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COMMUNICATIONS

Complete and selective nitration of tyrosine residue in peptides caused by ultraviolet matrix-assisted laser desorption/ionization

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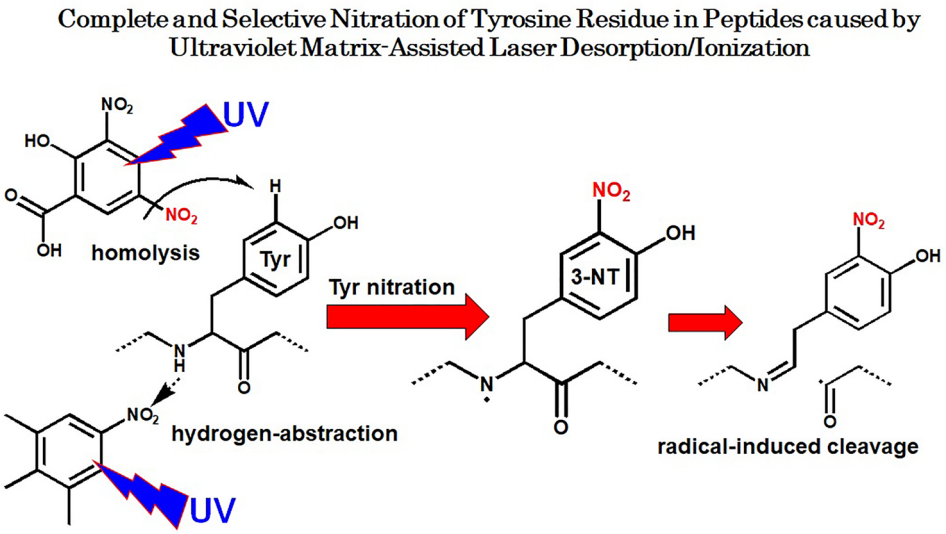
Abstract

Complete and highly selective nitration of tyrosine (Tyr) as a residue-specific modification in peptides was found without side reactions, using ultraviolet matrix-assisted laser desorption/ionization (UV-MALDI) with a nitroaromatic reagent 3, 5-dinitrosalicylic acid (3,5-DNSA). The tyrosine nitration supported two propositions, namely, the UV-induced. NO_2 attack reaction mechanism by Long et al. and the C–NO_2 homolysis as a thermal process by Wiik et al. and Furman et al. With the UV-MALDI of peptides, a residue-specific reaction was observed in glycine (Gly) residue, i.e., an oxidation of the alpha-carbon of Gly due to attack of hydroxyl radical (.OH).

Graphical abstract



Keywords Tyrosine nitration · Peptide · 3, 5-dinitrosalicylic acid · Matrix-assisted laser desorption/ionization · Oxidation

1 Introduction

Nitroaromatic compounds have provided unique aspects regarding the reactivities of nitro group (NO_2) such as intramolecular hydrogen transfer, isomerization and C–NO_2 bond homolysis caused by thermal and light stimuli [1, 2]. A nitroaromatic biological compound, 3-nitrotyrosine (3-NT) in peptides and proteins, is of interest in connection with

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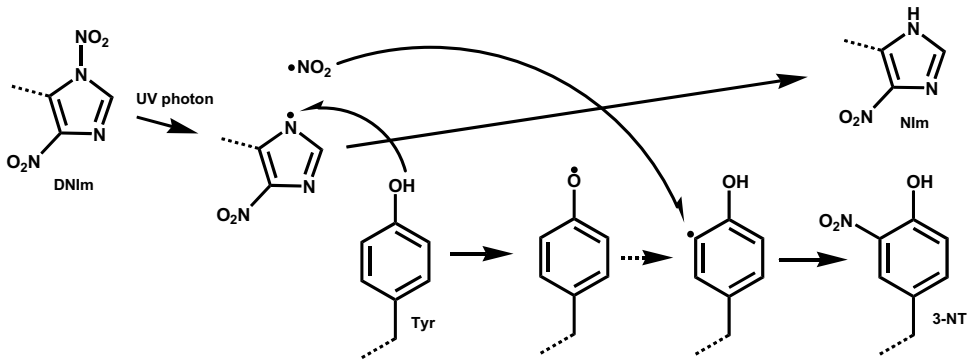


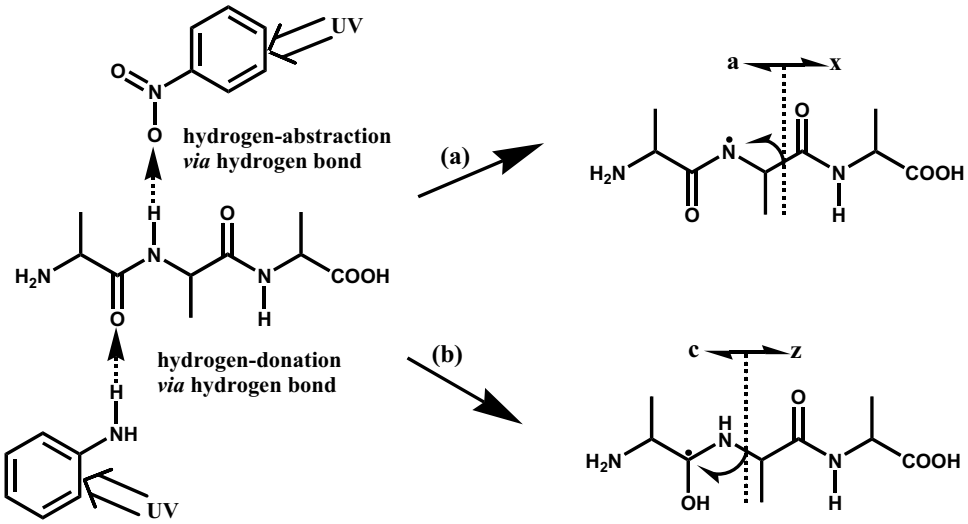
Scheme 1 Proposed reaction mechanism by Long et al. for tyrosine nitration of proteins by UV irradiation, modified from [10]

oxidative post-transnational modifications, signal transduction, pathogens of specific diseases, and a biomarker [3, 4]. Especially it is believed that post-translational modified proteins oxidized with strong one-electron oxidants such as $.NO_2$ and OH are often considered to be dead-end products resulting in degradation, apoptosis, and enzymatic deactivation [3–5]. Although mass spectrometry (MS) is often used for identifying the site of 3-NT in peptides and proteins [6–9], the use of ultraviolet (UV) MALDI results in photo-induced decompositions of the nitro group in analytes, giving the fragment ions $[M+H-16]^+$, $[M+H-30]^+$ and $[M+H-32]^+$ [4, 6]. It was recently reported by Long et al. [10], on the other hand, that the combination of UV light and 5-methyl-(2, 4)-dinitroimidazole (2,4-DNIm) as a nitration reagent resulted in the chemical nitration of tyrosine residues in proteins with high yields and without serious side reactions. Long et al. have proposed that tyrosine nitration occurs via radical reactions by attack of nitrogen dioxide radical ($.NO_2$) generated by UV photons (Scheme 1), and the production of $.NO_2$ radical originated from the $N–NO_2$ bond homolysis of the 2,4-DNIm excited with UV light is essentially of importance for the reaction. More recently Wiik et al. reported that using DFT calculations of substituted nitroaromatic compounds the $C–NO_2$ bond homolysis easily takes place as a thermal process, while in general the homolytic cleavage is a high-energy event [2]. It is also known that such a thermal decomposition of the $C–NO_2$ bond occurs in condensed phase with lower activation routes than the gas phase [11]. It is of importance for the tyrosine nitration coupled with the $C–NO_2$ homolysis to have evidence for thermal and radical reactions in condensed phase, because the methods for incorporating 3-NT residues into peptides and proteins with high yields and without side reactions are of importance for the study of the biological roles of 3-NT [3, 4, 10]. It is also of importance to consider that in the mechanism of tyrosine nitration (Scheme 1), electrophilic aromatic nitration occurs between ortho position of tyrosine as an electron donor and nitrogen dioxide $.NO_2$ as an acceptor [12]. Here the author reports that tyrosine residues of peptide molecules embedded in crystallized phase of 3, 5-dinitrosalicylic acid (3,5-DNSA) were completely and selectively nitrated with UV-MALDI.

2 Results and discussion

Nitroaromatic reagents 3,5-DNSA and 4-nitro-1-naphthol (4,1-NNL) have both hydrogen abstracting and electron withdrawing properties in nature, and it has been reported that the use of these reagents as the matrix of the MALDI analysis of peptides results in a/x-series ions [13, 14], while the use of hydrogen-donating reagents such as 5-amino-1-naphthol (5,1-ANL) [15] and 2,5-dihydroxybenzoic acid (2,5-DHB) [16, 17] results in c/z-series ions, as shown on the right side in Scheme 2. The cleavage site-specific fragment pairs, a/x and c/z, are produced by radical-initiated dissociation (RID) of the analyte ions with hydrogen-deficient $[M+H–H]^{\cdot+}$ and hydrogen-excess radicals $[M+H+H]^{\cdot+}$, respectively [15]. It is of importance to recognize that the site-selective intermolecular hydrogen transfer occurs via hydrogen bonding between analyte and matrix molecules [17]. The hydrogen bonding in the condensed phase samples in MALDI experiments is essentially of importance for generating the radical analyte ions (see Scheme 2). In the UV-MALDI with nitro-aromatic matrix reagents, the UV photons preferentially and electronically excite matrix molecules, and steadily the nitro group oxygens of the excited matrix molecules abstract amide hydrogen from the peptide backbone via hydrogen bonding, as shown on the left side in Scheme 2. As a result, the a/x-series pair ions are specifically produced as already reported [13, 14].

The MALDI mass spectra of a synthetic peptide ACTH18-39(Gly9-d2) containing aromatic Tyr6, Phe18, and Phe22 residues obtained with the nitroaromatic matrix reagents 3,5-DNSA and 4,1-NNL are shown in Fig. 1. As expected from Scheme 2a, the spectrum with 4, 1-NNL in Fig. 1b showed amino (N)-terminal a-series ions, although a9 ion originated from Cα–C bond cleavage at the Gyl(d2)–Ala residues was little observed as already reported [13]. Surprisingly, however, the spectrum with 3, 5-DNSA



Scheme 2 The specific cleavage at the Ca–C and N–Ca bond of the peptide backbone for the formation of a/x and c/z fragment pairs, through the site-specific hydrogen-bonding between matrix functional groups (–NO_2 and –NH_2) and the sites of amide region (NH and CO) of the peptide backbone

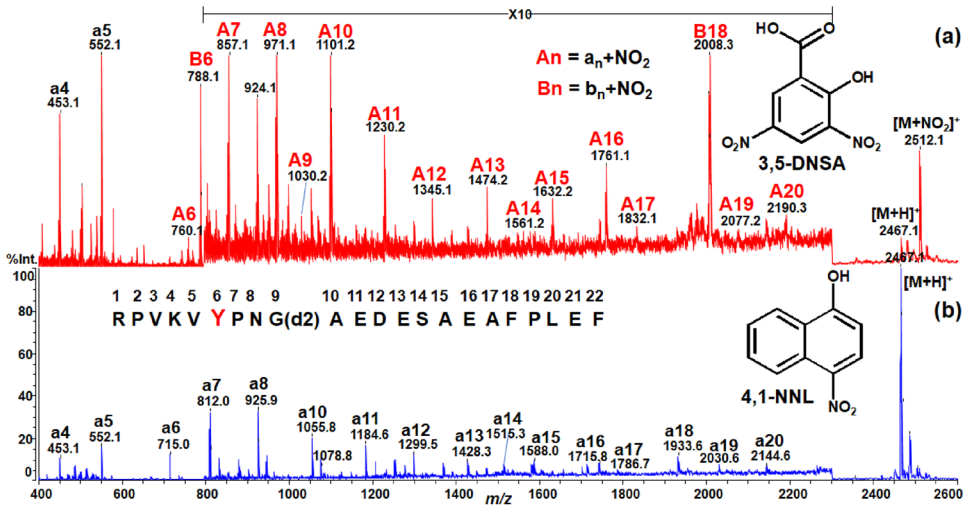


Fig. 1 MALDI mass spectra of ACTH18-39(Gly9-d2) with Tyr–Pro and Phe–Pro residues, obtained with hydrogen-abstracting oxidative matrices **a** 3,5-DNSA and **b** 4,1-NNL

did not show normal a-series ions but unexpected A-series ions corresponding to $[a+45]^+$ ions from A6 to A20, as well as preferential analyte ion $[M+H+45]^+$. The mass shift of 45 Da in a-series ions was observed in Tyr6 residue, while other N-terminal (RPVKV) and aromatic Phe residues did not show such a mass shift. This indicates that the complete and selective mass shift between Val5 and Tyr6 residues occurred in the Tyr6 residue using 3, 5-DNSA reagent, as shown in Fig. 2. The mass shift of 45 Da suggests that tyrosine nitration occurs in UV-MALDI with 3, 5-DNSA matrix reagent. The B6 and B18 ions observed in Fig. 1a and Fig. 2a are characteristic ions in cleavage at Xxx–Pro residues [18].

According to the reports of Long et al. [10] and Furman et al. [11], it is likely that the chemical nitration of tyrosine residue in peptides embedded in the condensed matrix crystal occurs by attack of the nitrogen dioxide radicals (.NO_2) on the peptide molecules (see Scheme 1), while the .NO_2 radicals are generated from 3, 5-DNSA molecules excited with UV photons. It is of importance to recognize that the .NO_2 and related oxidative radicals such as .OH can make it possible to access the backbone and sidechains of peptide molecules, and that in the final analysis the .NO_2 radicals selectively react with the phenol moiety of Tyr residue.

To have further evidence for the mass shift of 45 Da in the Tyr residue, the MALDI mass spectra of other synthetic peptides with and without Tyr residue were obtained with 3, 5-DNSA reagent, as shown in Fig. 3, 4, respectively. In the peptide with Tyr and six Gly residues (Fig. 3), the spectrum with 3, 5-DNSA showed the definite mass shift at 45 Da in the intense a5 to a10 ions and A11 to A15 ions, suggesting

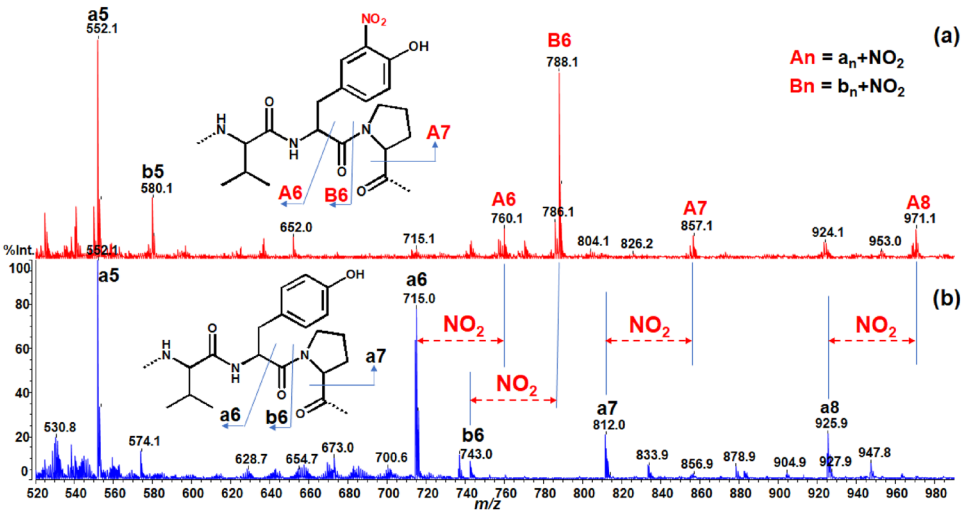


Fig. 2 Mass shift (45 Da) of the a and b ions by the tyrosine nitration at the Tyr6 residue in ACTH18-39(Gly9-d2) caused by the irradiation of UV photons to matrix crystals of **a** 3,5-DNSA and **b** 4,1-NNL

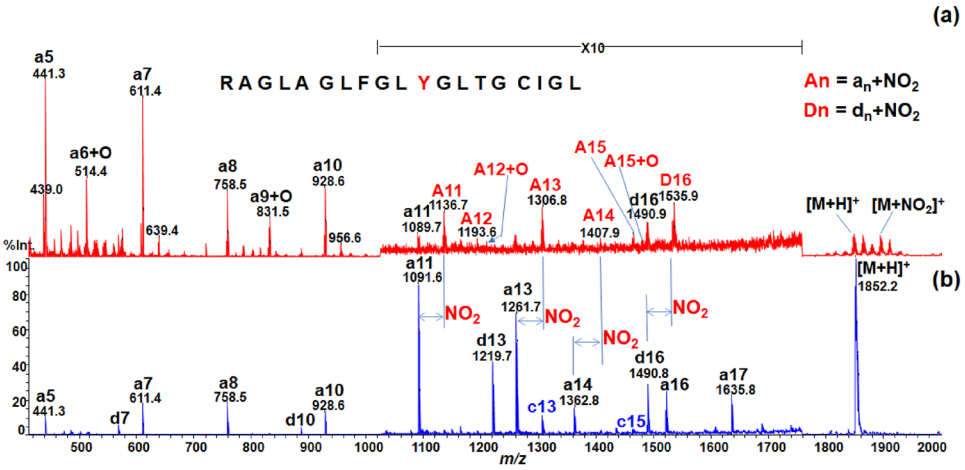


Fig. 3 MALDI mass spectra of RAGLA with Tyr–Gly and Phe–Gly residues, obtained with oxidative matrix **a** DNSA and **b** NNL

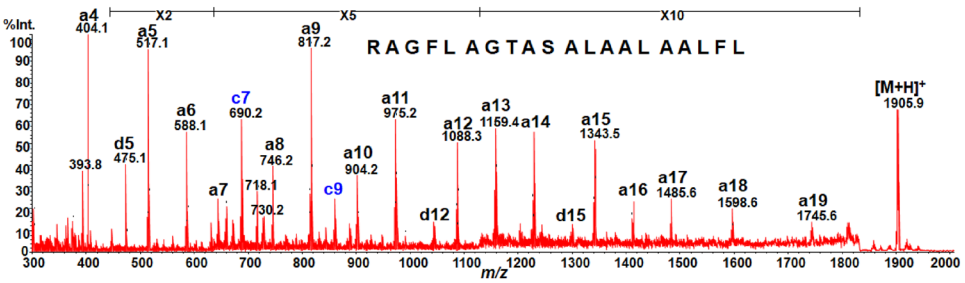
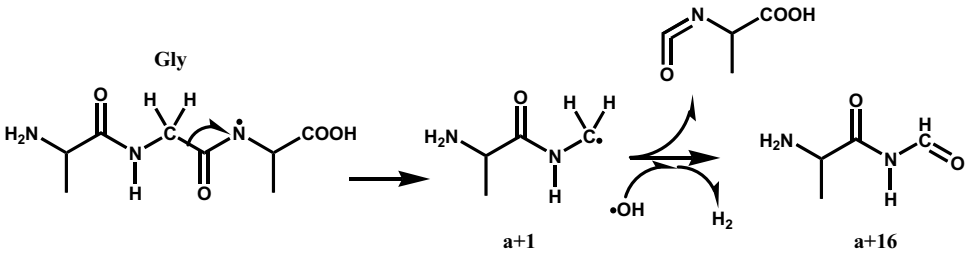


Fig. 4 MALDI mass spectra of a synthetic peptide RAGFL without Tyr residue, obtained with oxidative DNSA matrix

the nitration of Tyr11 residue of the peptide (Fig. 3a). The spectrum did not show A8 ion at an aromatic Phe8 residue but showed a8 ion originating from cleavage at the Cα-C bond of the Phe8–Gly9 residues. This indicates that selective nitration at the phenolic Tyr residue occurs in the UV-MALDI with 3, 5-DNSA. Interestingly, the spectrum with 3, 5-DNSA showed unexpected ion peaks corresponding to $[a+16]^+$ ion originating from cleavage at the Cα–C bond of the Gly–Xxx residues. The appearance of the definite peaks of $[a+16]^+$ and $[A+16]^+$ ions is important from the mechanistic implications regarding the radical-initiated dissociation (RID) in Scheme 2a, because it has been reported that a ion of the Gly–Xxx bond cleavage is very weak in this kind of the MALDI experiments [13]. The formation of the $[a+16]^+$ and $[A+16]^+$ ions suggests that oxidation reaction of the alpha-carbon (Cα) of the Gly residue selectively

Scheme 3 Proposed mechanism for $[a+16]^+$ ions originating from cleavage at the Cα–C bond of the Gly–Xxx residues



occurs by attack of hydroxyl radicals (.OH) generated by the photolysis of 3, 5-DNSA molecules [19, 20], as shown in Scheme 3. The generation of .OH radicals from nitroaromatic compounds can be strongly supported by the report of Nauser et al. [21]. The peak corresponding to D16 ion represents the loss of sidechain from Cys16 residue, which is independent of attack of the radicals [18]. In the peptide without Tyr and with alcoholic hydroxyl group containing serine (Ser)/threonine (Thr) residues (Fig. 4), the MALDI mass spectrum did not show the mass shifted A ions, but merely showed a-series ions and some d-series ions originating from the loss of sidechain. Notable peaks corresponding to the c ion may be produced by intramolecular hydrogen transfer and following RID reaction occurring independent of attack of the radicals, as shown in Scheme 4. The c ion peaks could be also observed in Fig. 3b, originating from cleavage at the N–Cα bond of the Leu–Thr and Gly–Cys residues.

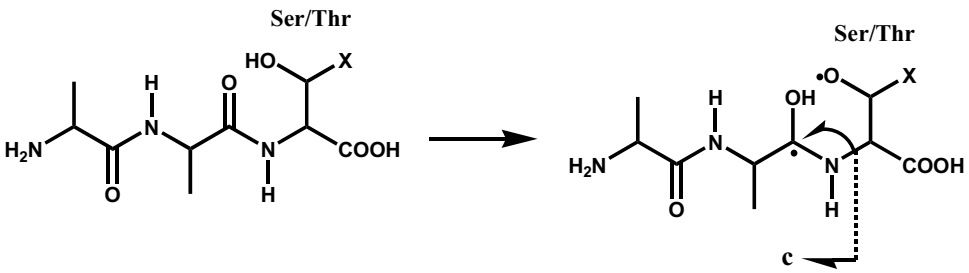
3 Experimental

UV-MALDI experiments were performed with an AXIMA-CFR time-of-flight (TOF) mass spectrometer (Shimadzu Co Ltd. Kyoto Japan). The MALDI MS experimental conditions and sample preparations are described elsewhere [11]. A difference in the sample preparation from the previous report [11] is that the molar ratio of matrix-to-analyte was three times larger than that of the report. 3, 5-Dinitrosalicylic acid (3,5-DNSA) and 4-nitro-1-naphothol (4,1-NNL) were purchased from Tokyo Chemical Industry (Tokyo, Japan). Synthetic peptides ACTH18-39(Gly9-d2) (Mr 2467.7), RAGLA (Mr 1852.2), and RAGFL (Mr 1905.3) were supplied from Peptide Institute (Minoh, Osaka, Japan).

4 Conclusions

Tyrosine nitration is an important event for research interests such as photo-induced radical reactions and the biological dead-end reaction or a signal of the oxidative degradation of proteins [4, 5], so that researchers need appropriate methods for incorporating 3-NT into peptides and proteins [10]. The use of a nitroaromatic reagent 3, 5-dinitrosalicylic acid (3,5-DNSA) in ultraviolet matrix-assisted laser desorption/ionization (UV-MALDI) experiments of peptides resulted in unexpected oxidation reactions, namely, the complete and selective nitration of tyrosine residue and the oxidation of alpha-carbon (Cα) of glycine (Gly) residue without serious side reactions. It was suggested that the oxidation reactions occurred by attack of the radicals $.NO_2$ and .OH on the backbone and sidechains of peptide molecules, and that the radicals were generated from the C–NO_2 homolysis and degradation of nitro groups of 3, 5-DNSA molecules in the condensed matrix crystal for UV-MALDI experiments. The tyrosine nitration observed completely supported the UV-induced reaction mechanism proposed by Long et al. [10] and the C–NO_2 homolysis as a thermal process proposed by Wiik et al. [2] and Furman et al. [11] because MALDI involves an explosive thermal process [22].

Scheme 4 Proposed mechanism for c ions originating from cleavage at the N–Cα bond of the Xxx–Ser/Thr residues



Declarations

Conflict of interest The author declares no competing financial interest.

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