MALDI In-Source Decay of Protein: The Mechanism of c-Ion Formation

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The in-source decay (ISD) phenomenon, the fragmentation at an N–Cα bond of a peptide backbone that occurs within several tens of nanoseconds in the ion-source in matrix-assisted laser desorption/ionization mass spectrometry (MALDI MS), is discussed from the standpoints of the discovery and early publications dealing with MALDI-ISD, the formation of c-ions in energy-sudden desorption/ionization methods, the formation of radical species in a MALDI, model construction for ISD, and matrix materials that are suitable for use in MALDI-ISD. The formation of c-ions derived from peptides and proteins in MALDI-ISD can be rationalized by a mechanism involving intramolecular hydrogen transfer, denoted as the "Takayama’s model" by De Pauw’s group (Anal. Chem. 79: 8678–8685, 2007). It should be emphasized that the model for MALDI-ISD was constructed on the basis of X-ray crystallography and scanning probe microscopy (SPM) analyses of matrix crystals, as well as the use of isotopically-labelled peptides.

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INTRODUCTION

Mass spectrometry (MS) is a scientific field that studies gas-phase ions of atoms, molecules and complex clusters. Clarifying the mechanism of ion formation and the fragmentation of gas-phase ions is major basic research interest in the field of MS, as well as having wide applicability to the material, biological, environmental, food, drug and omics sciences. Ionization and fragmentation in the ion-source continues to be one of the most interest events in gas-phase ion chemistry. The mechanism responsible for soft ionization in matrix-assisted laser desorption/ionization (MALDI)

and electrospray ionization (ESI)

remains unknown because of its complexity due to several factors such as the form of the matrix crystal being used, laser ablation within a very short timescale and photochemical and radical reactions in MALDI, and solvation between analyte and solvent molecules, ionic separation under a high electric field and droplet explosion in ESI. MALDI events are particularly complex and difficult to understand due to the numerous sequential ion–molecule and radical reactions that can occur in both the solid-phase and dense-gaseous MALDI plumes that are electronically excited with laser photons.

A feature of MALDI MS is radical-induced fragmentation (RIF), referred to as the “in-source decay (ISD)” of peptides and proteins. MALDI-ISD involves a prompt cleavage that occurs within several tens of nanoseconds (ns) in the MALDI ion-source, and ISD is caused by the radical-sites of neutral species independent of the charge-sites. Such radical species are generated by the transfer of hydrogen atoms from the matrix to the protein molecule. In general, the radical sites of organic chemicals are react easily with themselves or with other chemicals. Radical-induced unimolecular decay can be normally observed in analyte molecular ions A+ generated by the electron ionization (EI) of organic compounds. The analytic ions A+ degrade within 10−9 s in the ion-source, owing to the activity of radical sites. RIF plays major role in EI fragmentation such as simple (α)-cleavage. The high reactivity of radical-sites results in intramolecular rearrangements such as the McLafferty hydrogen rearrangement and the methylene chain rearrangement of fatty acid methyl esters. In those reactions, the radical-site located on the carbonyl oxygen can strongly initiate hydrogen rearrangements from γ-position methylene hydrogens to the oxygen via the formation of a six-membered ring transition state (Scheme 1(a)).
and methylene chain rearrangement from the methylene chain to the carbonyl oxygen via backbone ring transition states (Scheme 1(b)). It is very important to recognize that the formation of fragment ions with these rearrangements is complete within microseconds ($10^{-6}$ s) or shorter in the EI ion-source and those ring transition states can be attained within a short timescale ($10^{-14}$–$10^{-13}$ s) corresponding to molecular vibrational and rotational motions. Furthermore, intramolecular hydrogen and methylene chain rearrangements (Scheme 1) can take place without atomic motion through electron transfer on a very short timescale ($10^{-16}$ s) corresponding to vertical electronic excitation by means of the Frank–Condon principle. The timescales described above have important meanings in the case of ultraviolet (UV)-MALDI with photochemical processes, as are described in this review article.

The hydrogen atom ($H^\cdot$) is the smallest radical species that can participate in intramolecular rearrangements and scrambling in the mass spectrometry of organic molecules. The MALDI-ISD of proteins can start by intermolecular hydrogen transfer from photochemically excited matrix molecules ($M^\ast$) to protein analytes ($A$) via hydrogen-bonding between matrix active hydrogens (–OH and –NH$_2$) and backbone carbonyl oxygen in/on the matrix crystal (1), and the following radical-induced fragmentation (RIF) (2), ionization (3a) and radical combination/ionization (3b) occur independently of each other.

\[
M^\ast + A \rightarrow (M - H)^\cdot + (A + H) \quad (1)
\]
\[
(A + H) \rightarrow c + z' \quad (2)
\]
\[
c + H^\ast \rightarrow [c + H]^\ast \quad (3a)
\]
\[
z' + H^\ast + H^\cdot \rightarrow [z' + H]^\ast \quad (3b)
\]

The neutral fragments $c$ and $z'$ are ionized by proton transfer from the protonated matrix [$M + H]^\ast$ and to the deprotonated matrix [$M - H]^\ast$. The model of intermolecular hydrogen transfer (1) and the following RIF $\alpha$-cleavage (2) in MALDI-ISD was referred to as “Takayama’s model” by De Pauw’s group, and the model can explain the formation of ISD fragments and provides a powerful strategy for identifying and developing matrix materials that are suitable for use in MALDI-ISD.

In this review article, I discuss the discovery of ISD phenomenon in MALDI, radical formation in MALDI, the history of the observation of $c$-ions, the origin of hydrogen atoms to generate ISD fragments $c$ and $z'$, the model construction for ISD, and matrix materials suitable for MALDI-ISD. The nomenclature for the neutral fragments generated from MALDI-ISD with 2,5-DHB is shown in Scheme 2. Fragment ions observed in MALDI-ISD spectra can be understood by the protonation and deprotonation of neutral fragments.
DISCOVERY OF MALDI-ISD PHENOMENON AND EARLY RESEARCH PUBLICATIONS

The phenomenon of the ISD of peptides and proteins in MALDI MS was first reported by Brown and Lennon as “fast metastable ion fragmentation,” and they discovered this phenomenon in the course of the development of a time-of-flight mass spectrometer with a delayed extraction device. Subsequently, several groups reported on the MALDI-ISD data of peptides and proteins from the standpoints of practical and theoretical considerations of MALDI-ISD have been reviewed by Asakawa and Moon.

We also found fragment ions c and z+2 assigned by Biemann’s nomenclature, in the course of experiments directed at the influence of laser fluence on the appearance and peak resolution of an analyte signal. We understood that carboxyl (C)-terminal successive truncation occurred by unknown chemical processes in the MALDI ion-source, but immediately after the discovery of sequence-reflected fragment ions we concluded that the observed fragments were generated independent of ionization (protonation and deprotonation) under MALDI conditions, based on data from both positive- and negative-ion MALDI-ISD experiments of an amino acid peptide porcine pancreastatin33–49 (Pyr-EEEETAGAPQGLFRG-NH2). Positive- and negative-ion MALDI-ISD spectra of the peptide merely showed C-terminal side z′/y′-ions (m/z=16) and N-terminal side c′-ions (m/z=16), respectively. This suggested that c- and z′-fragments are generated via specific cleavage at the N–Cn bond of the backbone by the laser-induced excitation of neutral peptides (Scheme 2), while protonation and deprotonation of these fragments took place at the C-terminal side Arg residue and the N-terminal side Glu cluster, respectively.

That is, the neutral fragments c and z′ as the precursor of c- and z′-ions may be generated directly from neutral peptides, and the neutral fragments are ionized according to the acidic and basic characteristics of the constituent amino acid residues. Interestingly, c- and z′-ions were characterized observed when 2,5-dihydroxybenzoic acid (2,5-DHB) was used as the matrix, while the use of an α-cyano-4-hydroxycinnamic acid (CHCA) matrix resulted in the formation of α-, β- and γ-ions which are normally observed in collision-induced dissociation (CID) spectra of protonated peptides. The c- and z′-ions were rarely observed in energy-sudden desorption/ionization (ESDI) mass spectrometry.

**Table 1. Early reports of MALDI-ISD (1995–2007).**

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Scheme 3. Fragments originated from the N–Cn bond cleavage presented by Williams et al. 37

![Diagram of fragments from N-Cn bond cleavage](image)
c-ions can be observed in negative-ion FAB mass spectra of peptides with a glycerol matrix. Furthermore, the observation of c-ions was reported by Vorst et al. by using plasma desorption mass spectrometry (PD MS) with nitrocellulose matrices. FAB, LSIMS and PD can be referred to as ESDI, a type of high-translational energy particle impact method, and ESDI results in locally electronic excitation due to keV energy collision on the target molecules. Interestingly, the observation of c-ions in positive- and negative-ion FAB mass spectra of peptides suggests that FAB-ISD occurs independent of ionization, while a detailed mechanism was not presented by any of the above authors.

I also conducted FAB experiments of angiotensin I in an attempt to observe c-ions using a glycerol matrix. The resulting FAB mass spectrum gave peaks corresponding to a- and c-ions, as shown in Fig. 1 (unpublished data). Although the detailed mechanism responsible for this is not clear, the possibility that c-ions are formed by the N–Cα bond cleavage of peptide backbone via hydrogen transfer from matrix active hydrogens (hydroxyl groups of glycerol) to the carbonyl oxygens on the peptide backbone cannot be excluded. It has been reported that FAB with keV order particle beams brings about electronic excitations corresponding to electron impact energies of 30 eV or higher. Such a high-energy characteristic of FAB may result in the production of a- and c-ions in the FAB ion source.

According to Biemann’s nomenclature (Scheme 4), c-, y- and z+2 ions can be formed by intramolecular hydrogen rearrangements that occur during the CID experiments of peptide. This type of origin of hydrogen atoms is similar to the electron-capture dissociation (ECD) of multiply protonated proteins \([A+nH]^+\) leading to the formation of c- and z-ions by intramolecular hydrogen rearrangements via multiply-charged radical ions \([A+nH]^{(n-1)+}\) generated by electron-capture, although the production of hydrogen atoms essentially differs from CID experiments. It would be of interest to clarify the formation of hydrogen atoms and to determine the origin of hydrogens under ESDI conditions.

**HYDROGEN ATOM AND RADICAL SPECIES FORMATION IN MALDI WITH 2,5-DHB**

Scott et al. reported evidence that high levels of hydrogen atoms are generated from 2,5-DHB matrix when irradiated with nitrogen laser photons (337 nm) by performing the post-ionization (with a Nd:YAG laser) of neutral components in the MALDI plume ablated from a 2,5-DHB crystal surface. The experiments provided direct evidence for the presence of large amounts of hydrogen atoms in the MALDI plume with larger velocities (5,000 m/s) than the matrix species, as illustrated in Scheme 5. The formation of radical species such as hydrogen atoms (H·) and 2,5-DHB radicals (M·) has been also reported by the Hillenkamp group and Bourcier et al. in MALDI experiments with 2,5-DHB. When 2,5-DHB is used as a matrix, the MALDI plume generated with ablation is made up of large amounts of neutral species such as matrix molecules M, hydrogen atoms H· and DHB radicals M·, as well as small amounts of matrix ions \([M+H]^+\) and \([M−H]^−\) with the ratio of ions/neutrals in the range of \(10^{-9}\) to \(10^{-8}\).

Zubarev’s group pointed out that a combination of reactions of z’ radicals with radical species (H· and M·) occur in the MALDI plume. They reported that a C-terminal side peptide radical z’ binds to both a hydrogen atom H· and a 2,5-DHB radical M· to form z-related species \(z’(=z’+H\cdot)\) and a z-matrix \((=z’+M\cdot)\) in the MALDI plume. A similar mechanism regarding the production of z’ and z-matrix (Scheme 2) was reported by De Pauw’s group with 1,5-diaminonaphthalene (1,5-DAN) matrix. It is important to recognize
that a series of combination reactions of radical species can be completed within several tens or hundreds of nanoseconds (corresponding to delayed extraction time) in the ion source, due to the great amounts of radical and neutral species in the MALDI plume.

**MODEL CONSTRUCTION FOR MALDI-ISD OF PROTEIN**

1. **The origin of hydrogen atoms in forming c-ions**
   I attempted to construct a model for MALDI-ISD by using a model peptide RLG (RLGNQWAVGDLAE, $M_r$ 1428.6) and its corresponding deuterium-labeled peptide RLG(Ad3, Gd2) (RLGNQWA(d3)VG(d2)DLAE, $M_r$ 1433.6). It is most important to clarify the origin of hydrogen atoms to generate c-ions (Scheme 4) for a better understanding of the mechanism of the MALDI-ISD phenomenon. Although the formation of c- and z-ions in ECD can be explained by intramolecular hydrogen rearrangement, in MALDI-ISD there are at least three possible sources of hydrogen atoms from either protein ions or 2,5-DHB matrix as follows:
   1. Intramolecular rearrangement of active or non-active hydrogen atoms,
   2. Intramolecular rearrangement of hydrogens formed by electron capture in multiply protonated peptides under MALDI conditions,
   3. Intermolecular transfer of active hydrogens from 2,5-DHB matrix molecules.

   However, it is difficult to determine the origin of hydrogen atoms in hydrogen rearrangements, because active hydrogens can migrate from remote sites and some scrambling occurs. In this study, the ISD experiments were limited to three possible hydrogen sources to explain the c-ion formation:
   1-1. Hydrogen(s) of the alpha-carbon ($C_{\alpha}$) on the backbone (Scheme 6a),
   1-2. Hydrogen(s) of the side-chain beta-carbon ($C_{\beta}$) (Scheme 6a),
   3'. Active hydrogens (Ph–COOH and Ph–OH) of 2,5-DHB molecules (Scheme 6b).

   In order to confirm the possibility of intramolecular hydrogen rearrangements 1-1 and 1-2, the deuterium labeled peptide RLG(Ad3, Gd2) described above was used.

   Figure 2 shows MALDI-ISD spectra of the model peptides RLG and RLG(Ad3, Gd2). If the intramolecular hydrogen rearrangements occur as shown in Scheme 6(a), the $m/z$ value of the $c_6$ ion originating from the deuterated model RLG (Ad3, Gd2) shifts to 1 Da larger than that of the $c_6$ ion.
at \( m/z \) 773 of the model peptide RLG, by intramolecular deuterium rearrangement from the \( C_β \) methyl group of A(d3). Similarly, the \( c_5 \) ion of RLG (Ad3, Gd2) will shift to 4Da larger than that of \( c_5 \) ion at \( m/z \) 943 of RLG, as the result of the deuterium rearrangement from the \( C_α \) methylene of G(d2). However, no shifts in \( m/z \) values were observed in the ISD spectra, as shown in Fig. 2. Here it can be concluded that the hydrogen atom involved in the formation of \( c \)-ions did not originate at least from any hydrogens located on alpha- and beta-carbon positions. So that, it is most likely that the origin of hydrogen atoms in generating \( c \)-ions are from the 2,5-DHB matrix active hydrogens generated via a photochemically induced mechanism,9 as shown in Scheme 6(b).

According to the scenario regarding the origin of hydrogen atoms described above, MALDI-ISD experiments were performed for the deuterium labeled peptide RLG(A-d3, G-d2) deuterated with D2O, using a deuterium labeled matrix 2,5-DHB-d3 and deuterated solvents D2O and CH3COOD.9 The results obtained suggest that both a deuterium atom D\(^-\) and a deuteron D\(^+\) to generate \( c_3 \) (RLG-d11, \( m/z \) 355) and \( c_4 \) (RLGN-d14, \( m/z \) 472) ions were originated from the matrix 2,5-DHB-d3. Zubarev’s group also reported that an ISD hydrogen transfer is not mediated by the electron capture of multiply-charged peptides (Scheme 6(c)), but the origin of the hydrogen atom involved in generating \( c \)-ions in MALDI-ISD is from the matrix by the mechanism shown in Scheme 6(b).10 Consequently, it can be concluded that the origin of hydrogen atoms involved in forming \( c \)-ions in the MALDI-ISD of peptides and proteins are from the 2,5-DHB matrix.

**b) X-ray and scanning probe microscopy (SPM) analysis of 2,5-DHB crystal with and without protein incorporation**

It is important to note that only a couple of matrix materials produce analytes and ISD ions of peptides and proteins, and that 2,5-DHB is unique in its ability to form abundant ISD ions.20 In order to construct a model for
MALDI-ISD and to clarify the ISD phenomenon, it is necessary to understand the nature of the interactions between 2,5-DHB and protein molecules, based on appropriate information of the 2,5-DHB crystal (Fig. 3). Regarding this, Strupat et al. reported an X-ray crystallographic investigation of 2,5-DHB with and without the incorporation of protein into the matrix crystal.\textsuperscript{50)} They presented a concrete image for the crystal growth of 2,5-DHB. That is, the 2,5-DHB matrix without protein incorporation forms a hydrophobic planar network as the hydrophobic crystal plane (011) by intermolecular hydrogen-bonding, as shown in the illustration of Scheme 7, and furthermore, the stacking of the (011) crystal planes forms a (100) surface along the direction of crystal growth. The crystal surface plane (100), which is hydrophilic in nature due to phenolic and carboxylic hydroxyl groups, is normally irradiated by UV laser photons in MALDI experiments. This is of importance from the standpoint of the interaction between matrix and protein molecules that an abundance of phenolic hydroxyl groups (Ph–OH) are exposed from the 2,5-DHB matrix (100) crystal surface. Strupat et al. observed an important fact that the 2,5-DHB crystal structure did not change, even after protein incorporation, while the (100) crystal surface became smoother than that without protein incorporation.\textsuperscript{50)}

On the other hand, the structures of the crystal (100) surface plane of the 2,5-DHB matrix with and without peptide incorporation for ACTH18–39 were observed by using scanning probe microscopy (SPM).\textsuperscript{51)} In the case of the crystal surface without a peptide, right-angled layered steps along the crystal growth direction were observed (Fig. 4(a)), while the layered steps disappeared and the (100) crystal surface formed a smooth structure after peptide incorporation (Fig. 4(b)). From X-ray crystallographic data\textsuperscript{50)} and SPM data\textsuperscript{51)} of the 2,5-DHB crystals with and without analyte incorporation, it appears that the (100) crystal surface is covered with the analyte peptide or protein molecules. Therefore, intermolecular hydrogen-bonding between matrix active-hydrogens (Ph–OH and/or Ph–COOH) exposed from the (100) surface and carbonyl oxygens on the peptide backbone would be more likely, as illustrated in Scheme 8.
The formation of c fragments by intermolecular hydrogen transfer via hydrogen-bonding between matrix active hydrogens and backbone carbonyl oxygens

Hydrogen-bonding interactions between matrix active-hydrogens and backbone carbonyl oxygens (Scheme 8) can explain the MALDI-ISD phenomenon by assuming a photochemical intermolecular hydrogen transfer from the matrix to the analyte.9,10) The photochemical intermolecular hydrogen transfer in UV-MALDI can be understood by an electron transfer within a very short time without atomic motion (Scheme 1 left). Hydrogen transfer (rearrangement or abstraction) between a carbonyl oxygen and adjacent hydrogens results in the formation of radical species and subsequent degradation such as the Norrish Type II reaction.52) As a result, therefore, carbonyl oxygens of organic compounds have a high affinity for hydrogen atoms due to the radical characteristics of the photochemically excited state transition $n-\pi^*$, so that hydrogen atom abstraction from the matrix to the peptide backbone carbonyl oxygens can easily occur via hydrogen-bonding between matrix and peptide molecules that are excited with UV laser photons, as shown in Scheme 9. This type of hydrogen transfer may be due to a very short electron transfer time without any hydrogen atom movement, similar to a McLafferty hydrogen rearrangement (Scheme 1(a) left). The subsequent MALDI-ISD to form c-ions is completed within several tens of nanoseconds or less without intramolecular hydrogen scrambling in the MALDI ion-source.5,53)

According to the mechanism of intermolecular hydrogen transfer for MALDI-ISD presented in Schemes 8 and 9, Demeure et al. have attempted to present a strategy for the rational selection of the optimum MALDI-ISD matrix materials,18) and they determined the hydrogen-donating characteristics of several matrices and gave the order of the ability, i.e., picolinic acid (PA)>1,5-diaminonaphthalene (1,5-DAN)>2,5-DHB>sinapinic acid (SA)>α-cyano-4-hydroxy-cinnamic acid (CHCA). We also reported on the hydrogen-donating properties of matrix materials as follows, 1,5-DAN>5-amino-salicylic acid (5-ASA)>2,5-DHB>SA≒CHCA.54) Demeure et al. applied a mechanistic model (Scheme 9) to top-down proteomics using MALDI-ISD MS.18,55)

It should be emphasized from the experimental and rational results described above that the role of the X-ray crystallography and SPM analysis of matrix crystals is of great importance for developing a model construction of MALDI-ISD. Regarding this model, Asakawa et al. reported that the use of crystal phase matrices such as 2,5-DHB and 1,5-DAN resulted in abundant ISD ions via efficient hydrogen transfer, while hydrogen transfer was suppressed when a liquid or amorphous phase of a mixture of 2,5-DHB and 1,5-DAN

Scheme 8. Schematic illustration of intermolecular hydrogen-bonding between phenolic hydroxyl groups exposed from the hydrophilic (100) surface plane of the 2,5-DHB matrix crystal (lower) and the carbonyl oxygens of the peptide backbone (upper).

Scheme 9. MALDI-ISD processes involved in generating a c fragment. (a) Very fast electron transfer via hydrogen-bonding between matrix active hydrogen and the peptide backbone induced with laser photons, (b) the formation of 2,5-DHB phenoxy radicals, hydrogen atoms and hypervalent peptide radicals, and (c) the formation of ISD products $c$ and $z'$ by α-cleavage.9)
This observation strongly supports the model illustrated in Schemes 8 and 9.

**MATRIX MATERIALS SUITABLE FOR MALDI-ISD: 5-POSITION AS KEY FUNCTIONAL GROUPS OF ISD**

Brown et al. found that the most suitable matrix for the formation of ISD fragment ions was 2,5-DHB.\(^{30}\) I also attempted to estimate the ability of producing abundant ISD fragment ions of angiotensin I, substance P, and ACTH\(18–39\) by using dihydroxybenzoic acids (2,3-DHB, 2,4-DHB, 2,5-DHB (\(\epsilon_{337}: 4250\)), 2,6-DHB (\(\epsilon_{337}: 700\)), 3,4-DHB, 3,5-DHB (\(\epsilon_{337}: 100\)), trihydroxybenzoic acids (2,3,4-THB, 2,4,6-THB, 3,4,5-THB), and 2,5-HMBA (\(\epsilon_{337}: 5000\)).

\[\text{Scheme 10. Matrix materials (molar absorptivity for } \epsilon_{337} \text{ nm) used for the MALDI-ISD experiments of peptides.}\]  

\[\text{Dihydroxybenzoic acids (2,3-DHB, 2,4-DHB, 2,5-DHB (} \epsilon_{337}: 4250\), 2,6-DHB(} \epsilon_{337}: 700\), 3,4-DHB, 3,5-DHB (\(\epsilon_{337}: 100\)), trihydroxybenzoic acids (2,3,4-THB, 2,4,6-THB, 3,4,5-THB), and 2,5-HMBA(\(\epsilon_{337}: 5000\)).\]

![Diagram](image)

**Fig. 5.** MALDI-ISD spectra of RLG (RLGNQAVGDLAE, \(M_r 1428.6\)) obtained with (a) 2,5-DHB, and (b) 2,5-HMBA.

was used.\(^{36}\) This observation strongly supports the model illustrated in Schemes 8 and 9.

1. A hydroxyl group at the 2-position is an effective function for the formation of MALDI signals and ISD fragment ions, because the 2-position hydroxyl group can form a six-membered ring by hydrogen-bonding with the 1-position carboxyl group.
2. A large molar absorptivity may be a requirement for ISD fragment ion formation.
3. The 5-hydroxy group in 2,5-DHB has an important structural role in the formation of abundant ISD fragment ions. ISD experiments with a 5-methoxy group containing 2,5-HMBA matrix with a higher molar absorptivity of the nitrogen laser (337 nm) than that of 2,5-DHB did not show any ISD ion formation, as shown in Fig. 5.

It should be noted from the above descriptions that the hydroxyl group at the 5-position in 2,5-DHB has an especially important structural and functional role in the formation of abundant ISD fragment ions. The important points in identifying suitable matrix materials for MALDI-ISD are to consider both the high molar absorptivity (\(\epsilon_{337}\)) and the
appropriate positions of functional groups such as phenolic hydroxyl groups (Ph–OH) and anilinic amino groups (Ph–NH₂) which have a high capability for releasing hydrogen atoms. As a matter of fact, we identified matrix materials suitable for MALDI-ISD by considering such 5-position functional groups,54,57) as shown in Scheme 11 left. On the other hand, Smargiasso et al. reported that 2-aminobenzamide (2-AB) and 2-aminobenzoic acid (2-AA) were suitable for both positive- and negative-ion MALDI-ISD of peptides and proteins (Scheme 11 middle).58) This indicates that the presence of anilinic amino group(s) efficiently induces the release of hydrogen atoms from amino groups.

Furthermore, the recent report of a new matrix, 5-aminoo-1-naphthol (5,1-ANL),59,60) suitable for both positive- and negative-ion MALDI-ISD of intact proteins (Scheme 11 middle).59) This indicates that the presence of anilinic amino group(s) efficiently induces the release of hydrogen atoms from amino groups.

MALDI-ISD of proteins (Scheme 11 right). The 5,1-ANL matrix is particularly useful and has a superior capability for the comprehensive top-down analysis of intact proteins.14) Crystals of 5,1-ANL, 1,5-DAN, and 1,5-DHN with and without cytochrome c incorporation are shown in Fig. 6.

CONCLUSION

The MALDI-ISD of peptides and proteins can be understood by dividing the overall process into three steps. The first step can be considered to be the photochemical intermolecular abstraction of hydrogen by carbonyl oxygens of the peptide backbone from environmental matrix chemicals via hydrogen-bonding (Scheme 9(a)). The second step is the prompt simple (α)-cleavage of neutral radical analytes (Scheme 9(b)). The last step, the formation of ISD products (Scheme 9(c)) and ionization of the neutral species takes place via proton transfer reactions between neutral ISD fragments c, z', z-M (Scheme 2) and matrix ions ([M+H]+ and [M–H]−) in the MALDI-plume. The ionization occurs
at both basic and acidic functional groups according to the gas-phase basicity and acidity of the neutral peptide fragments. In the case of intact proteins, both positive- and negative-ion ISD spectra merely produce N-terminal side c-ions. Therefore, MALDI-ISD can be used for the top-down sequencing of unknown and post-translational modified proteins. It should be stressed that the model for MALDI-ISD was constructed on the basis of X-ray crystallography findings and SPM analyses of matrix crystals, as well as the use of isotope-labelled peptides.

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