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Relative quantification of enantiomers of chiral amines by high-throughput LC–ESI-MS/MS using isotopic variants of light and heavy L-pyroglutamic acids as the derivatization reagents

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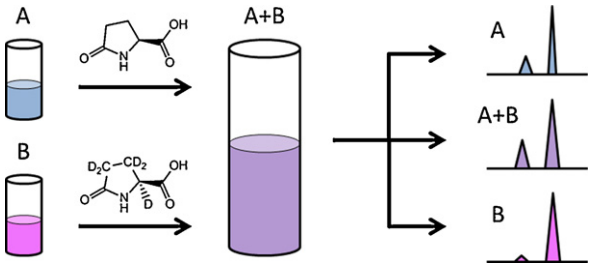
HIGHLIGHTS

- Development of chiral labeling reagent for a pair of amine enantiomers.
- High-throughput analysis of diastereomers by UPLC–ESI-MS/MS.
- Highly efficient separation and detection of the enantiomers.
- Differential analysis of enantiomer ratio in different sample groups using light and heavy labeling reagents.

GRAPHICAL ABSTRACT



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ABSTRACT

L-Pyroglutamic acid (L-PGA) was evaluated as a chiral labeling reagent for the enantioseparation of chiral amines in terms of separation efficiency by reversed-phase chromatography and detection sensitivity by ESI-MS/MS. Several amines and amino acid methyl esters were used as typical representatives of the chiral amines. Both enantiomers of the chiral amines were easily labeled with L-PGAs at room temperature for 60 min in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide and 1-hydroxy-1*H*-benzotriazole as the activation reagents. The resulting diastereomers were completely separated by reversed-phase chromatography using the small particle (1.7 μm) ODS column (Rs = 1.6–6.8). A highly sensitive detection at a low-fmol level (1–4 fmol) was also obtained from the multiple reaction monitoring (MRM) chromatograms. Therefore, a high-throughput determination was achieved by the present UPLC–ESI-MS/MS method.

An isotope labeling strategy using light and heavy L-PGAs for the differential analysis of chiral amines in different sample groups was also proposed in this paper. As a model study, the differential analysis of the R and S ratio of 1-phenylethylamine (PEA) was performed according to the proposed procedure using light and heavy reagents, i.e., L-PGA and L-PGA-d_5. The R/S ratio of PEA, spiked at the different concentrations in rat plasma, was almost similar to the theoretical values. Consequently, the proposed strategy using light and heavy chiral labeling reagents seems to be applicable for the differential analysis of chiral amine enantiomers in different sample groups, such as healthy persons and disease patients.

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1. Introduction

Mass spectrometry (MS) has become a popular analytical technique because of its high sensitivity and specificity compared to other detection techniques such as UV and FL. Various applications,

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e.g., impurity analysis and metabolite identification, have been carried out by the LC–MS method [1]. Besides, the isotope dilution method has been widely adopted for the quantitative analysis of target molecules, such as drug metabolites [2]. The use of an isotope internal standard for the molecule of interest provides a high accuracy and precision for the quantitative determination by overcoming matrix effects such as ion suppression [3]. However, the use of individual isotope standards for all the target molecules is not practical. An alternative approach is to introduce a mass tag to a series of molecules in the sample by chemical reaction [4–7]. By mixing of the labeled sample and the mass-different labeled standards, the identification and quantification of molecules can be attained. This method is also applicable to the differential analysis of two sample groups such as healthy persons and diseased patients [8,9]. The strategy could be adopted for the relative quantification of enantiomers of chiral molecules in two sample groups.

The separation of a pair of enantiomers of a chiral molecule is generally difficult using a conventional ODS column without chiral recognition. In such a situation, the chemical derivatization technique is sometimes adopted to improve the separation efficiency of the analytes as the diastereomers. The derivatization usually reduces the polarity of the target molecule, which prevents co-elution with high polar endogenous materials in the complex matrices. Furthermore, the derivatization increases the molecular mass of the relatively small molecules. As a result, the interference of endogenous materials is prevented by increasing the retention times during the reversed-phase chromatographic run. Many derivatization reagents [10–19], such as 1-pyrenesulfonyl chloride and 3-aminopyridyl-*N*-hydroxysuccinimidyl carbamate, can successfully determine the target molecules possessing various functional groups by LC–MS. However, the development of chiral derivatization reagents for highly sensitive MS detection is still in progress. Based on these observations, we are studying the development of novel chiral derivatization reagents for various functional groups such as the amine and carboxyl. Several chiral derivatization reagents for the carboxylic acid moiety have been developed in our laboratory [20,21].

Metabolomics is a rapidly evolving field for studying biological systems and discovering potential disease markers [22]. The metabolome analysis with an adequate sensitivity and specificity is essential in defining the metabolomics. However, only a fraction of metabolites are currently analyzed by LC–MS due to technical limitations. The detection of all metabolites at once is very challenging due to the significant diversity in the physicochemical properties of the metabolites. One strategy to solve the diversity issue is to fractionate the metabolome into several groups according to the chemical structure, hydrophobicity, etc. We are currently pursuing an analytical strategy of selectively labeled molecules containing a certain chemical moiety, followed by LC–MS/MS analysis for the metabolite identification and quantification.

Amines are the major components of metabolites in a metabolome, the same as carboxylic acids [23,24]. Quantitative profiling of amine-containing metabolites in complex biological samples is important for biological studies and disease biomarker discovery using metabolomics. For example, amino acids [25,26] and polyamines [27,28] are common biomarkers for human physiological processes. Recently, naturally occurring D-amino acids (D-AA), such as D-Ser and D-Asp, were found in a wide variety of living organisms both in their free form and as the isomeric residues in many proteins [29]. The D-AA is now considered as novel physiologically active substances and biomarkers even in mammals. However, a chiral metabolomics study, except for D-AA, is very limited due to technical difficulties. Thus, the determination of the enantiomers of chiral molecules is an important issue for the metabolome study.

In this study, L-pyroglutamic acid (L-PGA) was evaluated as the chiral labeling reagent for primary and secondary amines in terms of separation efficiency by reversed-phase chromatography and detection sensitivity by ESI-MS/MS. Several chiral amines and amino acid methyl esters were used for the evaluation as typical representatives of the chiral amines. We also report an isotope labeling strategy using light and heavy L-PGAs for the chiral amine moiety in combination with UPLC–ESI-MS/MS. L-PGA and L-PGA-d_5 were adopted for the labeling of two different sample groups. The differential analysis of the enantiomers of 1-phenylethylamine (PEA) using light and heavy L-PGAs is demonstrated as a model of the chiral biomarker in patients.

2. Experimental

2.1. Materials and chemicals

L-Pyroglutamic acid (L-PGA), (S)(−)- and (R)(+)-1-phenylethylamine (SR-PEA), (S)(−)-(1-naphthyl)ethylamine (S-NEA), D- and L-valine methyl ester hydrochloride (DL-ValME), D- and L-phenylalanine methyl ester hydrochloride (DL-PheME), and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) were purchased from Tokyo Kasei (Tokyo, Japan). (S)(+)- and (R)(−)-1-aminoindan (SR-AI) and (R)(+)-(1-naphthyl)ethylamine (R-NEA) were obtained from Sigma–Aldrich (St. Louis, MO, USA). 1-Hydroxy-1*H*-benzotriazole monohydrate (HOBt; Dojindo Laboratory, Kumamoto, Japan) and L-2-pyrrolidinone-3,3,4,4,5-d_5-5-carboxylic acid (L-PGA-d_5; C/D/N Isotopes, Quebec, Canada) were used. Acetonitrile (CH_3CN) and formic acid (HCOOH) of LC–MS grade were purchased from Kanto Chemicals (Tokyo, Japan). Deionized and distilled water (H_2O) was used throughout the study (Aquarius PWU-200 automatic water distillation apparatus, Advantec, Tokyo, Japan). All other reagents and solvents were of analytical grade.

A 10 mM solution of amines (SR-PEA, SR-AI, SR-NEA, DL-ValME, DL-PheME) in acetonitrile was prepared as the stock solution. The working solutions were prepared by sequential dilutions with acetonitrile.

2.2. UPLC–ESI-MS/MS

The UPLC–ESI-MS/MS analysis was performed using a Xevo^{TM} TQ-S triple quadrupole mass spectrometer (Waters, Milford, MA) connected to an ACQUITY ultraperformance liquid chromatograph (UPLC I-class, Waters). An ACQUITY UPLC BEH C18 column (1.7 μm, 100 mm × 2.1 mm i.d.; Waters) was used at the flow rate of 0.4 mL min^{-1} and 40 °C. The diastereomers derived from L-PGA or L-PGA-d_5 were analyzed by UPLC–ESI-MS/MS in the positive-ion mode unless otherwise stated. The separation and detection conditions were as follows: mobile phase, acetonitrile–water mixture containing 0.1% (v/v) formic acid; capillary voltage, 3.00 kV; cone voltage, 50 V; desolvation gas flow, 1000 L h^{-1}; cone gas flow, 150 L h^{-1}; nebulizer gas flow, 7.0 L h^{-1}; collision gas flow, 0.15 mL min^{-1}; collision energy, 20 eV; collision cell exit potential, 5 V; desolvation temp, 500 °C. The analytical software (MassLynx, version 4.1) was used for the system control and data processing.

2.3. Recommended reaction conditions for chiral amines with L-PGA or L-PGA-d_5

The freshly prepared solutions of EDC (10 mM) in acetonitrile (100 μL), HOBt (10 mM) in acetonitrile (100 μL), and L-PGA or L-PGA-d_5 (1 mM) in acetonitrile (100 μL) were vigorously mixed with chiral amines in acetonitrile (100 μL). The reaction mixtures were stored at the room temperature for 60 min. After removal of the

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