Chemistry and Physics of Lipids 216 (2018) 9–16

Contents lists available at ScienceDirect

Chemistry and Physics of Lipids

journal homepage: www.elsevier.com/locate/chemphyslip

Examining the effect of regioisomerism on the physico-chemical properties of lysophosphatidylethanolamine-containing liposomes using fluoro probes

Yusuke Yamamoto[1], Takayuki Furukawa[1], Seiji Takeda, Hiroyuki Kashida, Hitoshi Chiba, Shu-Ping Hui*

Graduate School of Health Science, Hokkaido University, North 12, West 5, Kita-ku, Sapporo, 060-0812 Japan

ARTICLE INFO

Keywords:
Lysophosphatidylethanolamine
Acyl migration
Fluoro analogue
Chemical synthesis
Phase-transition temperature

ABSTRACT

Lysophospholipids (LysoPLs) receive steadily increasing attention in the area of lipid chemistry and biology. However, the physico-chemical properties of individual LysoPL regioisomers have not yet been investigated. Herein, we report the synthesis of fluoro analogues of lysophosphatidylethanolamines (LPEs) and examine the physico-chemical properties of the LPE regioisomers using chemically synthesized fluoro probes.

1. Introduction

Lysophospholipids (LysoPLs) represent a subclass of phospholipids (PLs) and contain only one fatty acid on the glycerol backbone. LysoPLs have been attracting much interest in recent years on account of their unique bioactivity (Kihara et al., 2015; Makide et al., 2009; Taniguchi et al., 2017), e.g. in signal transduction, platelet aggregation, cell proliferation, and cell migration. However, the impact of LysoPL regioisomerism (e.g. 1-acyl- vs. 2-acyl-LysoPL) on their physico-chemical properties remains to be determined. This dearth should be ascribed to the fact that native LysoPL regioisomers are in equilibrium with each other via acyl migration (Plückthun et al., 1985; Liu et al., 2014; Stafford and Dennis, 1987), which hampers an investigation of their individual properties. (Fig. 1).

In this study, we focused on lysophosphatidylethanolamines (LPEs) as representative LysoPLs, given that we have previously reported that the thermodynamically disfavored 2-acyl-1-lysoPE isomer, which bears a PUFA chain, is preferred in murine liver tissue and shows a significant decrease in NASH model mice compared to control mice (Furukawa et al., 2016). Moreover, Ekroos has demonstrated that the ratio of LPE regioisomers differs substantially in skin and plasma (Koistinen et al., 2015), while Aoki has reported similar results in the context of comprehensive lipid analysis (Makide et al., 2014). In their entirety, these reports suggest that the LPE regioisomers may play different roles in our body. Even so, LPE regioisomers have not yet been investigated systematically, which stands in sharp contrast to other LysoPL classes (Kihara et al., 2015). In order to obtain a better understanding of the regioisomerism and its effects on LPEs, we employed fluorine substitution (Fig. 1), which is a widely accepted strategy in drug development and diagnostic technology. The reasons for this substitution are based on some similar chemical properties between the hydroxyl group and the fluorine atom: i) both groups are electron-withdrawing groups, ii) both groups can contribute to hydrogen bonding, and iii) both groups share a similar ionic radius. Furthermore, this methodology represents an emerging approach in LysoPL research on e.g. lysophosphatidic acid (, 2003a,b, Xu and Prestwich, 2002) and lysophosphatidylserine (Ikubo et al., 2015) to eliminate the influence of acyl migration. Unfortunately, authentic protocols towards fluorinated lysophosphatidic acid or lysophosphatidylserine are relatively laborious. Moreover, these synthetic strategies may require some modifications, depending on the PL head group (D'Arrigo and Servi, 2010). Thus, less laborious synthetic protocols to LPEs should represent a desirable research target. Herein, we describe a short synthesis of racemic 1-deoxy-1-fluoro- and 2-deoxy-2-fluoro-LPEs.

Apart from attracting significant attention in biology, LysoPLs have garnered interest from physical chemists. The physico-chemical property of LysoPLs should be helpful for the understanding of the lipid behavior in membranes, cells, or the body (Stafford et al., 1989; Arouri

Abbreviations: DAST, *N*,*N*-diethylaminosulfur trifluoride; DCM, dichloromethane; DMAP, *N*,*N*-dimethylaminopyridine; DMF, *N*,*N*-dimethylformamide; EDC, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride; EPA, eicosapentaenoic acid; LysoPLs, lysophospholipids; LPE, lysophosphatidylethanolamine; NASH, non-alcoholic steatohepatitis; PLs, phospholipids; PUFA, polyunsaturated fatty acid; TBDPS, *tert*-butyldiphenylsilyl; TFA, trifluoroacetic acid; THF, tetrahydrofuran

* Corresponding author.
E-mail address: keino@hs.hokudai.ac.jp (S.-P. Hui).
[1] Contributed equally to this work.

https://doi.org/10.1016/j.chemphyslip.2018.08.001
Received 16 April 2018; Received in revised form 2 August 2018; Accepted 2 August 2018
Available online 03 August 2018
0009-3084/

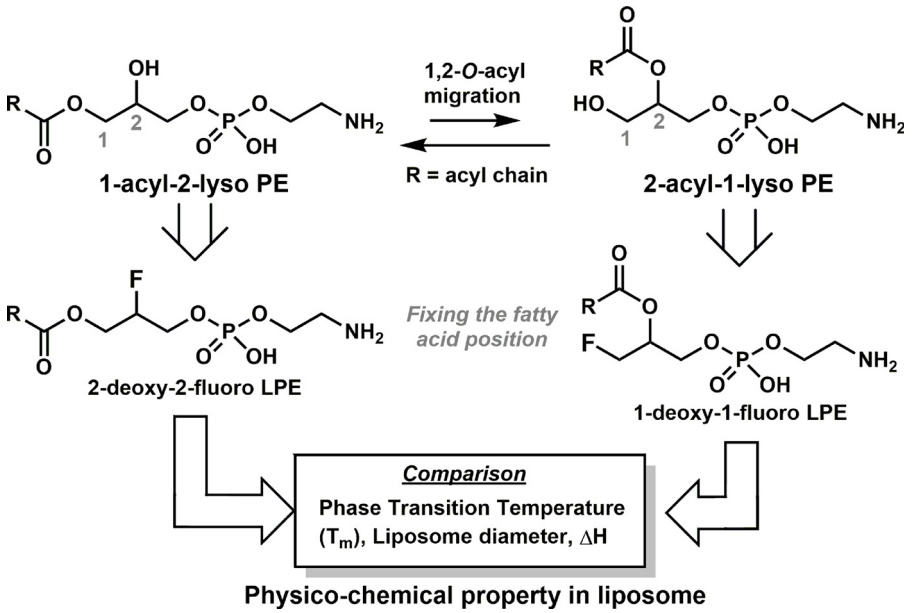

Fig. 1. Schematic illustration of the scope of this study.

and Mouritsen, 2013; Marsh, 2013). Moreover, the liposome architecture is considered to be promising in the context of drug-delivery systems and LysoPLs have already been used to modify the membrane fluidity (Tilcock et al., 1986; Slater et al., 1989). So far, the effect of the head group, pH value, and ion strength have been investigated. However, studies focusing on the influence of the esterified position remain elusive. Herein, we examine the physico-chemical properties of the regioisomers of fluoro-substituted LPEs. Specifically, we examined three parameters in this study; i) the phase-transition temperature (T_m); ii) the van't Hoff enthalpy (ΔH_{vH}) and calorimetric enthalpy (ΔH) obtained from differential scanning calorimetry (DSC), which represent useful parameters to understand thermal or polymorphic behavior of lipid membranes (Demetzos, 2008; McElhaney, 1982); iii) the liposome diameter by dynamic light scattering (DLS).

2. Materials and methods

2.1. General information

Unless otherwise noted, all reagents were purchased from Wako Pure Chemical, Tokyo Chemical Industry, or Sigma-Aldrich. TLC was performed on Merck pre-coated plates (20 cm × 20 cm; layer thickness: 0.25 mm; Silica Gel $^{60}F_{254}$); spots were visualized by an ethanol solution of phosphomolybdic acid or aqueous solution of phosphomolybdic acid, conc.H_2SO_4 and phosphoric acid with heating at 250 °C for ~1/2 min or by UV light (254 nm) where applicable. Column chromatography was performed on silica gel N60 (spherical type; particle size: 40–50 μm; Kanto Chemical Industry) with the solvent systems specified, whereby the ratio of solvent systems is given in v/v. ^1H and ^{13}C NMR spectra were recorded on a JNM-ECP400 (JEOL, Japan; ^1H: 400 MHz, ^{13}C: 100 MHz, ^{19}F: 376 MHz, ^{31}P: 162 MHz) spectrometer. Chemical shifts are given in ppm and referenced to internal 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) (Fulmer et al., 2010). TFA was used as an external standard for the ^{19}F NMR measurements (δ_F -78.50), while trimethylphosphine was used as an external standard for the ^{31}P NMR measurements (δ_P 36.20). Assignments in the ^1H NMR spectra were made based on a first-order analysis of the spectra using the ACD/NMR processor software (Advanced Chemistry Development, Inc.). High-resolution electrospray ionization mass spectra (ESI-HRMS) were recorded on a LTQ Orbitrap XL (Thermo Fisher Scientific), while low-resolution electrospray ionization mass spectra (ESI-LRMS) were recorded on a LXQ (Thermo Fisher Scientific).

2.2. Preparation of liposomes

Stock solution (chloroform: methanol = 3:1, v/v) of each fluorinated LPEs (2 mg/mL) and chloroform solution of DMPC (1 mg/mL) were mixed in a 30 mL round -bottom flask and evaporated. The residue was freeze-dried for at least 3 h in order to remove all solvents completely. Then, PBS (pH = 7.35) was added to the film, and the mixture was vortexed for 2 min (and sonicated when necessary). Then, the samples were extruded (10 times) using an extruder (Avanti Polar Lipids) equipped with a membrane (100 nm). Then, samples were collected in Eppendorf tubes.

2.3. Dynamic light scattering (DLS)

The size (diameter) of the liposomes was measured using a Zetasizer nano ZEN3600 (MALVERN), equipped with a laser (λ = 633 nm) set at 37 °C. Prior to the measurements, liposome samples were diluted twentyfold in degassed and filtered PBS (pH = 7.35).

2.4. Differential scanning calorimetry (DSC)

The phase-transition behavior of different liposomes was analyzed using a NANO DSC (TA Instruments). 650 μL of the samples (lipid concentration: 2.0 mg/mL) were measured at a scan rate of 0.5 °C/min, and degassed PBS (pH = 7.35) was used as a reference. The thus obtained data was analyzed using the NanoAnalyze software (TA Instruments) and fitted by a two-state-scaled model in order to estimate the phase-transition temperature (T_m), calorimetric enthalpy (ΔH), and van't Hoff enthalpy (ΔH_{vH}). The cooperative units (CU) were calculated as the ratio between the van't Hoff enthalpy and the calorimetric enthalpy.

3. Results and discussion

3.1. Synthesis of 1-deoxy-1-fluoro-LPEs and 2-deoxy-2-fluoro-LPEs

The synthesis of 1-deoxy-1-fluoro-LPEs (**6a-c**) starts from readily available protected glycerol **1** (Sax et al., 2006) (Fig. 2). Unfortunately,