Biochimica et Biophysica Acta 1848 (2015) 434–443

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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

Effect of glycyrrhetinic acid on lipid raft model at the air/water interface

Seiichi Sakamoto [a,*], Takuhiro Uto [b], Yukihiro Shoyama [b]

[a] Department of Pharmacognosy, Graduate School of Pharmaceutical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812–8582, Japan
[b] Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Nagasaki International University, 2825-7 Huis Ten Bosch, Sasebo, Nagasaki 859-3298, Japan

ARTICLE INFO

Article history:
Received 18 September 2014
Received in revised form 24 October 2014
Accepted 13 November 2014
Available online 20 November 2014

Keywords:
Glycyrrhetinic acid (GA)
Glycyrrhizin (GC)
Langmuir monolayer
Lipid raft
Membrane-disrupting activity

ABSTRACT

To investigate an interfacial behavior of the aglycon of glycyrrhizin (GC), glycyrrhetinic acid (GA), with a lipid raft model consisting of equimolar ternary mixtures of *N*-palmitoyl sphingomyelin (PSM), dioleoylphosphatidylcholine (DOPC), and cholesterol (CHOL), Langmuir monolayer techniques were systematically conducted. Surface pressure (π)–molecular area (A) and surface potential (ΔV)–A isotherms showed that the adsorbed GA at the air/water interface was desorbed into the bulk upon compression of the lipid monolayer. In situ morphological analysis by Brewster angle microscopy and fluorescence microscopy revealed that the raft domains became smaller as the concentrations of GA in the subphase (C_{GA}) increased, suggesting that GA promotes the formation of fluid networks related to various cellular processes via lipid rafts. In addition, ex situ morphological analysis by atomic force microscopy revealed that GA interacts with lipid raft by lying down at the surface. Interestingly, the distinctive striped regions were formed at $C_{GA} = 5.0$ μM. This phenomenon was observed to be induced by the interaction of CHOL with adsorbed GA and is involved in the membrane-disrupting activity of saponin and its aglycon. A quantitative comparison of GA with GC (Sakamoto et al., 2013) revealed that GA interacts more strongly with the raft model than GC in the monolayer state. Various biological activities of GA are known to be stronger than those of GC. This fact allows us to hypothesize that differences in the interactions of GA/GC with the model monolayer correlate to their degree of exertion for numerous activities.

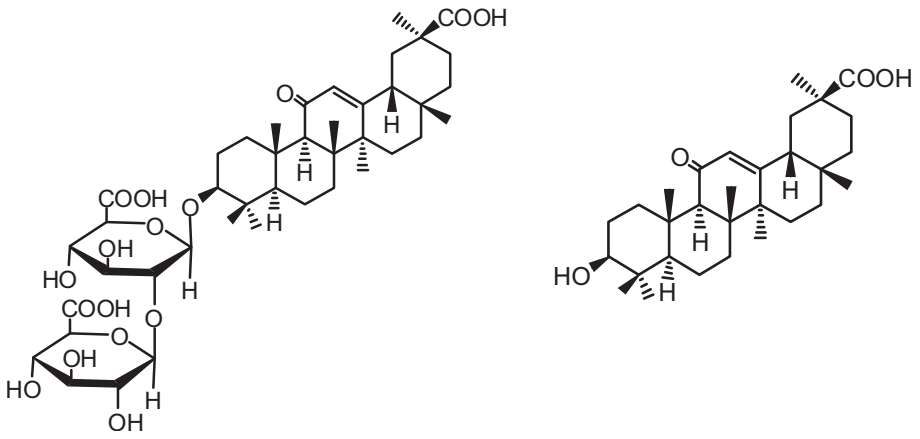
1. Introduction

The root and stolon of the genus *Glycyrrhiza* (licorice) are the most frequently used natural resources in Japanese traditional Kampo medicines and are known to exhibit various pharmacological activities, including hepatoprotective [1,2], anti-*Helicobacter pylori* [3], anti-inflammatory [4,5], anti-ulcer [6], expectorant [7], anti-microbial [8–10], and memory enhancing [11] activities. Because of its outstanding activities, more than 70% of prescribed Kampo medicines contain licorice. In addition, licorice has also been used worldwide as a sweetener, tobacco flavoring agent, and food additive. In addition, it is used in cosmetics and confectionery foods [12]; therefore, it was recently selected as the "Medicinal plant of the year 2012" by the University of Würzburg, WWF, TRAFFIC. The major bioactive compound of licorice is glycyrrhizin (GC), which is structurally classified as an oleanane-type triterpenoid saponin that possesses two glucuronic acid groups in a molecule. Because GC is metabolized to glycyrrhetinic acid (GA; Fig. 1) by human intestinal flora after being administered orally [13], GA, rather than GC, is expected to be the active form that exerts numerous biological activities. Indeed, GA as well as GC have been reported to have a large number of biological activities, which include anti-estrogenic [14], anti-inflammatory [15], anti-ulcer [16], anti-asthmatic [17], inhibitory activity of human complement [18] and 11β-hydroxysteroid dehydrogenase type 2 (11βHSD2) [19], and anti-viral activity [20,21]. In addition, GA has been shown to exhibit various anti-tumor activities against leukemia cells [22], the human hepatoma cell line HepG2 [23,24], breast cancer cell lines MCF-7 and ZR-75-1 [23,25,26], and androgen-dependent prostate cancer cell line LNCaP [27]. Recently, fitting analysis by a molecular modeling method of various triterpenoids to 11βHSD2 was performed to predict the toxic effects of triterpenoids on tumor cells by 11βHSD2 inhibition. This study revealed that GA was the best-fitted triterpenoid to the ligand binding site in 11βHSD2, which led to the apoptosis of the tumor cells [28].

Microdomains enriched in sphingolipids (sphingomyelin (SM) and glycosphingolipids) and cholesterol (CHOL) have recently been a subject of great interest in cell biology because they provide dynamic scaffolding for a variety of different and crucial cellular processes including protein trafficking [29], signal transduction [30,31], CHOL and membrane transport [32–34], and calcium homeostasis [35]. The long saturated acyl chains in sphingolipids are thought to strongly interact with CHOL to form a liquid-ordered (l_o) phase, whereas unsaturated phospholipids are loosely packed to form a liquid-disordered (l_d) phase [36–39]. These different properties in packing states induce phase separation to organize microdomains so-called lipid rafts [40]. So far, numerous studies related to the organization of lipid rafts have been physicochemically conducted by using lipid mixtures that mimic the components of the outer leaflets of plasma membranes, where the

* Corresponding author. Tel./fax: +81 92 642 6581.
E-mail address: s.sakamoto@phar.kyushu-u.ac.jp (S. Sakamoto).

http://dx.doi.org/10.1016/j.bbamem.2014.11.014
0005-2736/



Glycyrrhizin (GC) Glycyrrhetinic acid(GA)

Fig. 1. Structures of glycyrrhizin (GC) and glycyrrhetinic acid (GA).

combination of sphingolipids, phospholipids, and CHOL has been most widely utilized. To directly address the lateral organization of lipid rafts, the Langmuir monolayer and Langmuir–Blodgett (LB) techniques, atomic force microscopy (AFM) [41–44], Brewster angle microscopy (BAM) [45,46], and fluorescence microscopy (FM) [47–54] have been used to investigate the raft-mimicking lipid mixtures. In particular, the Langmuir monolayer behavior during compression and expansion well reproduces dynamic formation and destruction of the lipid rafts. These methods enable us to visually provide information regarding the phase variations (or lateral diffusion) on micro- and nano-scales.

In a previous study, the interfacial behavior of GC with a lipid raft model of equimolar ternary *N*-palmitoyl sphingomyelin (PSM), dioleoylphosphatidylcholine (DOPC), and CHOL monolayers has been systematically explored [55]. GC was observed to regulate the size of the raft domains by interacting from the bulk to increase a liquid-expanded (LE) network, which is considered to play an important role in the substance transportation via lipid rafts. Moreover, GC-derived distinctive stripped regions resembling the feature of membrane disruption so-called hemolytic activity were successfully observed at a GC concentration of 50 μM in the subphase (C_{GC} = 50 μM), despite the fact that the degree of GC's hemolytic activity is relatively low.

Here, we investigated the interaction of GA, which is the hydrolysate of GC by human intestinal flora, with the equimolar ternary PSM/DOPC/CHOL monolayer to gain insight into the interfacial behavior of raft domains in the presence of GA. GA was dissolved in the subphase solution, where the pH and ionic strength were adjusted to physiological conditions, to clarify the interaction of adsorbed GA from the bulk with the raft model. Moreover, in this study, AFM observation was conducted to visually investigate the interaction manner between GA and the lipid raft model at the air/water interface. By comparing the interfacial behaviors of GA with those of GC described in our previous study [55], the effect of glucuronic acid groups on the lipid raft model and hemolytic activity were also discussed.

2. Materials and methods

2.1. Materials

Glycyrrhetinic acid (GA; ≥99%) was obtained from Nagara Science Co. (Gifu, Japan). *N*-Palmitoyl-D-*erythro*-sphingosylphosphorylcholine (*N*-palmitoyl sphingomyelin, PSM; >99%), 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (dioleoylphosphatidylcholine, DOPC; >99%), and 1-palmitoyl-2-{6-[(7-nitro-2-1,3-benzoxadiazol-4-yl)amino]hexanoyl}-*sn*-glycero-3-phosphocholine (NBD-PC; >99%) were purchased from Avanti Polar Lipids (Alabaster, AL, USA). Cholesterol (CHOL; ≥99%) was obtained from Sigma Chemical Co. (St. Louis, MO, USA). These lipids and GA were used without further purification. Chloroform (99.7%) and methanol (99.8%), which were used as spreading solvents, were purchased from Kanto Chemical Co. (Tokyo, Japan) and Nacalai Tesque (Kyoto, Japan), respectively. The chloroform/methanol (2/1, v/v) mixture was used for the preparation of stock solutions of PSM (0.5 mM), DOPC (0.5 mM), and CHOL (1.0 mM) monolayers. Tris(hydroxymethyl) aminomethane (Tris), NaCl, and acetic acid were obtained from Nacalai Tesque. NaCl was heated at 1023 K for 24 h before use to remove all surface-active organic impurities. Triply distilled water (surface tension = 72.0 mN m^{-1} at 298.2 K and the electrical resistivity = 18 MΩ cm) was used for the preparation of the subphase solution.

2.2. Surface pressure–area isotherms

The surface pressure (π) of Langmuir monolayers was measured with an automated homemade Wilhelmy balance (Mettler Toledo, AG-245) with a surface pressure resolution of 0.01 mN m^{-1}. The pressure-measuring system was equipped with a filter paper (Whatman 541, periphery = 4 cm). The trough was made from Teflon-coated brass (area = 750 cm^2), and both hydrophobic and lipophobic Teflon barriers were used in this study. π–molecular area (A) isotherms were recorded at a temperature of 298.2 ± 0.1 K, which was thermostatically controlled by a circulating water system. After the sample was spread onto the surface, monolayers were allowed to compress for 15 min to evaporate the spreading solvent. The monolayer was compressed at a speed of ~0.10 nm^2 per molecule per minute. Standard deviations (SD) for A and π were ~0.01 nm^2 and ~0.1 mN m^{-1}, respectively. The subphase was prepared at 0.02 M Tris buffer with 0.13 M NaCl containing different concentrations of GA. Its pH was adjusted to 7.4 with an adequate volume of acetic acid.

2.3. Surface potential–area isotherms

The measurement of surface potential (ΔV) was performed simultaneously with the π measurement when the monolayer was compressed at the air/water interface. It was monitored using ionizing an ^{241}Am electrode placed 1–2 mm above the interface while a reference electrode was dipped into the subphase. An electrometer (Keithley 614) was used to measure the ΔV. The SD for ΔV was ~5 mV.