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Rapid tin-mediated access to a lysophosphatidylethanolamine (LPE) library: Application to positional LC/MS analysis for hepatic LPEs in non-alcoholic steatohepatitis model mice

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ABSTRACT

Even though lysophospholipids have attracted much interest in recent years on account of their unique bioactivity, research related to lysophospholipids is usually hampered by problems associated with standard sample preparation and discrimination of regioisomers. Herein, we demonstrate a quick tin-chemistry-based synthetic route to lysophosphatidylethanolamines (LPEs) and its application in the positional analysis of hepatic LPEs in non-alcoholic steatohepatitis (NASH) model mice. We found that the preference of hepatic LPE regioisomer largely depends on the unsaturation of acyl chain in both control and NASH model mice. In addition, hepatic C18:2-LPE and C20:5-LPE levels were significantly lower in the NASH model mice than those in the control. The LC/MS technique based on the library of LPE regioisomers allows an accurate observation of hepatic LPE metabolism and might provide useful information to elucidate yet ambiguous pathogenesis of NASH.

1. Introduction

Phospholipids (PLs) are fundamental biomolecules that consist of a polar head group, a glycerol backbone, and fatty acids. Their importance in membrane assembly and molecular signaling as e.g. lipid mediators has been well documented (Aoki et al., 2015). Lysophospholipids (LPLs) represent a PL subclass and contain only one fatty acid on the glycerol backbone. Recent studies revealed that these unique PLs, especially lysophosphatidic acids (LPAs), contribute to an array of biological responses such as platelet aggregation, smooth muscle contraction, cell proliferation, and cell migration (Mills and Moolenaar, 2003) via binding to the corresponding receptors. Compared to LPAs or lysophosphatidylserines (LPSs) (Makide et al., 2014) the functions of lysophosphatidylethanolamines (LPEs) still remain undiscovered, which is mostly due to the limited commercial availability of LPEs.

Another frequently encountered problem in LPE and LPL research is 1,2-*O*-acyl migration (Fig. 1) (Plückthun and Dennis, 1982; Liu et al., 2014), as this equilibrium furnishes 1-LPE and 2-LPE regioisomers, which exhibit different physico-chemical (Okudaira et al., 2014) and biological (Xu et al., 2005) properties. Unfortunately, suppression of this migration still remains difficult, and accordingly the precise determination of the regioisomer ratio prior to a use of the mixture is of paramount importance. NMR spectroscopy can be a powerful tool for this purpose (Medina and Sacchi, 1994), even though it requires relatively large sample quantities and is thus not ideally suited for the analysis of biological samples. Conversely, mass spectrometry (MS) is more advantageous in terms of its detection limit, and has already been applied to the characterization of various LPLs (Hsu and Turk, 2009). Nevertheless, their complete identification still requires the use of standard samples.

Herein, we report a new short synthetic route to LPEs, which is suitable for the construction of an LPE library. Moreover, we applied this synthetic library into the positional analysis of LPEs in murine liver tissues by using LC–MS platform. In this study, we employed a non-alcoholic steatohepatitis (NASH) model mice developed before (Yimin et al., 2011), and compared the hepatic

Abbreviations: Tr, triphenylmethyl; DPPE, 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine; PE, phosphatidylethanolamine; LPL, lysophospholipid; LPE, lysophosphatidylethanolamine; LPC, lysophosphatidylcholine; LPI, lysophosphatidylinositol; LPA, lysophosphatidic acid; LPS, lysophosphatidylserine; PL, phospholipid; HG, head group; iPrOH, isopropanol; DMF, dimethylformamide; Bu_2SnO, dibutyltinoxide; Teoc, 2-(trimethylsilyl)ethoxycarbonyl; TFA, trifluoroacetic acid; FA, fatty acid; PUFA, polyunsaturated fatty acid; NASH, non-alcoholic steatohepatitis; NAFLD, non-alcoholic fatty liver disease.

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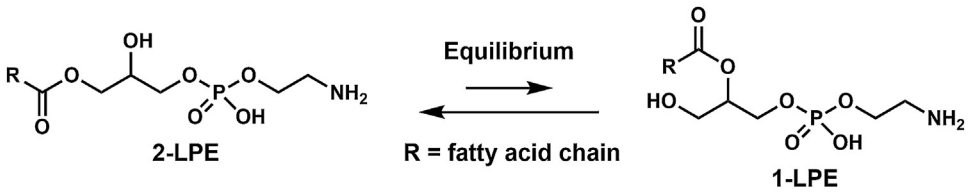

Fig. 1. 1,2-*O*-acyl migration in LPEs.

levels of LPE regioisomers between the NASH model and the control mice. NASH is a progressive hepatic disease developed in association with fatty liver and multiple risk factors such as oxidative stress and proinflammatory factors. The pathogenesis of NASH is yet ambiguous, and therefore, no specific diagnosis/treatment/prevention method is available. This study aims to obtain useful information for understanding of the pathogenic processes of NASH from the viewpoint of hepatic LPE metabolism.

2. Experimental

2.1. Materials and methods

Unless otherwise noted, all reagents were purchased from Wako Pure Chemical Industries Ltd. (Tokyo, Japan) or Sigma-Aldrich. TLC was performed on pre-coated plates (20 cm × 20 cm; layer thickness: 0.25 mm; silica gel 60F_{254}; Merck), and spots were visualized by spraying with an ethanol solution of Ninhydrin, followed by heating to 250 °C for ~0.5 min or by exposure to UV light (λ = 254 nm) when applicable. Column chromatography on silica gel (N60; spherical type; particle size: 40–50 μm; Kanto Chemical Industry) was carried out using the specified solvent systems, whereby the solvent ratio is given in v/v. ^{1}H and ^{13}C NMR spectra were recorded on a 400 MHz JNM-ECP400 spectrometer (JEOL, Japan; ^{1}H: 400 MHz, ^{13}C: 100 MHz), and multiplicities are given as singlet (s), broad (br), doublet (d), doublet of doublets (dd), triplet of doublets (td), triplet (t), quintet (q), or multiplet (m). Chemical shifts are expressed in ppm and referenced to TMS (δ_H 0.00), $CHCl_3$ (δ_H 7.26), CH_3OD (δ_H 3.31), $CDCl_3$ (δ_C 77.16), or CD_3OD (δ_C 49.00) as the internal standard (Fulmer et al., 2010). Assignments in the ^{1}H NMR spectra were made by first-order analysis using the ACD/NMR processing software (Advanced Chemistry Development, Inc.). High-resolution electrospray ionization mass spectra (HR-ESI-MS) and liquid chromatography mass spectra (LC–MS) were recorded on an LTQ Orbitrap XL (Thermo Fisher Scientific), equipped with a Shimadzu Prominence HPLC system.

2.2. Liver tissues and lipid extraction

Murine liver tissues were prepared as we reported previously (Yimin et al., 2011). Briefly, NASH model mice were produced by the combination of high-fat diet for 23 weeks and several intravenous injections of oxidized low-density lipoproteins. This model shows histhopathological and metabolic features similar to human NASH (Yimin et al., 2011). Mice fed a regular diet were used for control study. Hepatic lipids were extracted from a liver tissue (~50 mg wet weight) using a conventional Folch method (Folch et al., 1957). Animal experiments were conducted according to the Regulations for the Care and Use of Laboratory Animals of Hokkaido University. Our experimental protocol was approved by the Institutional Animal Care and Use Committee (IACUC) at Hokkaido University (Approval No. 10-0028).

2.3. LC-ESI-MS/MS conditions and procedures

LPE regioisomers were separated on an HPLC with an Atlantis T3 column (3 μm × 2.1 mm × 150 mm; Waters) using an aqueous AcOH (0.1%)/acetonitrile gradient system (flow rate: 0.3 mL min^{-1}; for details, see: Supplementary information). The following ESI conditions were applied: capillary voltage = 4000 V, desolvation temperature = 350 °C, sheath gas = 50 psi, and auxiliary gas = 10 psi. LPE precursor ions and product ions were detected in positive ion mode; for LPE precursor ions: FTMS (resolution: 60,000); for product ions: ITMS (CID normalized collision energy = 35 V). Obtained data were analyzed using the Qual browser (Thermo Fisher Scientific). 10 μL of hepatic lipid extracts was injected, and extracted ion chromatogram (EIC) was obtained on the basis of theoretical molecular weight (10 ppm error range) for each LPEs. Regioisomer ratios were calculated based on integrated peak areas. All assays were done in five replications: livers from five different NASH model and control mice were analyzed.

2.4. Data processing

Relative intensity was calculated by integrated peak area over liver weight. Statistical differences were analyzed by two-tailed Mann-Whitney *U* test (Prism 6, GraphPad Software Inc.) and a value of $P < 0.05$ was regarded as significant.

3. Results and discussion

3.1. Chemical synthesis of an LPE library

Although the chemical synthesis of several LPEs has already been reported (D'Arrigo, 2010), most of these examples represent a 'total synthesis' approach. Even though such a strategy is undoubtedly useful, it is not suitable for the construction of a library, as it is simply too time consuming. We envisaged that a semi-synthetic approach should be more favorable for the construction of such a library, as it may potentially require significantly less steps. It should also be noted here that the chemical semi-synthesis of LPEs still remains unexplored.

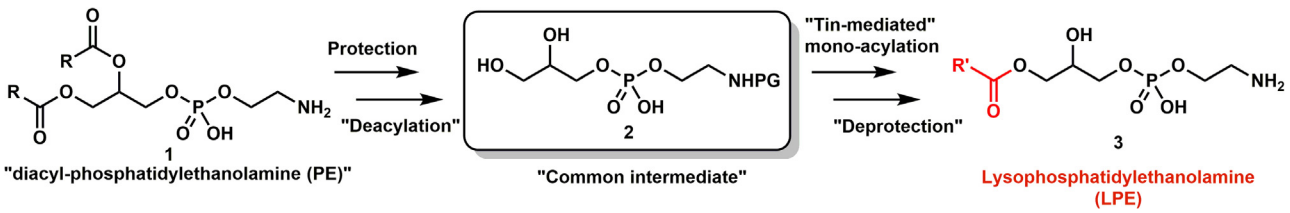

Fig. 2. Synthetic strategy towards LPEs in this study; R, R' = fatty acid chains; PG = protecting group.