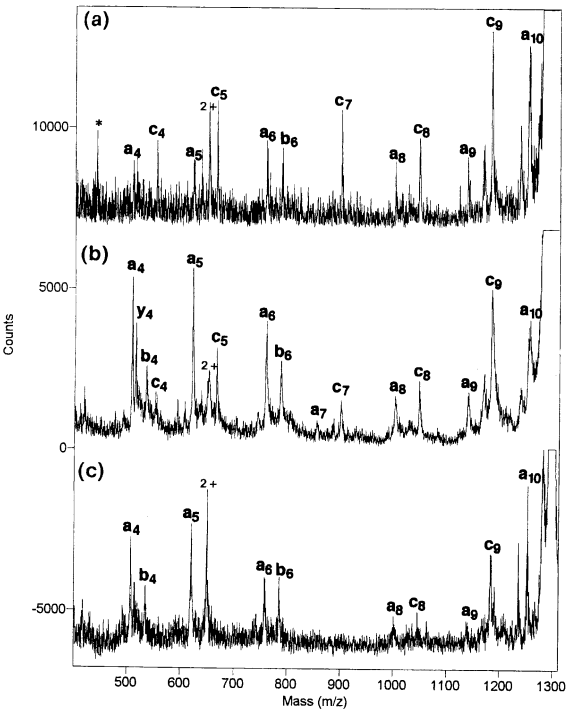


Fig. 2. Positive-ion MALDI/TOF-ISD spectra of the angiotensin I, DRVYIHPFHL (Mr 1296), obtained with (a) a 2,5-dihydroxybenzoic acid matrix utilizing a moderate laser fluence 2200, and (b) α-cyano-4-hydroxycinnamic acid matrix utilizing a moderate laser fluence 1900 in fluence value. The asterisk indicates the peak originating from the matrix used. A 2+ represents the peak corresponding to the doubly protonated molecule $[M + 2H]^{2+}$.

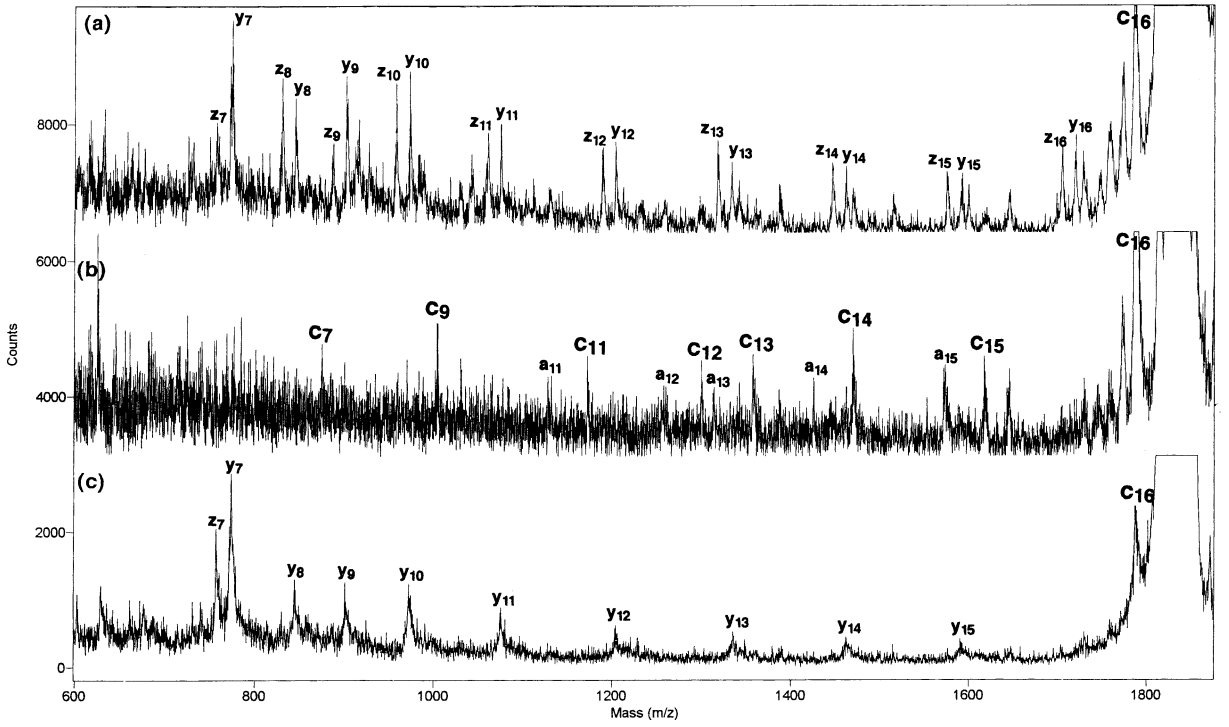


Fig. 3. MALDI/TOF-ISD spectra of the porcine pancreastatin (33–49), Pyr-EEEEETAGAPQGLFRG-NH_2 (Mr 1830), in (a) positive-ion and (b) negative-ion modes obtained with a 2,5-dihydroxybenzoic acid matrix utilizing a moderate laser fluence 2200, and in (c) positive-ion mode with an α-cyano-4-hydroxycinnamic acid matrix utilizing a moderate laser fluence 2000.

dently. This statement is strongly supported by the FAB mass spectrum [2] and PD mass spectra [9] that show c_n- and a_n-series ions characteristically. Further, the explanation (2) documents that the degradation may occur independently of the presence and site of charge as in the $[M + H]^+$ ion. This means that a positive-ion c_n-series in the ISD does not necessarily take a proton H^+ of the amide nitrogen at the C-terminus.

In order to obtain evidence for the specific degradation to form c_n-series ions induced by laser irradiation, positive- and negative-ion MALDI/TOF-ISD spectra of a peptide [porcine pancreastatin (33–49)] that has the five acidic amino acid cluster at the N-terminus (Pyr-EEEEETAGAPQGLFRG-NH_2, Mr 1830) were compared to each other. The positive-ion ISD spectrum with a DHB matrix specifically showed y_n- and z_n-series ions, as shown in Fig. 3(a), whereas the negative-ion ISD spectrum showed c_n- and a_n-series ions [Fig. 3(b)]. It is important to give the positive z_n- and negative c_n-series ions, indicating that cleavage occurs at the NH–C^α bonds in both negative- and positive-ion modes. The intrinsic cleavage at the NH–C^α bonds relates to the formation of z_n-series ions in negative-ion mode, whereas the cleavage accounts for the formation of c_n-series ions in positive-ion mode. The use of a CHCA matrix gave only y_n-series ions in the positive-ion ISD spectrum except for the z_7 ion [Fig. 3(c)]. The matrix effect, in which DHB effectively cleaved at the NH–C^α bonds compared with CHCA, was observed again. The observation of the c_n- and a_n-series ions in the negative-ion mode indicates that the formation of those series ions is strongly dependent on the presence of acidic amino acid residues. This suggests that an ISD process occurs specifically at the NH–C^α bonds

independent of the charge state of molecular-related ions, $[M + H]^+$ and $[M - H]^-$, and independent of the site of protonation.

On the other hand, the appearance of y_n-series ions in positive-ion ISD spectra [Figs. 3(a) and 3(c)] indicates that the cleavage at the CO–NH bonds of peptide backborne is also an intrinsic process in the ISD phenomenon. The cleavage at the CO–NH bonds relates to the formation of b_n-series ions, as shown in Scheme 1. The production of the y_n- and/or z_n-series ions in the positive-ion ISD spectra (Fig. 3) may be attributed to the presence of a basic Arg residue near the C-terminus. This indicates that the production of fragment ions (c_n-, a_n-, y_n-, and z_n-series) strongly depends on basic and acidic characters of the constituent amino acid residues. Further, the matrix effect, in which the use of DHB results in y_n- and z_n-series ions and the use of CHCA results in y_n-series ions, suggests that the cleavages at the NH–C^α and CO–NH bonds occur independently. The cleavages at the NH–C^α and CO–NH bonds may relate to the production of c_n- and z_n-series ions and b_n-, a_n-, and y_n-series ions, respectively. From the fact that x_n-series ions were not observed, it is suggested that ISD does not occur at the C^α–CO bond. Effective cleavages at the NH–C^α bonds in both positive- and negative-ion modes when the DHB matrix is used may attribute to the hydrogen-bonding interaction between matrix and analyte molecules, as pointed out by Brown and his co-workers [4]. This interpretation seems to be convenient for us to explain the matrix effect.

The results obtained here are summarized as follows: (1) ISD processes occur intrinsically at NH–C^α and/or CO–NH bonds according to the matrix used; (2) the bond cleavages may occur independent of the formation of molecular-related ions, $[M + H]^+$ and $[M - H]^-$; (3) the production of c_n-, a_n-, y_n-, and z_n-series ions depends upon the characters of the constituent amino acid residues.

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