Available online at www.sciencedirect.com
SCIENCE DIRECT®
Colloids and Surfaces B: Biointerfaces 42 (2005) 157–174
COLLOIDS AND SURFACES B
www.elsevier.com/locate/colsurfb

Cerebroside Langmuir monolayers originated from the echinoderms I. Binary systems of cerebrosides and phospholipids

Hiromichi Nakahara [a], Shohei Nakamura [a], Kazufumi Nakamura [b], Masanori Inagaki [b], Mariko Aso [c], Ryuichi Higuchi [b], Osamu Shibata [a,*]

[a] *Division of Biointerfacial Science, Graduate School of Pharmaceutical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan*
[b] *Natural Products Chemistry, Graduate School of Pharmaceutical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan*
[c] *Pharmaceutical Synthetic Chemistry, Graduate School of Pharmaceutical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan*

Received 10 November 2004; accepted 29 January 2005
Available online 16 March 2005

Abstract

The surface pressure (π)–area (A), the surface potential (ΔV)–A and the dipole moment ($\mu_\perp$)–A isotherms were obtained for two-component monolayers of two different cerebrosides (LMC-1 and LMC-2) with phospholipids of dipalmitoylphosphatidylcholine (DPPC) and with dipalmitoylphosphatidylethanolamine (DPPE) on a subphase of 0.5 M sodium chloride solution as a function of phospholipid compositions by employing the Langmuir method, the ionizing electrode method, and the fluorescence microscopy. Surface potentials (ΔV) of pure components were analyzed using the three-layer model proposed by Demchak and Fort [J. Colloid Interf. Sci. 46 (1974) 191–202]. The contributions of the hydrophilic saccharide group and the head group to the vertical component of the dipole moment ($\mu_\perp$) were estimated. The miscibility of cerebroside and phospholipid in the two-component monolayers was examined by plotting the variation of the molecular area and the surface potential as a function of the phospholipid molar fraction ($X_{\text{phospholipid}}$), using the additivity rule. From the A–$X_{\text{phospholipid}}$ and ΔV_{m}–$X_{\text{phospholipid}}$ plots, partial molecular surface area (PMA) and apparent partial molecular surface potential (APSP) were determined at the discrete surface pressure. The PMA and APSP with the mole fraction were extensively discussed for the miscible system. Judging from the two-dimensional phase diagrams, these can be classified into two types. The first is a positive azeotropic type; the combinations of cerebrosides with DPPC are miscible with each other. The second is a completely immiscible type: the combination of cerebrosides with DPPE. Furthermore, a regular surface mixture, for which the Joos equation was used for the analysis of the collapse pressure of two-component monolayers, allowed calculation of the interaction parameter (ξ) and the interaction energy ($-\Delta\varepsilon$) between the cerebrosides and DPPC component. The miscibility of cerebroside and phospholipid components in the monolayer state was also supported by fluorescence microscopy.

Keywords: Langmuir monolayer; Glycosphingolipids; Cerebrosides; Phospholipids; Surface dipole moment ($\mu_\perp$); π–A isotherm; ΔV–A isotherm; Two-dimensional phase diagram; Fluorescence microscopy

1. Introduction

The functions of animal cells at their surfaces regulate such fundamental biological processes as growth, differentiation, and motility. Although the nature of the functions is not understood at the molecular level, it is understood that the complex glycolipid and glycoprotein molecules sitting at the outer surface of the cells are involved. Lipid molecules containing sugar groups are called glycosphingolipids. Glycosphingolipids (GSLs) are present in most animal cell plasma membranes and are thought to play a role in a number of cellular functions, including cell recognition, adhesion, regulation, signal transduction, and development of tissues. They predominantly locate on the outer leaflet of the membrane and may act to protect the membrane from harsh conditions such

DOI of original article:10.1016/j.colsurfb.2005.01.013.
* Corresponding author. Tel.: +81 92 642 6669; fax: +81 92 642 6669.
E-mail address: shibata@phar.kyushu-u.ac.jp (O. Shibata).
URL: http://kaimen.phar.kyushu-u.ac.jp/.

0927-7765/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.colsurfb.2005.01.012

as a low pH or degradative enzymes [1]. A detailed description of the chemical, structural, and functional properties of glycolipids in general can be found in a review article by Mggio [2].

Glycosphingolipids (cerebrosides) are amphiphilic compounds consisting of saccharide and ceramide moieties and are ubiquitous components of the plasma membrane of all eucaryotic cells [3,4]. Glycosphingolipids are considered to be receptors for microorganisms and their toxins, modulators of cell growth, and differentiation, and organizers of cellular attachment to matrices [5,6]. Recent cell biological studies show that cerebrosides in plasma membranes form clusters, the so-called rafts, with cholesterol and are relatively less phospholipids than other areas of plasma membrane. Glycosphingolipids could mediate the signal transduction pathway through interaction with these signaling proteins and not only circulate between the plasma membrane and intracellular organs but also move laterally over the exoplasmic membrane. Such migration could be conducted by raft [7,8]. Glycosphingolipids [9–13] are a major component of the myelin sheath [14–16]. Glucocerebrosides and lactosylceramide are the major extraneural glycosphingolipids [17–19]. GSLs with tri- and tetrasaccharide-containing head groups, known as globosides, are found in the erythrocyte membrane [20]. GSLs show heterogeneity not only in their saccharide head group, but also in their ceramide moieties. The biological significance of ceramide heterogeneity is not still understood well. However, especially the structure of ceramide for the fatty acid moieties could influence the localization and functions of GSLs on the plasma membrane, possibly by direct interaction with cholesterol, phospholipids, and the transmembrane domains of receptor proteins [21–24]. Unusual structures of GSLs will be revealed in future through further technological innovation.

However, the organization of cerebroside–phospholipid mixtures is quite unclear. As long as we know, reports on cerebrosides are still much fewer in number than reports on gangliosides [25]. In the previous studies, we have only restricted a two-component monolayer system of sphingolipid (cereboroside:LMC-2), cholesteryl sodium sulfate (Ch-S), and cholesterol and their combinations as to monolayer properties of surface pressure– and surface potential–surface area at the air/water interface without fluorescence microscopy measurements [26].

Here, we have focused on characterizing the Langmuir behavior of some pure cerebrosides, phospholipids, and their two-component systems at air/water interface. Surface pressure (π)–, surface potential (ΔV)–, and dipole moment ($\mu_{\perp}$)–A isotherms were obtained for the pure compounds and their two-component systems. The surface potentials were analyzed using the three-layer model proposed by Demchak and Fort [27]. The phase behavior of two-component monolayers was examined in terms of additivity of molecular surface area and of surface potential. Furthermore, it was analyzed employing the partial molar molecular area (PMA) and apparent partial molar surface potential (APSP). The molecular interaction between monolayer components was investigated using the Joos equation. Finally, the monolayers were examined by fluorescence microscopy. Similar analyses are reported for binary cerebroside – steroid monolayers in the following articles in series.

2. Experimental

2.1. Materials

The cerebrosides (PA-0-5, LMC-1, and LMC-2) possess β-*O*-glucosyl head group linked to the terminal hydroxyl group of ceramide. These compounds were obtained from the less polar fraction of the extract of the echinoderms. PA-0-5 was extracted from the sea cucumber *Pentacta australis* (Gokakukinko in Japanese). The chemical structure of this compound has been already identified [28]. On the other hand, LMC-1 and LMC-2 were obtained from the starfish *Luidia maculata* (Yatsudesunahitode in Japanese). These compounds (LMC-1 and LMC-2) were molecular species [19]. LMC-1 has double bonds in the hydrophobic chains. By hydrogenation with Pd/C in *n*-hexane/EtOH (1:1, v/v), LMC-1 was converted into sphinganine (LMC-1-H) that has two saturated hydrophobic chains. All cerebrosides were checked by ^{1}H- and ^{13}C-NMR spectra after purification by TLC and HPLC. The compositions of the hydrophobic acyl chain and long chain base (LCB) are given in Table 1. In Fig. 1, *n* and *m* are the number of carbon atoms of the acyl chain and long chain base, respectively. Dipalmitoylphosphatidylcholine(L-α-1-palmitoyl-2-hydroxy-*sn*-glycero-3-phosphocholine, DPPC) was purchased from Avanti Polar Lipids, Inc. (Birmingham, Alabama, U.S.A.) and dipalmitoylphosphatidylethanolamine(L-α-1-palmitoyl-2-hydroxy-*sn*-glycero-3-phosphoethanolamine, DPPE) was obtained from NOF Corporation (Japan). Their purity was >99%. All phospholipids were checked by TLC just before their use and used without further purification. The chemical structures of the cerebrosides used are shown in Fig. 1.

Table 1
Acyl chain and long chain base (LCB) compositions of cerebrosides

	Composition (%)		
	PA-0-5	LMC-1	LMC-2
Acyl chain			
22	100 (22:0)	56.8	56.0
23		34.7	35.5
24		8.5	8.5
LCB part			
16 (m = 11)		9.6	
17 (m = 12)	100 (17:1)	38.5	5.3
18 (m = 13)		16.0	12.2
19 (m = 14)		35.9	82.5