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Comparative Analysis of Derivatization Reagents for Catecholamines and Amino Acids

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Abstract: We compared four derivatization reagents to analyze catecholamines and amino acids by matrix-assisted laser desorption/ionization (MALDI) mass spectrometry. 2,4,6-Trimethylpyrylium tetrafluoroborate (TMPy), 2,4-diphenyl-pyranylium tetrafluoroborate (DPP-TFB), 4-(anthracen-9-yl)-2-fluoro-1-methylpyridin-1-ium iodide (FMP-10), and triphenyl pyrilium (TPP) were used as derivatization reagents that can specifically modify primary amines or hydroxy groups in target molecules. Three derivatization reagents, not including TPP, reacted with all target molecules. The derived catecholamines dopamine and L-DOPA, and the amino acids GABA and glycine, were efficiently ionized in comparison with non-derivatized targets. Comparative analysis indicated that TMPy and FMP-10 produced general increases in signal-to-noise ratios (S/N), whereas DPP and TPP produced specific increases in the S/N of GABA and DA. Notably, TMPy is a small molecule that efficiently reacts with target molecules due to the absence of high bulk and steric hinderance.

Keywords: derivatization; mass spectrometry; dopamine; L-DOPA; GABA; 2,4,6-trimethylpyrylium tetrafluoroborate

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1. Introduction

Derivatization methods are commonly used for chromatography analysis to change the physical characteristics of target molecules, namely altering volatility temperatures, thermal stability, and avoidance of column adsorption in gas chromatography (GC), and to lower the detection limits in liquid chromatography (LC). Recent advances in mass spectrometry (MS) have been realized by implementing derivatization methods for multiplex protein profiling [1] so that protein content quantitation can be comprehensively compared among multiple samples. Other common derivatization reagents include ICAT (isotope coded affinity tag) [2] and stable isotope labeling by/with amino acids in cell culture (SILAC) [3]. A third approach allows analysis of multiplex protein profiling using isobaric tags for relative and absolute quantitation (iTRAQ) [4,5]. However, improving the ionization efficiency of small molecules by using derivatized reagents has not received much attention.

For instance, the small molecules L-dihydroxyphenylalanine (L-DOPA) [6], dopamine (DA), glycine, and γ-aminobutyric acid (GABA) are important chemical messengers involved in intraneuronal signaling in the central nervous system. Unfortunately, the native structures of these target molecules are not efficiently ionized and detected.

To efficiently ionize target molecules, several polyaromatic hydrocarbon (PAH)-based derivatization reagents were recently developed for use in MS. 2,4-diphenyl-pyranylium

tetrafluoroborate (DPP-TFB) [7] and 2,4,6-triphenylpyrylium tetrafluoroborate (TPP) [8] can selectively react with primary amine groups. 2-Fluoro-1-methyl pyridinium (FMP)-based derivatization reagents target phenolic and primary amine groups [9]. In this study, the utility of these common derivatization reagents, along with the newly developed reagent 2,4,6-trimethylpyrylium tetrafluoroborate (TMPy) as a monoaromatic hydrocarbon, are evaluated for their utility in detecting catecholamines and amino acids. TMPy can also selectively target primary amine groups and can yield ions that are detectable in positive mode due to the positive ions in its structure. The primary difference between TMPy and other reagents is the size of the molecule (Figure 1). We investigated whether four target samples are capable of being derivatized by several reagents, and subsequently compared ionization efficiency by evaluating signal-to-noise (S/N) ratios.

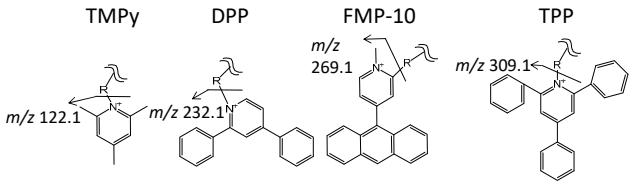

Figure 1. Chemical structure of derivatization reagents and fragmentation of derivatized reagents.

2. Materials and Methods

MALDI TOF-MS

2,4,6-Trimethylpyrylium tetrafluoroborate (TMPy) (Taiyo Nippon Sanso Co., Tokyo, Japan) and triphenyl pyrilium (TPP) (Sigma-Aldrich, St. Louis, MO, USA) were dissolved in 950 µL of 70% methanol alkalified with 50 µL of triethyl amine (TEA) to obtain 4.3 mM derivatization solutions. 2,4-diphenyl-pyranylium tetrafluoroborate (DPP-TFB) (Sigma-Aldrich, USA) was dissolved in 1.0 mL of 75% methanol alkalified with 0.5 µL of TEA to obtain a 4.3 mM derivatization solution. 4-(anthracen-9-yl)-2-fluoro-1-methylpyridin-1-ium iodide (FMP-10) (HTX-imaging, USA) was dissolved in 1.0 mL of 70% acetonitrile to obtain a 4.3 mM derivatization solution. The following four derivatization reagent-labeled catecholamine solutions were prepared: L-DOPA: 10 pmol/µL, dopamine (DA): 10 pmol/µL and norepinephrine (NE): 10 pmol/µL (Sigma-Aldrich, USA) and L-Dopa-(phenyl-d_3) (Taiyo Nippon Sanso Co., Japan): 10 pmol/µL. A 2.5 µL aliquot of each sample solution was mixed with 7.5 µL of each derivatization reagent in a sealed 0.2 mL PCR test tube, respectively. The mixture was then heated at 60 °C for 10 min. A 0.5 µL aliquot of formic acid was then added, and the solution was stored in a tightly sealed container at 4 °C. A suspension containing a 1.0 µL aliquot of derivatization reagent-labeled catecholamine and a-cyano-4-hydroxycinamic acid (CHCA) (10 mg/mL) as an ionization-assisting reagent were placed on a target plate using a pipette. Ionization of standard L-DOPA, DA, NE, and L-Dopa-(phenyl-d_3) were confirmed by MALDI-TOF-MS (rapifleX, Bruker Daltonik GmbH). The analyte surface was irradiated with 1,000 laser shots and TOF spectra were acquired in positive ion detection mode.

3. Results

The detected masses of TMPy-labeled standard DA (*m/z* 258.2), L-DOPA (*m/z* 302.2), L-Dopa-(phenyl-d_3) (*m/z* 305.2), GABA (*m/z* 208.1), and glycine (*m/z* 180.1) increased by 105.0 Da compared with their original masses (MW 153.1, 197.1, 200.1, 103.1, and 75.0) (Figure 2a–e). The detected masses of DPP-labeled standard DA (*m/z* 368.2), L-DOPA (*m/z* 412.2), L-Dopa-(phenyl-d_3) (*m/z* 415.2), GABA (*m/z* 318.2), and glycine (*m/z* 290.1) increased by 215.1 Da compared with their original masses (Figure 2f,j). The detected masses of FMP-10-labeled standard DA (*m/z* 421.2), L-DOPA (*m/z* 465.2), L-Dopa-(phenyl-d_3) (*m/z* 468.2), GABA (*m/z* 371.2), and glycine (*m/z* 343.1) increased by 268.1 Da compared with their original masses (Figure 2k–o). The detected masses of TPP-labeled standard DA (*m/z* 444.2) and GABA (*m/z* 394.1) increased by 291.1 Da compared with their original masses (Figure 2p,s).

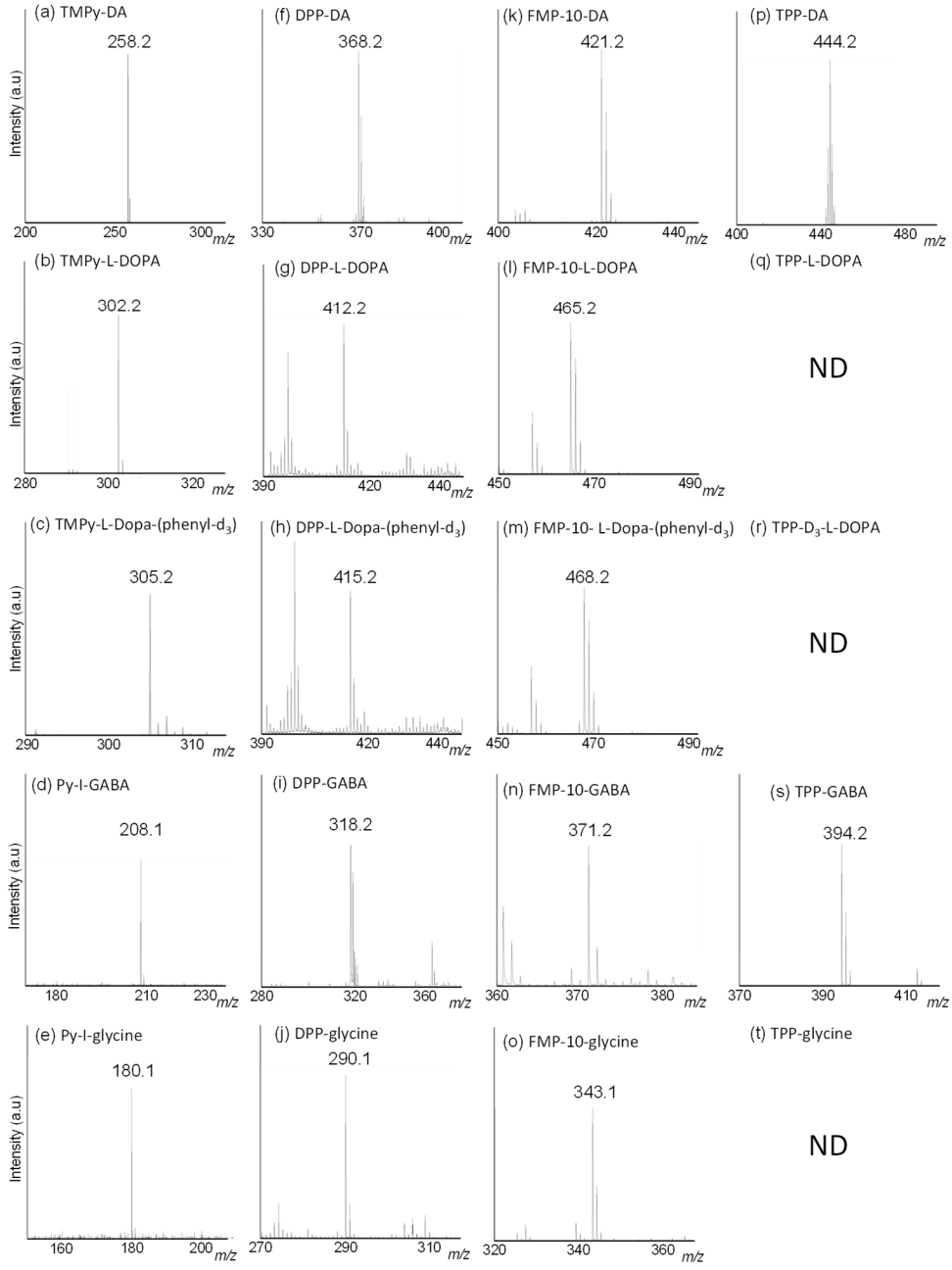

Figure 2. Mass spectra of TMPy-labeled dopamine (DA) (**a**), L-DOPA (**b**), L-Dopa-(phenyl-d_3) (**c**), GABA (**d**), and glycine (**e**). DPP-labeled DA (**f**), L-DOPA (**g**), L-Dopa-(phenyl-d_3) (**h**), GABA (**i**), and glycine (**j**). FMP-10-labeled DA (**k**), L-DOPA (**l**), L-Dopa-(phenyl-d_3) (**m**), GABA n), and glycine (**o**). TPP-labeled DA (**p**), L-DOPA (**q**), L-Dopa-(phenyl-d_3) (**r**), GABA (**s**), and glycine (**t**). ND = not detected.

Theoretically, the signals of TPP-labeled L-DOPA, L-Dopa-(phenyl-d_3), and glycine are *m/z* 488.1, 501.1, and 366.1, respectively. However, these signals were not observed, indicating that TPP did not react with L-DOPA, L-Dopa-(phenyl-d_3), and glycine due to steric hinderance. We confirmed reproducibility six times for the derivatized reaction. When derivatization was not used, dopamine and L-DOPA were detected in negative ion mode, while GABA and glycine were detected in positive ion mode (data not shown). Thus, one of the benefits of using derivatization reagents is that all derivatized target molecules can be detected in positive mode, thereby eliminating the requirement to perform measurements under both polarities. The MS spectra of TMPy-derived targets (Figure 2a–e) showed sharp peaks and low minor peaks compared with those of the other derivatization

reagents. Thus, we evaluated detection efficiency using S/N values. An additional merit of using derivatized reagents is that specific fragmented ions can be detected in tandem MS. Thus, tandem MS confirmed the fragmentation ions of TMPy, DPP, FMP-10, and TPP, respectively. Fragmented ions of the pyridine ring (*m/z* 122.1) moiety from TMPy, 2,4-diphenyl-pyranylium (*m/z* 232.1) moiety from DPP, anthranene-9-yl pyridinium moiety (*m/z* 269.1) from FMP-10 and triphenyl-pyranylium (*m/z* 309.1) moiety from TPP were cleaved and observed from all derived target molecules (Figure 1). The S/N ratios of derivatized signals were improved relative to the non-derivatized targets (Table 1).

Table 1. Comparison of derivatization reagents for reactive efficiency to target molecules.

	Signal-to-Noise Value for Derivatized Standard Catechol Amines				
Target	**Non-Derivatization**	**TMPy**	**DPP**	**FMP-10**	**TPP**
DA	16	187	164	43	785
L-DOPA	13	661	20	49	N.D.
D_3-L-DOPA	11	532	30	61	N.D.
GABA	39	119	710	52	942
Glycine	10	19	10	21	N.D.

Signal-to-Noise value was determined by fragment ion of derivatization reagent, respectively.

4. Discussion

Generally, the S/N values of TMPy were greater than the other derivatized targets. In particular, the S/N ratios were improved more than 10 times for catecholamines such as DA and L-DOPA. For the amino acids, the S/N of GABA was slightly improved (three times) and the S/N of glycine was marginally improved (two times). The modest effect of TMPy on glycine may be due to steric hinderance related to the small structure of the glycine molecule. DPP derivatization resulted in the efficient ionization of DA and GABA, whereas L-DOPA and glycine were only marginally ionized. FMP-10 derivatization also produced a general increase in the ionization of all targets. TPP derivatization resulted in the most efficient ionization of DA and GABA. On the other hand, other targets were not detected following TPP derivatization which may be due to failure of the derivatization reaction. Overall, TMPy can be utilized to derive target molecules such as catecholamines and amino acids. Notably, the S/N value of TMPy-labeled L-DOPA was exceptionally improved relative to the other reagents. In L-DOPA, the position of the amino group adjoins a carboxy group, which renders it unable to react with polyaromatic derivatization reagents. The structures of the derivatization reagents used herein contain polyaromatic moieties, except for TMPy, which is considerably smaller than the other derivatization reagents (Figure 2). Thus, TMPy is not sterically hindered and easily reacts with the primary amine group of L-DOPA. Glycine could not be derivatized effectively with any of the reagents. Glycine is constructed by an amino group and carboxy group through a single carbon. Thus, the degree of freedom of the amino group in glycine is too small to react with derivatization reagents. In addition, originally, the reaction mechanism was different between pyrylium salts (TMPy, DPP, and TPP) an FMP-10. TMPy, DPP, and TPP react with the primary amino group. On the other hand, FMP-10 reacts with the hydroxy group. As a result, comparatively, TMPy improved the S/N ratio of glycine more than the other reagents. TMPy is suitable in terms of reproducibility and cost in practical use.

5. Conclusions

In conclusion, using derivatization reagents to ionize catecholamines and amino acids improved ionization efficiency and furthered our understanding of which derivatization reagents are optimal for these target molecules.

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