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NEW TYPE OF MATRIX FOR ANALYSIS OF LOW MOLECULAR WEIGHT COMPOUNDS BY MALDI-TOF MS

Masamichi Nakakoshi,[a]* Natsumi Funakoshi,[b] Hiroki Nakajima,[b] and Masayoshi Tsubuki[a]*

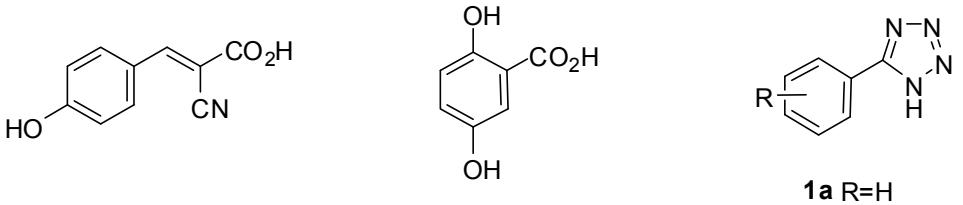
[a] Institute of Medicinal Chemistry, Hoshi University, 2-4-41 Ebara, Shinagawa, Tokyo 142-8501, Japan
[b] Life Science Business Unit, Shimadzu Corporation, 1 Nishinokyo-Kuwabaracho, Nakagyo-ku, Kyoto 604-8511, Japan

Abstract – Matrix for analysis of low molecular weight compound by MALDI-TOF MS was investigated. We found the new type of matrix, 5-aryl-1*H*-tetrazoles, which are suitable for the detection of low molecular weight compound ions. When propranolol hydrochloride was measured with 5-aryl-1*H*-tetrazoles, the strong matrix-derived peaks are not detected in low mass range.

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has been shown to be an especially useful tool for the characterization of high molecular weight molecules.[1] The results obtained for high-mass molecule from MALDI mass spectrometry experiments give not only molecular weight information but also structural information.[2] In addition, the advantage of MALDI mass spectrometry is higher availability to high-throughput analysis of a lot of samples compared with other mass spectrometry. However, the strong peaks based on matrix molecule derivative always are detected in the low mass range and cause interference to detect the small molecule ions because of ion suppression and overlapping matrix peaks. The better compound for low molecular measurement is not yet found though the matrix of an organic compound is variously examined.[3] This destined feature of MALDI-TOF MS makes the analysis of small molecules difficult. For avoiding this disadvantage for the analysis of low molecular weight compounds, following four strategies have already studied:

a) No matrix compound (Laser desorption/ionization-MS)
b) Development new type sample target for suppressing the matrix molecule ions
c) Using steady polymer matrix
d) The matrix of inorganic compounds [4-6]

The surface assisted laser desorption/ionization (SALDI), was developed as a solution of a) and b) types strategies.[7,8] Soltzberg *et al.*[9] reported one method of type c) that the matrix derivative ions were not observed in Mass spectra when poly(3-octylpolythiophene2,5-diyl) was used as matrix. Schlosser *et al*. proposed to added the ammonium citrate and KCl as a method of d)[10] and can reduce the amount of matrix derivative ion peaks in using the α-cyano-4-hydoxycinnamic acid (CHCA)[11] / 2,5-dihydoxybenzoic acid (DHB)[12] for detection of the peptide compound. However, the price of commercial SALDI target is too expensive for routine screening analysis and the methods of other strategies don't have enough sensitivity. Since CHCA and DHB, standard matrices, are aromatic carboxylic acids, we were intended to investigate organic matrices by replacement of carboxylic acid functionality. We report here 5-aryl-1*H*-tetrazole derivatives **1a-c** as a new type of matrix for low molecular weight compound analysis (Figure 1). The derivative ion peaks of these organic compounds were less observed in Mass spectra and didn't suppress the detection of sample ions.



CHCA
Mw=189.17

DHB
Mw=154.12

1a R=H
1b R=2-Br
1c R=4-OH

Figure1. CHCA, DHB, and 5-aryl-1*H*-tetrazoles **1a-c**

We chose tetrazole moiety as an isoster of carboxylic acid functionality according to bioisosters in medicinal chemistry.[13] The mass spectra of 5-phenyl-1*H*-tetrazole **1a** (Mw=146.15) under positive and negative mode conditions are shown in Figure 2. A relatively weak peak of *m/z* at 168 could be assigned to the Na^+ adduct ion for **1a** under positive mode, whereas an obvious peak for **1a** (145, $[M - H]^-$ under negative mode was observed. Regardless of the ratio of the sample and the matrix, the advantage of the tetrazole **1a** is not to be observed on $[M + H]^+$ by measurement of the positive ion detection mode but detected on the $[M - H]^-$ by negative ion detection mode.

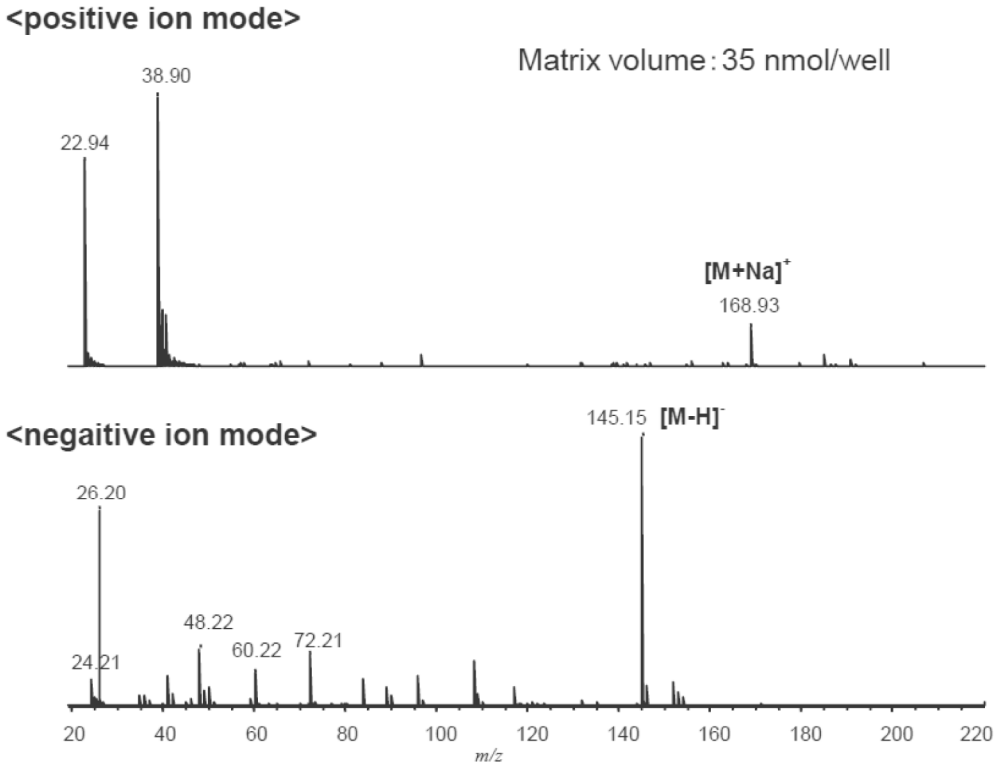


Figure 2. MALDI-TOF mass (positive and negative modes) spectra of 5-phenyl-1*H*-tetrazole **1a** (35 nmol/well) on stainless plate

We next compared 5-phenyl-1*H*-tetrazole **1a** with both CHCA and DHB in MALDI-TOF MS analysis of propranolol hydrochloride as a sample (Figure 3). When **1a** was used as a matrix, the peaks derived from **1a** were not detected in Mass spectra under positive ion mode. Propranolol (Mw=259.34) was detected as 260, $[M + H]^+$; 282, $[M + Na]^+$; and 298, $[M + K]^+$. In the case of CHCA as a matrix, peaks of *m/z* at 171, $[matrix + H - H_2O]^+$; 189, $[matrix + H]^+$; 211, and $[matrix + Na]^+$ were detected. DHB as a matrix resulted in the detection at 153, $[matrix]^+$. These peaks derived from CHCA and DHB were detected in the low mass field by the positive ion mode. These peaks would make the analysis of low weight molecular compounds difficult. In cases of both CHCA and DHB, only peak for propranolol 260 $[M + H]^+$ was observed.

Having a candidate for a new type of matrix in hand, we further tested 5-(2-bromophenyl)-1*H*-tetrazole **1b** (Mw=225.05) and 5-(4-hydroxyphenyl)-1*H*-tetrazole **1c** (Mw=162.15). Mass spectra for propranolol hydrochloride with **1b** and **1c** under positive mode condition are shown in Figure 4. It is noted that there were no signals, $[matrix + H]^+$, of the matrices in both cases. The peak for propranolol (260, $[M + H]^+$) was clearly detected in both cases.

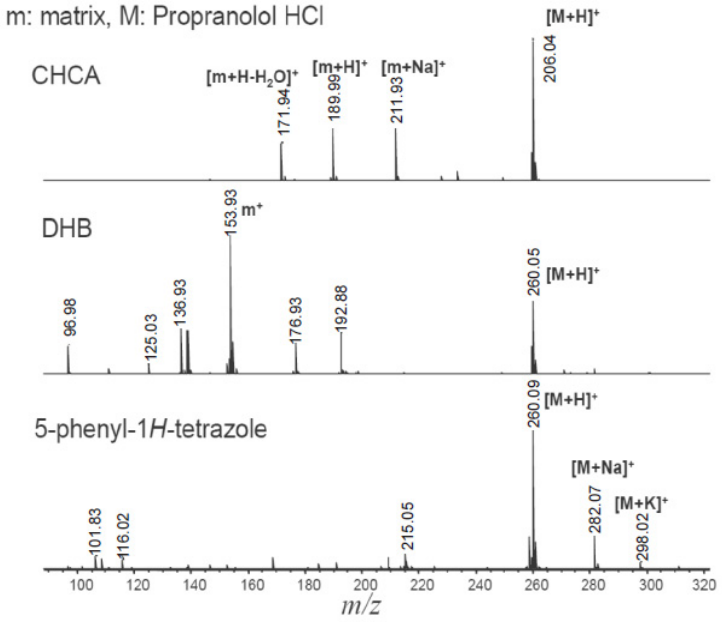


Figure 3. MALDI-TOF mass (positive mode) spectra of propranolol HCl (30 nmol) using matrix (CHCA, DHB and 5-phenyl-1*H*-tetrazole **1a**; 30 nmol) on stainless plate

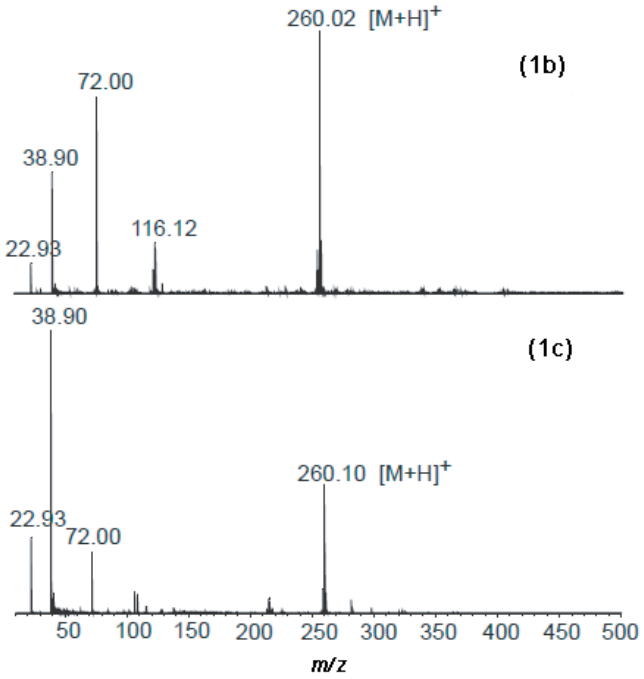


Figure 4. MALDI-TOF mass (positive mode) spectra of propranolol HCl (30 nmol) using 5-aryl-1*H*-tetrazoles **1b** and **1c** (30 nmol) per well on stainless plate

Figure 5 shows the S/N in each sample volume. 5-(2-Bromophenyl)-1*H*-tetrazole **1b** showed S/N equal with DHB and CHCA. Other tetrazoles **1a** and **1c** were low S/N ratio. In the amount of 30 nmol, the detection of matrices peaks are not detected as for the all matrices, and only the sample peaks are detected.

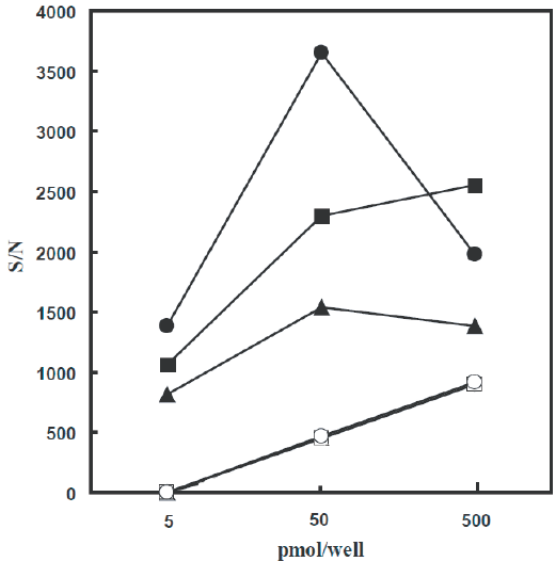


Figure 5. Sample/Noise in each sample volumes with propranolol HCl in matrices under positive mode. Closed square: CHCA, Closed triangle: DHB, Open square: 5-phenyl-1*H*-tetrazole **1a**, Closed circle: 5-(2-bromophenyl)-1*H*-tetrazole **1b**, Open circle: 5-(4-hydroxyphenyl)-1*H*-tetrazole **1c**

The tetrazole derivatives have an excellent character that only the peak of the sample can be detected. Therefore, when the low molecular weight compound is measured, it turned out that this matrix is extremely effective. Since these matrices do not ionize the biological macromolecule compounds such as the proteins, these matrices could be effective for the analysis of the drug distribution throughout the body and the drug metabolism.

In conclusion, we found a new type of matrix, 5-aryl-1*H*-tetrazole, for the analysis of low molecular weight compounds by MALDI-TOF MS. The advantage of the tetrazole matrix in comparison with CHCA and DHB was demonstrated by MALDI-TOF MS analysis of propranolol. Noteworthy that there were no signals of the matrix and peaks for propranolol ($[M+H]^+$, $[M+Na]^+$ and $[M+K]^+$) were detected under positive mode condition. Moreover, this matrix can be measured in stainless plate usually used. This result shows the possibility of new strategy of MALDI-TOF MS satisfied cheaper running cost and enough usefulness of low molecular weight compound analysis.

EXPERIMENTAL

5-Phenyl-1*H*-tetrazole **1a** and propranolol hydrochloride were purchased from Sigma-Aldrich Co. 5-Aryl-1*H*-terazole derivatives **1b** and **1c** were synthesized from the corresponding nitrile derivatives.[14]

Measurement of MALDI-TOF MS

The measurement of Mass spectra was performed by MALDI-reflectron-TOF MS (AXIMA-CFR, Shimazu Kyoto Japan). An equal amount of sample and matrix solutions (30 mmol/L) in MeCN were mixed for preparing equimolar mixture solution. 1μL of the above mixture solution was applied on a sample target. Finally, 30 nmol of sample and matrix was used for measuring Mass spectra. CHCA and DHBA were used as standard matrices.

REFERENCES

1. R. Arakawa, S. Okuno, and Y. Wada, *J. Mass. Spectrom. Soc. Jpn.*, 2004, **52**, 33.
2. S. Sekiya, Y. Yamaguchi, K. Kato, and K. Tanaka, *Rapid Commun. Mass Spectrom.*, 2005, **19**, 3607.
3. S. Martic, J. D. Brennan, M. A. Brook, S. Ackloo, and N. Nagy, *Can. J. Chem.*, 2007, **85**, 66.
4. R. Smyth, J. A. Turton, C. J. Clarke, M. J. Yorkc, T. O. Dare, C. S. Lane, and M. R. Munday, *Food Chem. Toxicol.*, 2008, **46**, 2972.
5. X. Dong, J. Chen, J. Li, and Y. Wang, *Anal. Chem.,* 2010, **82**, 6208.
6. R. P. Obena, P.-C. Lin, Y.-W. Lu, I.-C. Li, F. del Mundo, S. dR. Arco, G. M. Nuesca, C.-C. Lin, and Y.-J. Chen, *Anal. Chem.*, 2011, **83**, 9337.
7. A. Kraj, T. Dylag1, A. Gorecka-Drzazga, S. B. J. Dziuban, and J. Silberring, *Acta Biochim. Polonica*, 2003, **50**, 783.
8. J. Wei, J. M. Buriak, and G. Sizudak, *Nature*, 1999, **399**, 243.
9. L. J. Soltzberg and P. Patel, *Rapid Commun. Mass Spectrom.,* 2004, **18**, 1455.
10. G. Schlosser, G. Pocsfalvi, E. Huszar, A. Malorni, and F. Hudecz1, *J. Mass Spectrom.,* 2005, **40**, 1590.
11. R. C. Beavis, T. Chaudhary, and B. T. Chait, *Org. Mass Spectrom.,* 1992, **27**, 156.
12. K. Strupat, M. Karas, and F. Hillenkamp, *Int. J. Mass Spectrom. Ion Processes*, 1991, **111**, 89.
13. A. Burger, *Prog. Drug. Res.*, 1991, **37**, 287.
14. K. Masao, N. Ohtani, F. Kai, I. Moriguchi, and S. Inouye, *J. Med. Chem.*, 1987, **30**, 552.