

# Oxidation of Ferrocene Derivatives in Desorption/Ionization on Porous Silicon

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In matrix-assisted laser desorption/ionization (MALDI), the true molecular structures of some analytes are not represented by the observed ions due to a redox reaction. In earlier reports, electron transfer from analyte to chemical matrix has been proposed for the oxidation of ferrocene derivatives in MALDI. To address such a redox phenomenon in laser desorption/ionization processes, two ferrocene derivatives,  $\text{FcCH}_2\text{CH}_2\text{Fc}$  and  $\text{FcCH}_2\text{NMe}_2$  [ $\text{Fc}:(\text{C}_5\text{H}_5)\text{Fe}(\text{C}_5\text{H}_4)$ ], were analyzed by a matrix-free method, desorption/ionization on porous silicon (DIOS). The oxidized species,  $\text{Fc}^+\text{CH}_2\text{NMe}_2$  and  $\text{FcCH}_2\text{CH}_2\text{Fc}^+$ , were detected in the DIOS mass spectra. The results suggested that electron transfer from the analytes to the sample target occurs during the ionization process.

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## Introduction

Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) is currently an indispensable method for analyzing biomolecules and synthetic polymers.<sup>1-6</sup> While the mechanism of ionization is largely unknown, physical data on the ionization potentials and the gas-phase acidity or basicity have been accumulating, and the roles of the matrix–matrix, matrix–analyte and analyte–analyte reactions in both primary and secondary ionization events have been evaluated in some detail.<sup>7-16</sup> These studies have suggested the secondary ion-molecule reactions in the plume to be essential to understanding the process of ionization and the mass spectrum.<sup>7,9,11</sup>

Some analytes are reduced by these reactions as follows: Copper(II) ions are reduced by laser desorption/ionization (LDI) with or without a chemical matrix.<sup>16</sup> Flavin-containing compounds, such as riboflavin, riboflavin 5'-phosphate and flavin-adenine dinucleotide, are reduced in MALDI and fast atom bombardment (FAB) ionization, but not in electrospray ionization, as reported by Itoh *et al.*, who also found that the reduction levels depend on the matrix molecule types.<sup>17</sup> In the case of nitrotyrosine, the  $\text{NO}_2$  moiety is partially converted into  $\text{NH}_2$ , probably due to reduction after photo-induced dissociation by UV laser irradiation.<sup>18,19</sup> Similarly, the S-nitrosylated cysteine, Cys(SNO), in peptides is completely converted to cysteine, Cys(SH), in MALDI.<sup>20,21</sup>

Different mechanisms, such as electron capture and charge exchange with the chemical matrix, have been proposed to account for the reductions in MALDI.<sup>8,9</sup> A line of studies has indicated that free electrons are formed by photoelectric emission from the metal/dielectric-substance interface.<sup>22-25</sup> The metal sample target is not the photoelectron source, because the

work function of metals is greater than the photon energy.<sup>23</sup> The presence of matrix molecules or analytes placed on a metal target enhances electron emission *via* band bending and the associated reduction in work function.<sup>22,23</sup> Thus, electron transfer from the sample target would be essential for understanding the analyte reduction in LDI.

In desorption/ionization on porous silicon, termed DIOS,<sup>26</sup> analytes are deposited on a silicon chip, which is prepared by electrochemical anodization or chemical etching of crystalline silicon, for LDI without the addition of a chemical matrix. The DIOS mass spectra are thus free of cluster ions of the matrix, facilitating identification of the ions of small analyte molecules. DIOS-MS has been applied to various kinds of compounds, such as peptides, natural products, small organic molecules and synthetic polymers.<sup>26-36</sup> The ionization efficiency of DIOS relies on a porous scaffold providing a sufficient surface area to retain analytes, and on the UV-absorptive property, which affords the transfer of laser energy to analytes.<sup>26,27</sup> In contrast to MALDI, chemical reactions in a plume composed of analytes and the sample matrix do not need to be considered in the ionization mechanism of DIOS. It is thus intriguing to investigate analyte reduction in DIOS. In our previous study, copper(II) and riboflavin were reduced in DIOS.<sup>34</sup> Furthermore, Cu(II) ion reduction was inversely proportional to the concentration of the analytes, and thus the thickness of the deposited analyte would affect the reduction, as in the case of MALDI.<sup>16,25</sup> These findings suggest that electron transfer from the sample target to analytes is likely in DIOS as well, although the ionization mechanism of DIOS is unknown.

Juhász and Costello reported on the oxidation of oligomers and polymers containing ferrocene and ruthenocene moieties in MALDI measurements.<sup>37</sup> They described that either electron transfer from the analyte to the chemical matrix or direct photoionization of the analyte could account for their results. Limbach and co-workers presented a systematic study of MALDI measurements of some analytes with different

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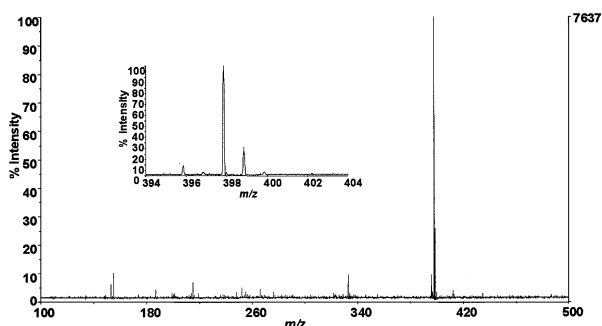


Fig. 1 DIOS mass spectrum of 1,2-diferrocenylethane.

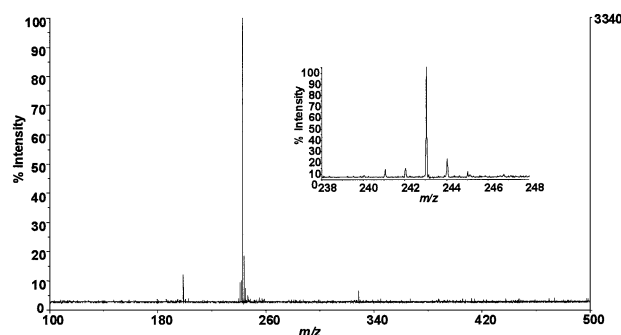


Fig. 2 DIOS mass spectrum of *N,N*-dimethylaminomethylferrocene.

ionization potentials.<sup>38,39</sup> Their results suggested that electron transfer from the analytes to the matrices has a great effect on the production of radical molecular cations. In this work, some ferrocene derivatives were measured by DIOS to investigate the oxidation of the analytes in DIOS.

## Experimental

### Materials

Methanol, ethanol, and 2-propanol, chloroform, and 46% hydrofluoric acid (HF) were obtained from Wako Pure Chemicals (Osaka, Japan), and 1,2-diferrocenylethane [ $\text{FcCH}_2\text{CH}_2\text{Fc}$ ;  $\text{Fc}:(\text{C}_5\text{H}_5)\text{Fe}(\text{C}_5\text{H}_4)$ ] and  $\alpha$ -cyano-4-hydroxycinnamic acid (CHCA) were from Sigma-Aldrich (Milwaukee, WI, USA). *N,N*-Dimethylaminomethylferrocene [ $\text{FcCH}_2\text{NMe}_2$ ] was purchased from Tokyo Kasei (Tokyo, Japan). Silicon wafers were from Sumitomo Mitsubishi Silicon Corporation (Tokyo, Japan).

### Preparation of DIOS chip

DIOS chips were prepared as described previously.<sup>26,27</sup> Briefly, a silicon (100) wafer of n-type and  $\rho = 0.5 - 1.5 \Omega \text{ cm}$  resistivity was anodically etched ( $4 \text{ mA/cm}^2$ ) in a 1:1 (v/v) solution of ethanol-HF (46%) under exposure to white light ( $70 \text{ mW/cm}^2$ ) for 1 min. After etching, the DIOS chips were washed in methanol, then in 2-propanol, and finally in ethanol, and dried *in vacuo*.

### Mass spectrometry

$\text{FcCH}_2\text{CH}_2\text{Fc}$  was dissolved at 1 mg/mL in chloroform, and  $\text{FcCH}_2\text{NMe}_2$  was dissolved at 1 mg/mL in methanol. Each 0.5  $\mu\text{L}$  aliquot of these analyte solutions was deposited on a DIOS chip and dried at room temperature.

DIOS-MS was performed with a Voyager DE Pro time-of-flight mass spectrometer (Applied Biosystems, Foster City, CA, USA) equipped with a 337 nm nitrogen laser. The DIOS chip was taped onto a stainless stage, which was prepared in-house to offset the thickness of the chip. The analyte ions were accelerated at 20 kV under delayed extraction conditions in the reflectron positive ion mode. Monoisotopic peaks of the ion species of CHCA,  $[\text{M}+\text{H}]^+$  at  $m/z$  190.05,  $[2\text{M}+\text{H}]^+$  at  $m/z$  379.09,  $[3\text{M}-3\text{H}+4\text{Na}]^+$  at  $m/z$  656.06, were used as calibrants.

## Results and Discussion

Ferrocene derivatives undergo electron-transfer reactions in

solution to form chemically stable radical cations, and have relatively low gas-phase ionization potentials.<sup>40,41</sup> Although the proton affinity of ferrocene is relatively high, the rate of proton transfer to ferrocene in the gas phase is slow.<sup>42</sup> Limbach and co-workers reported on the oxidation of ferrocene derivatives (ferrocene, 1,2-diferrocenylethane, and decamethylferrocene) in MALDI.<sup>38,39</sup> They proposed resonant two-photon ionization of the chemical matrix, followed by electron transfer from the analyte to the matrix molecule. Therefore, it was interesting to examine the oxidation of ferrocene derivatives by DIOS, since chemical matrices are not used in DIOS.

In the DIOS mass spectrum of 1,2-diferrocenylethane ( $\text{FcCH}_2\text{CH}_2\text{Fc}$ ,  $M = 398$ ), the oxidized ion,  $\text{FcCH}_2\text{CH}_2\text{Fc}^+$ , was observed at  $m/z$  398, as reported in the MALDI measurements (Fig. 1).<sup>38,39</sup> The observed isotopic pattern ( $M-2$ : 11;  $M-1$ : 3;  $M$ : 100;  $M+1$ : 29;  $M+2$ : 6) agrees well with the theoretical isotopic peak pattern ( $M-2$ : 13;  $M-1$ : 3;  $M$ : 100;  $M+1$ : 29;  $M+2$ : 5) of  $\text{FcCH}_2\text{CH}_2\text{Fc}^+$  ion, indicating that a protonated molecule of 1,2-diferrocenylethane was not formed. *N,N*-Dimethylaminomethylferrocene ( $\text{FcCH}_2\text{NMe}_2$ ,  $M = 243$ ) has an amino group as well as a Fc center. Therefore, it is expected that the molecule can catch a proton easily compared to 1,2-diferrocenylethane. In the DIOS mass spectrum of  $\text{FcCH}_2\text{NMe}_2$  (Fig. 2), an intense molecular ion of  $\text{Fc}^+\text{CH}_2\text{NMe}_2$  was observed at  $m/z$  243 without the  $\text{Fc}^+\text{CH}_2\text{NHMe}_2^+$  ion, and the isotopic ratios of the ion indicated that the protonated molecular ion  $\text{FcCH}_2\text{NHMe}_2^+$  was not formed (theoretical:  $M-2$ , 6;  $M$ , 100;  $M+1$ , 17;  $M+2$ , 2; DIOS:  $M-2$ , 9;  $M$ , 100;  $M+1$ , 17;  $M+2$ , 4).

Previous reports suggested that electron transfer from analytes to matrices has a large effect on the oxidation of analytes in MALDI.<sup>37-39</sup> Although a chemical matrix was not used, the Fc centers of  $\text{FcCH}_2\text{CH}_2\text{Fc}$  and  $\text{FcCH}_2\text{NMe}_2$  were oxidized in DIOS. Furthermore, direct laser desorption/ionization of these ferrocene derivatives in the absence of a matrix on a stainless-steel sample target did not result in the production of the molecular ion. These results suggest that electron transfers from analytes to the target in the DIOS ionization process. Sunner *et al.* suggested that the ionization mechanism of DIOS is similar to surface-assisted laser desorption/ionization (SALDI), and the formation of gas-phase ions was initiated on the surface of sharp crystal tips and edges that protrude out of the sample surface.<sup>43,44</sup> On the other hand, many researchers suggested that the secondary ion-molecule reactions in the plume are essential to understanding the MALDI ionization process.<sup>7,9,11</sup> The ionization mechanism of DIOS may differ much from that of MALDI.

## Conclusions

Two ferrocene derivatives,  $\text{FcCH}_2\text{NMe}_2$  and  $\text{FcCH}_2\text{CH}_2\text{Fc}$ , were analyzed by DIOS. The oxidized species,  $\text{Fc}^+\text{CH}_2\text{NMe}_2$  and  $\text{FcCH}_2\text{CH}_2\text{Fc}^+$ , were detected in DIOS, although a sample matrix was not used. The results indicated that electron transfer from analyte to sample target occurred in the ionization process of DIOS.

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