Optimization in Detecting Multiply-charged Protein Ions using MALDI TOF-MS

Jihyeon Lee, Jangmi Hong, Taehee Kim, and Jeongkwon Kim*

Department of Chemistry, Chungnam National University, Daejeon 305-764, Korea

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Abstract: The effects of trifluoroacetic acid (TFA) were evaluated on the generation of multiply charged ions of cytochrome *c* in a 2-nitrophloroglucinol (2-NPG) matrix in high-vacuum, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). The presence of 1% TFA in the 2-NPG matrix solution was more effective in generating multiply charged protein ions than matrix solutions containing 0.1% or 0% TFA. Regarding the matrix itself, with 1% TFA, 2-NPG was significantly more effective in generating multiply charged ions than 2,5-dihydroxybenzoic acid (2,5-DHB). The maximum charge state of cytochrome *c* was +8 when using a 2-NPG matrix containing 1% TFA.

Key words: MALDI-TOF MS, 2-nitrophloroglucinol, Multiply charged ions, Laser spray ionization

Introduction

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) is a soft ionization technique frequently used for the analysis of proteins. MALDI matrices are effective energy mediators that facilitate analyte ionization. In sample preparations for MALDI-TOF MS, the analyte is dispersed in the matrix solution and deposited as a single sample spot. A pulsed laser beam then strikes the sample spot, causing desorption of both the matrix and analyte. MALDI-TOF MS typically provides a mass spectrum of mostly singly charged proteins in a vacuum system, while electrospray ionization MS typically yields multiply charged protein ion peaks.[1,2]

Generally, conventional matrices such as 2,5-dihydroxybenzoic acid (2,5-DHB), sinapic acid, and α-cyano-4-hydroxycinnamic acid generate mostly singly charged protein ions in high-vacuum MALDI systems. Recently, however, atmospheric pressure MALDI reportedly can produce multiply charged protein ions under certain conditions.[3] This technique is referred to as laser spray ionization (LSI). More recently, the employment of a new matrix, 2-nitrophloroglucinol (2-NPG), was shown to yield multiply charged protein ions even in high vacuum.[4] Since most commercial MALDI-TOF MS instruments operate in high vacuum, the production of multiply charged protein ions, facilitated by the 2-NPG matrix, would significantly reduce the *m/z* scan range and allow high mass accuracy analyses with enhanced sensitivity.

The current study investigated the efficiency of generating multiply charged protein ions under high-vacuum MALDI-TOF MS conditions using 2-NPG as a sample matrix, where various levels of trifluoroacetic acid (TFA) were added to the sample matrix to enhance and optimize the generation of multiply charged protein ions. This is the first systematic investigation on the effects of acid additives in a MALDI sample matrix on the generation of multiply charged protein ions.

Experimental

2,5-DHB, 2-NPG, TFA, acetonitrile (ACN), and horse heart cytochrome *c* were purchased from Sigma-Aldrich (St. Louis, MO, USA).

All mass spectra were obtained on a Voyager MALDI-TOF mass spectrometer (Applied Biosystems, Foster City, CA, USA) equipped with a nitrogen laser (337 nm, 3 ns pulse, 20 Hz repetition rate) in linear ion mode for a mass range of 500~15,000 Da.

To prepare the 2,5-DHB matrix solutions, 10 mg of 2,5-DHB was dissolved in 1 mL of aqueous 50% ACN/1% TFA. The 2-NPG matrix solution was prepared by dissolving 10 mg of NPG into 1 mL of aqueous 50% ACN, 50% ACN/0.1% TFA, or 50% ACN/1% TFA solutions. To make a protein stock solution, 10 mg of cytochrome *c* was dissolved in 1 mL of distilled water. The stock solution was then diluted with distilled water to make a 10 pmol/μL cytochrome *c* sample solution.

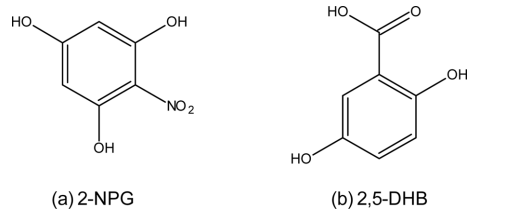
A dried-droplet sample preparation method was adopted for MALDI-TOF MS analyses. A 1-μL droplet of the mixture solution (matrix:sample = 1:1 by volume) was placed on a MALDI plate and allowed to dry, forming a microcrystal layer.

*Reprint requests to Dr. Jeongkwon Kim
E-mail: jkkim48105@cnu.ac.kr

Results and Discussion

Molecular structures of 2-NPG and 2,5-DHB are shown in Figure 1. Note that 2-NPG is a novel matrix used to enhance the generation of multiply charged protein ions in high-vacuum MALDI-MS analysis. 2,5-DHB is one of the most commonly used matrix materials in MALDI-MS analyses.[5]

Figure 2 shows MALDI mass spectra of cytochrome *c* using the 2-NPG and 2,5-DHB matrices. The theoretical average *m/z* values for the individual charge states are given in Table 1. The MALDI mass spectrum of cytochrome *c* using the 2-NPG matrix without any TFA contains peaks representing three charge states: +1, +2, and +3 (Figure 2(a)). The presence of 0.1% TFA provided additional higher charge states up to +5 (Figure 2(b)). The presence of 1% TFA generated even higher charge states up to +8 (Figure 2(c)). For comparison, the MALDI mass spectrum of cytochrome *c* in the 2,5-DHB matrix with 1% TFA is shown in Figure 2(d), where only a dominant singly charged ion peak and a minor doubly charged ion peak were observed.



(a) 2-NPG (b) 2,5-DHB

Figure 1. The molecular structures of (a) 2-NPG and (b) 2,5-DHB.

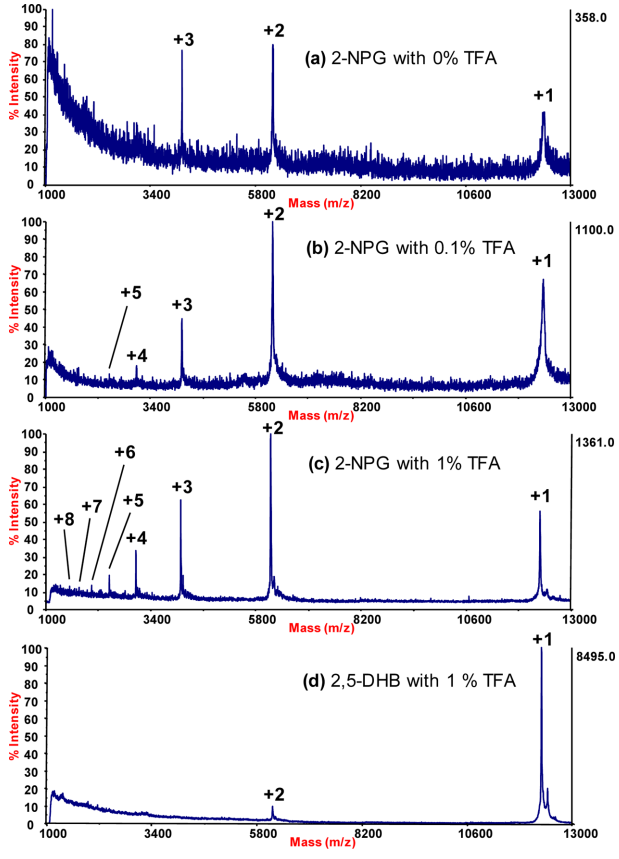


Figure 2. MALDI mass spectra of cytochrome *c* using (a) 2-NPG in 50% ACN, (b) 2-NPG in 50%ACN/0.1% TFA, (c) 2-NPG in 50% ACN/1% TFA, and (d) 2,5-DHB matrix in 50% ACN/1% TFA. Charge state of each protein peak is labeled.

Table 1. The theoretical average *m/z* value for each charge state of cytochrome *c*[a)]

charge	*m/z* value
1	12362
2	6181
3	4121
4	3091
5	2473
6	2061
7	1767
8	1546
9	1374
10	1237

[a)]The molar mass of cytochrome *c* was calculated by adding the mass of amino acid sequences from 2-105 of horse heart cytochrome *c* (Swiss-Prot accession number/entry name = P00004/CYC_HORSE) ($C_{524}H_{841}N_{144}O_{151}S_4$), the mass of a heme group ($C_{34}H_{32}O_4N_4Fe_1$), and the mass of N-terminal acetylation ($C_2H_2O_1$).

Higher concentrations of TFA (> 1%) were also evaluated, but no improvements over the performance of matrices containing 1% TFA were observed. The 2-NPG matrix containing 1% TFA provided the highest abundance of multiply charged cytochrome *c* ions, yielding both the highest charge states and the highest signal-to-noise ratios.

Trimpin and coworkers[4] were the first to report the use of a 2-NPG matrix to enhance the generation of multiply charged protein ions in high-vacuum MALDI analyses. However, no mention was made as to the amount of acid, if any, added to the matrix solution. A 2-NPG matrix in 50:50 ACN:H_2O was also used without acid in a recent high-vacuum application of LSI.[6] The current analysis demonstrates that the addition of a small amount of TFA (1.0%) to the 2-NPG matrix solution can significantly improve the detection of multiply charged protein ions.

Conclusion

The generation of multiply charged protein ions using a 2-NPG matrix containing TFA was evaluated and optimized in a high-vacuum MALDI system. A 2-NPG matrix containing 1% TFA was optimal in terms of signal-to-noise and peak abundance for creating multiply charged protein

ions. These results are a significant improvement over analogous analyses using a 2,5-DHB matrix. The generation of multiply charged protein ions shifts larger molecules into a lower *m/z* range and results in higher-quality mass spectra.

Acknowledgements

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