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Influence of initial velocity of analytes on in-source decay products in MALDI mass spectrometry using salicylic acid derivative matrices

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ABSTRACT

The fragmentation and recombination process of peptide radicals during matrix-assisted laser desorption/ionization (MALDI) in-source decay (ISD) with salicylic acid derivative matrices has been described. MALDI-ISD is initiated by hydrogen transfer between peptide and matrix. The use of reducing and oxidizing matrix leads to the formation of $c'/z^{\bullet}$ and $a^{\bullet}/x$ fragment pairs, respectively. The fragmentation of analyte radicals occurs via a uni-molecular dissociation processes and independently of the collision in MALDI plume. In contrast, hydrogen transfer between analyte radicals and matrix molecules occurs by collision in MALDI plume as a bi-molecular process. The rate of collision in MALDI plume could be estimated by initial velocity of analyte ions and it affect on the yields of ISD fragments. Considering the initial velocity of analyte ions allows better understanding the MALDI-ISD process.

Introduction

Matrix-assisted laser desorption/ionization in-source decay (MALDI-ISD) has been used to determine the sequence of peptides and proteins [1,2]. 2,5-Dihydroxyl benzoic acid (2,5-DHB) was efficient to induce MALDI-ISD and was widely used for MALDI-ISD experiment [1]. Recently, it was found that the choice of matrix can dramatically affect the ISD fragmentation and a number of matrices have been developed to enhance the quality of MALDI-ISD spectra [3]. In particular, the use of 1,5-diaminonapthalene (1,5-DAN) [4], 5-aminosalicylic acid (5-ASA) [5], 2-aminobenzoic acid (2-AA) [6] and 5-amino-1-naphthol (5,1-ANL) [7] produced strong intensity of ISD fragment ions.

MALDI-ISD is initiated by the hydrogen transfer from matrix molecule to the carbonyl oxygen of peptide backbone [8]. Subsequently, $c'/z^{\bullet}$ fragment pair are formed by the radical-induced cleavage at the N—C_{α} bonds [9]. However, $z^{\bullet}$ radical fragments were not observed in MALDI-ISD spectra, and instead z', matrix adduct on z and w fragment ions were detected. It suggests that radical $z^{\bullet}$ fragments undergo either gain of a hydrogen atom or loss of side-chain after the backbone cleavage [9,10]. The hydrogen and matrix attachment for $z^{\bullet}$ radicals forming z' and (z + matrix) fragments occur during the collision between $z^{\bullet}$ radicals and matrix molecules, whereas the side-chain loss from $z^{\bullet}$ radical fragment leads to w fragment, occurring by uni-molecular dissociation. It has been reported that those reactions competitively occur and are dependent on the initial velocity of analyte ions [10], which is proportional to collision rate in MALDI plume [11]. The formation rate of z' fragments were decreased by lower collision rate in MALDI plume. As a result, it allows forming the w fragments due to a longer lifetime of the $z^{\bullet}$ radical fragments [10]. In contrast, it has been previously reported that the collisional activation of $z^{\bullet}$ radical fragments can lead to side-chain loss [12,13]. Therefore, the existence of $z^{\bullet}$ radical fragments containing enough internal energy for side-chain loss would be expected to give strong intensity of w fragment ions. The internal energy of analyte ions in MALDI-ISD increases with the decreasing of the matrix proton affinity, as described by Demeure et al. [14]. However, the abundance of z' and w ions did not show any correlation with the proton affinity of matrix and it cannot be explained from the standpoint of internal energy of analyte ions [10].

Recently, we found that the oxidizing matrices, 5-formylsalicylic acid (5-FSA) [15], 5-nitrosalicylic acid (5-NSA) [15] and 2,5-bis(2-hydroxyethoxy)-7,7,8,8-tetracyanoquinodimethane (bisHE-TCNQ) [16] for MALDI-ISD resulted in the generation of a and x fragments by cleavage of the C_{α}—C bond. The oxidizing matrices provides useful complementary information to the conventional matrices for the MALDI-ISD analysis of amino acid sequencing and site localization of phosphorylation in peptides [17]. MALDI-ISD with an oxidizing matrix is initiated by hydrogen abstraction from amide

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Table 1
Monoisotopic mass (M_m) and sequence of analyte peptides used.

Analyte peptide	M_m	Sequence
Fiblinopeptide A	1535.69	ADSGEGDFLAEGGGVR
ACTH18-35	1977.95	RPVKVYPNGAEDESAEAF
[Arg^{18}]-ACTH19-36	1977.95	PVKVYPNGAEDESAEAFR
Bradykinin potentiator B	1181.69	Pyr-GLPPRPKIPP

nitrogen of the peptide backbone onto the matrix, leading to the formation of hydrogen-deficient peptide radical [15]. Subsequently, hydrogen-deficient peptide radicals undergo either C_α—C bond cleavage or further loss of a hydrogen atom. The C_α—C bond cleavage leads to $a^\bullet/x$ fragment pair and a fragments were generated by further hydrogen abstraction from $a^\bullet$ radical fragment after the backbone cleavage [18]. An ab initio calculation showed that formation of an $a^\bullet/x$ fragment pair from hydrogen-deficient peptide radical is nearly thermoneutral [19]. Therefore, the C_α—C bond cleavage probably occurs by uni-molecular dissociation. In contrast, the collision between hydrogen-deficient peptide radicals and matrix molecules resulted in an oxidized species $[M-2H+H]^+$ by further hydrogen abstraction. The use of bisHE-TCNQ gave the higher intensity of a fragment ions and lower initial velocity of analyte ions compared with the use of 5-NSA and it can be explain from the standpoint of collision rate in MALDI plume [16].

In the present study, we focus our attention on influence of initial velocities of analyte ions using salicylic acid derivatives. We confirm that the ISD fragmentation is dependent upon the initial velocity of analyte ion.

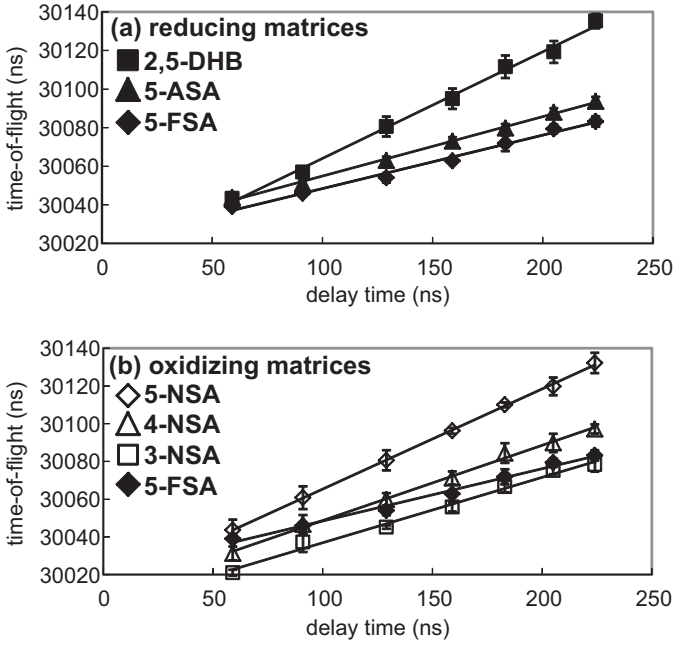

Fig. 1. The variation of the time-of-flight of protonated ACTH18-35 with the delay time, for (a) three different reducing matrices 2,5-DHB [16], 5-ASA and 5-FSA, and (b) four different oxidizing matrices 5-NSA [16], 4-NSA, 3-NSA and 5-FSA.

Experimental

Materials

The peptides, ACTH18-35, [Arg^{18}]-ACTH19-36, fibrinopeptide A and bradykinin potentiator B were purchased from Peptide Institute (Osaka, Japan). The sequences of peptides used are summarized in Table 1. 2,5-DHB, 3-NSA and 4-NSA was purchased from Sigma-Aldrich (St. Louise, MO, USA). 5-NSA, 5-ASA and 5-FSA were purchased from Tokyo Chemical Industry (Tokyo, Japan). Trifluoroacetic acid and acetonitrile were purchased from Wako Pure Chemicals (Osaka, Japan). All reagents were used without further purification except for water, which was purified through a MilliQ water purification system (Millipore; Billerica, MA, USA).

Sample preparation

Analyte peptides were dissolved in water at concentration of 20 pmol/μL. NSA isomers (10 mg/mL) and 2,5-DHB (20 mg/mL) were dissolved in water/acetonitrile (1:1, v/v) with 0.1% TFA.

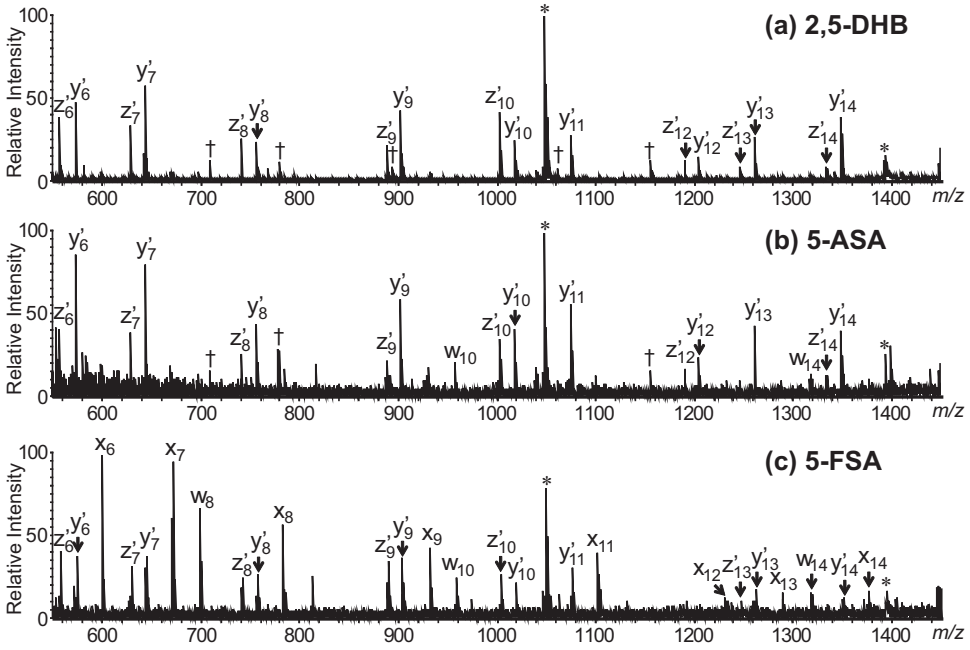

Fig. 2. MALDI-ISD spectra of fibrinopeptide A obtained with (a) 2,5-DHB, (b) 5-ASA and (c) 5-FSA. Asterisk and dagger indicate metastable ions and matrix adduct of z fragments, respectively.