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A highly sensitive isotope-coded derivatization method and its application for the mass spectrometric analysis of analytes containing the carboxyl group

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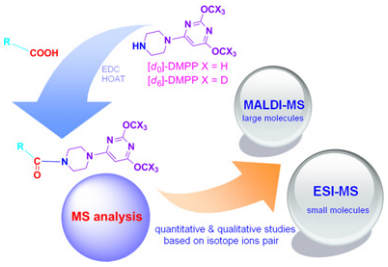
HIGHLIGHTS

- A novel MS-based isotope reagent DMPP was developed for the derivatization toward carboxyl group.
- The simple and rapid labeling reaction was carried out under mild conditions with high specificity.
- The LODs of analytes containing the carboxyl and excess labeling reagent were greatly reduced.
- Enhanced accuracy in peptide sequencing was achieved by the introduction of isotope-coded DMPP.

GRAPHICAL ABSTRACT



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ABSTRACT

A novel isotope labeling reagent d_0-/d_6-2, 4-dimethoxy-6-piperazin-1-yl pyrimidine (DMPP) has been developed for derivatization toward the carboxyl group based on carbodiimide chemistry for mass spectrometry (MS) analysis. The strengths of this derivatization strategy involve fast labeling (15 s), low chemical background and general access to most carboxylic analytes. This has been demonstrated using a series of compounds containing carboxylic acids, including peptides and proteins. To enhance the MS response of the derivatized analytes, the design of DMPP has been based on integration of the theoretical consideration of high gas-phase hydrogenation capacity and hydrophobicity. In addition, the high abundance product ions at m/z 225 and m/z 231 from d_0-/d_6-DMPP labeled carboxylic acids indicate high efficiency of the gas-phase cleavage induced by the labeling reagent. Quantitative determination of these ions can also be used in single reaction monitoring to achieve extremely high sensitivity toward the target analytes. This has subsequently been used to determine trace free fatty acids in human urine. Furthermore, the DMPP labeled peptides also provide additional sequence information in MALDI–MS/MS because of the formation of sequence-related isotope fragment ions. This DMPP-oriented labeling technique is expected to be a promising tool for the MS detection of many varieties of compounds containing carboxyl groups.

1. Introduction

Carboxylic compounds form a large group of biologically significant molecules [1,2], including small molecular organic acids, fatty acids, amino acids, peptides and proteins, which have proved to be of crucial significance to a number of disorders and diseases [3–7]. Thus, sensitive and accurate detection of these compounds

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provides a great opportunity for monitoring the progress of some diseases. Mass spectrometry (MS) is treated as a powerful tool for the analysis of numerous analytes with high sensitivity and specificity. The technique offers alternative ways of ionization, including electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI), and electron ionization (EI). However, carboxylic compounds tend to show a low ESI–MS response in positive ion mode, due to the poor gas-phase proton affinity of the carboxyl group. In some cases, it is still difficult to satisfy the normal requirements for trace analysis despite the relatively high ESI–MS signal of carboxylic compounds in negative ion mode [8–11]. Carboxylic compounds also have a relatively low vapor pressure because of the interaction of active hydrogen bonds, which is a major drawback for direct GC–EI–MS analysis. In addition, the lack of characteristic product ions [8,9] means that the determination of many fatty acids (FAs) is easily affected by the matrix effect, thus showing weak detection specificity. It is therefore of great importance to develop an effective method to enhance the effectiveness of MS for the analysis of carboxylic compounds.

Chemical derivatization [12–15] plays a crucial role in the use of MS-based detection in solving quantitative and qualitative problems with various compounds. Attention has been paid to new labeling methods for the carboxyl group in previous studies [16–29]. For example, since they form a very important component of carboxylic compounds, FAs have been derivatized, using a variety of techniques, to improve the analytical performance of MS [20–29]. Traditionally, the FAs were transformed by methylation, then combining GC–MS analysis [25–29] but this involved tedious sample pretreatment and gave limited sensitivity. In recent years, better labeling methods have been applied for FA detection using ESI–MS [20–23]. For instance, Franke et al. derivatized FAs using a straightforward one-pot two-step reaction procedure, which gave good sensitivity in obitrap MS [21]. Regnier and co-workers attached a quaternary amine to the analytes, which enabled the identification and quantification of FAs in positive ion mode [22]. Unfortunately, the methods still showed limited efficiency in signal enhancement and required a time-consuming labeling procedure.

The detection of larger compounds containing carboxyl groups, such as peptides and proteins, can be achieved by chemical labeling, which also serves as a versatile tool since it can also be used to tag a variety of reactive groups [30–43]. However, previous studies have mainly concentrated on the labeling of nucleophilic groups, such as the amino [30–34] and mercapto groups [34–37]. There have been a few reports describing isotope-coded methods for the derivatization of electron-deficient groups, such as the carboxyl group [38–43].

To address the challenges described above, here, taking into consideration of the gas-phase hydrogenation capacity and hydrophobicity, an isotope-coded labeling reagent d_0-/d_6-2,4-dimethoxy-6-piperazin-1-yl pyrimidine (DMPP) was designed for carboxyl group derivatization and examined with a series of FAs, organic acids, peptides, and proteins. The potential of the developed method was further demonstrated by the analysis of free FAs in urine samples. Moreover, the power of DMPP for promoting the accuracy of peptide sequencing was also fully investigated.

2. Experimental

2.1. Chemicals and reagents

Tert-butyl-1-piperazinecarboxylate, 2,4,6-trichloropyrimidine, lysozyme, β-casein and Bovine serum albumin (BSA) were purchased from Sigma–Aldrich (St. Louis, MO). HPLC grade acetonitrile (ACN), methanol, ethanol and dimethylformamide (DMF) were obtained from Merck (Darmstadt, Germany). All the fatty acids including the other six organic carboxylic acids (≥98%) were purchased from Shanghai Aladdin reagent Co., Ltd. (shown in supplementary data Table S1). Methanol-d3 was a product of Cambridge Isotope Laboratories (Andover, MA). Deionized water was produced by a Direct-Q water purification system (Millipore, ELPaso, TX). Other chemicals were all of analytical grade and used without further purification. 732-strong-acid cation exchange resin (gel-type) was obtained from Shanghai Resin Factory Co., Ltd.

2.2. Computational methods

Hartree–Fock 6-31G* (equilibrium geometry) in Spartan 06 software was employed to optimize the geometries of the DMPP-derivatives with and without protons, and used to calculate their corresponding energies. For each DMPP-derivative, the average energy differences between the state of each protonated site and unprotonated state plus proton ($E = 0$) was considered as the gas-phase hydrogenation capacity. In addition, the log P value of each compound was calculated using ACD/labs 6.0.

2.3. Synthesis of DMPP

The procedure for synthesis of DMPP is provided in supplementary data Section 1.

2.4. Derivatization procedure

Solutions of [d_0]-/[d_6]-DMPP (30 nmol μL^{-1}) in methanol (8 μL) and HOAt (15 nmol μL^{-1}) in methanol (3 μL) and 70 μL methanol were sequentially added to a solution of FAs (200 pmol μL^{-1}) in methanol (5 μL). EDC (15 nmol μL^{-1}) in methanol (7 μL) was then added to the solution to initiate the reaction. After mixing for 15 s at room temperature, the solvent was immediately removed by evaporation with nitrogen to terminate the derivatization. The residue was redissolved in 20 μL solution of 50% ACN in water containing 0.1% TFA. An equal volume of resin was then added to the solution, with slight agitation for 10 s, to specifically absorb any excess labeling reagent. The standard pretreatment of the resin is described in supplementary data Section 2. The resulting resin was further washed with 600 μL ethanol, together with 5 min ultrasound (100 W, 40 kHz), three times at room temperature. For further ESI–MS experiments the combined washing solution was dried completely using nitrogen for longer term storage at −4 °C until redissolved in 50% ACN in water. The derivatization procedure of other analytes is shown in supplementary data Section 3.

2.5. LC–ESI–MS/MS

The ESI–MS^1 experiments were performed using a system consisting of a HPLC system (1260 Series LC, Agilent) and an ESI source triple-quadrupole time-of flight (Q-TOF) mass spectrometer (G6500, Agilent). The system was controlled by Q-TOF MassHunter software (B.04.00 Agilent). All of the SIM and SRM experiments were performed with an ESI source triple-quadrupole mass spectrometer (G6410A, Agilent) that was controlled by QQQ MassHunter software (B.01.03 Agilent). LC/ESI–MS chromatograms were acquired in positive/negative ion mode with the capillary voltage set at 4000 V and fragmentor at 125 V while the drying gas flow was maintained at 8 L min^{-1} and the gas temperature at 350 °C. A delta EMV was set at 400. The precursor ion was subjected to collision induced dissociation (CID) by nitrogen with optimized collision voltage. The injection volume was set at 10 μL. All of the 18 FA separations were performed with deionized water (solvent A) and ACN (solvent B) using the column (Agilent Zorbax SB-C8, 2.1 mm × 100 mm, 1.8 μm, 80 Å, USA). The elution condition (0.3 mL min^{-1}) consisted of increasing solvent B (ACN) from 75% to