Available online at www.sciencedirect.com
SCIENCE DIRECT®
Colloids and Surfaces B: Biointerfaces 41 (2005) 223–231
COLLOIDS AND SURFACES B
www.elsevier.com/locate/colsurfb

Properties of galactocerebroside layers transferred to glassy carbon electrodes: effect of an applied electric field

N. Wilke[b,*], A.M. Baruzzi[a], B. Maggio[b], M.A. Pérez[a], M. López Teijelo[a]

[a] *Instituto de Investigaciones en Fisicoquímica de Córdoba (INFIQC), Departamento de Fisicoquímica, Universidad Nacional de Córdoba, 5000 Córdoba, Argentina*
[b] *Centro de Investigaciones en Química Biológica de Córdoba (CIQuiBiC), Departamento Química Biológica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, 5000 Córdoba, Argentina*

Accepted 5 January 2005

Abstract

Galactocerebroside films deposited onto glassy carbon electrodes have been previously studied through the electrochemical response of a redox couple present in solution. Those experiments indicated that the film is inhomogeneous and that there are lipid-free places. In this work, we present experimental results indicating that those bare regions are formed when the electrode is introduced in an aqueous solution, and that the size and/or amount of uncovered domains increase when negative potentials are applied to the film. The experimental techniques employed for these findings are epifluorescence microscopy and ellipsometry.

Keywords: Covered electrodes; Lipids; Epifluorescence microscopy; Ellipsometry; Electrochemistry

1. Introduction

Monolayers formed at the air–water interface and transferred to solid substrates are used to study the properties and supramolecular structure of layers formed by lipids constituent of membranes [1]. When the substrate of the transferred film is an electrode, electrochemistry offers a good possibility to obtain information concerning the interaction among the components, its electrical properties and permeability to different species of interest. Surfactant covered electrodes are widely studied in electrochemistry for applications in the biosensors field [2–5], as electronic devices [6,7], to avoid metal corrosion [8–10], to study very fast heterogeneous reactions [11–13] and as biomembrane models [14–20]. The coupling of electrochemistry with optical techniques provides the possibility of analyzing the effects of an external electric field on the film structure. The application of electric fields has varied effects on organized biosystems in terms of cellular function, membrane stability–instability processes, membrane channel conductance and activity of membrane enzymes [21]. Studies done many years ago showed that phospholipid head groups can act as sensors for the electrical properties of the membrane interface [22]. Also, for glycosphingolipids in particular, it has been shown that the resultant molecular dipole moment perpendicular to the interface can compensate or enhance effects on the membrane organization of externally applied electrostatic field across the interface and regulate the activity of amphitropic membrane-associated enzymes such as phospholipase A_2 involved in lipid-mediated signal transduction [23].

Surfactant monolayers on solid surfaces generally form inhomogeneous lattice structures, leaving packing defects in the substrate [11–13]. In this sense, the analysis of redox reactions at covered electrodes gives information about the efficiency of the transfer process onto the solid substrate. In general, it is observed that the redox reaction occurs through pits and holes present in the lipid layer [24–26].

* Corresponding author. Fax: +54 351 4334074.
E-mail address: wilke@mail.fcq.unc.edu.ar (N. Wilke).

0927-7765/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.colsurfb.2005.01.001

Galactocerebroside (galcer) forms a condensed film at the air–water interface. It is a neutral sphingolipid whose polar head group is a galactose moiety and the hydrocarbon tail is in average 20 carbon atoms long. In a previous paper we found that for electrodes covered with a series of neutral lipids, the surface coverage depends on the compactness of the monolayer at the aqueous phase–air interface [24]. In this sense, galcer should form compact films with few holes of small size. However, we found that galcer films on glassy carbon electrodes are inhomogeneous [24–26] and, from the comparison of the results with theoretical curves, a fractional coverage of 0.90 ± 0.02 was calculated [26].

Ellipsometry is a very convenient non-destructive optical method that has been successfully used to obtain information about thickness, porosity and optical properties of organic adsorbed layers. Many examples are found in the literature, which show the usefulness of this technique to study layers on air–water or solid–air interfaces [27–34].

There is an increasing attention devoted to the properties and structure of supported lipid layer films. In this sense, films transferred from the air–water interface provide a controlled way of producing supported lipid layers. In addition, the understanding of the effect of the external conditions on the film structure is an important attainment.

The aim of this paper is to analyze the structure of galcer layers transferred to a solid substrate in different environments (at the air–solid and aqueous solution–solid interfaces) and the effect promoted by an electric field. For this purpose, we analyze the properties of galcer films transferred onto glassy carbon electrodes by the use of epifluorescence microscopy and ellipsometry and, in this way; we try to confirm the presence of holes at the electrode–aqueous phase interface and to find out when they are formed. In this sense, the applied electric field could modify the film structure, and could be involved in the formation of holes.

2. Experimental

2.1. Materials

Glassy carbon, used as substrate, was commercially available (0.033 cm^2 of area). The surface was polished with alumina of 1, 0.5 and 0.03 μm, and rinsed with ultrapure water immediately before lipid transfer. The electric field was applied using a platinum mesh as counter-electrode and a $Ag|AgCl|Cl^-$ (3 M) as reference electrode. The electrochemical experiments coupled to ellipsometric measurements were carried out using a three-electrode cell adapted for optical measurements. These experiments were performed in a solution containing 20 mM of $NaNO_3$ as supporting electrolyte (analytical reagent, Mallinckrodt) with ultra-pure water produced by a Milli-Q system (18 MΩ). Galactocerebroside (galcer) was from Avanti Polar Lipids and $DilC_{18}$, the fluorescent probe, was from Molecular Probes.

A Zeiss Axioplan (Carl Zeiss, Obcrkochem, Germany) epifluorescence microscope, with a source of UV radiation consisting of a mercury lamp HBO 50 and an objective of 20× was used. Exposure times were typically between 0.1 and 1 s. The pictures were recorded by a CCD video camera (Micromax, Princeton Instruments Inc., USA) commanded through Metamorph 3.0 software (Universal Imaging Corporation, PA, USA).

A Rudolph Research rotating-analyzer automatic ellipsometer (vertical type, 2000FT model) provided with a 75 W tungsten–halogen lamp as light source and a filter (546.1 nm) was used. All measurements were made at an incidence angle of 70.00°.

2.2. Methods

2.2.1. Galactocerebroside (galcer) L–B layers

Galcer was spread on ultrapure water from solutions approximately 1 nmol μL^{-1} prepared in chloroform:methanol (2:1). Details of the equipment used have been given in previous publications [35,36]. The subphase temperature was kept at (25 ± 1) °C with a refrigerated Haake F3C thermocirculator. Surface pressure was measured with a platinized platinum plate. The monolayers were taken to the transfer pressure. Film transfer was accomplished by the touching method, that is, by gently allowing horizontal contact of the electrode surface with the lipid monolayer with a precision step motor. The electrode was maintained in that position for 30 s before lifting and the procedure was repeated. Every film was allowed to dry out in air for 30 min. The decrease in the total monolayer area as the electrode was lifted was measured as a control, although the change cannot be used for calculating a transfer rate because the electrode is embedded in support, onto whose surface the lipid is also adsorbed.

2.2.2. Epifluorescence microscopy measurements

The lipid solutions were doped with 1 mol% of the fluorescent probe $DilC_{18}$ and transferred to the glassy carbon electrode as described in Section 2.2.1. The observations were carried out at room temperature.

2.2.3. Application of electric fields

A triangular potential wave at 2 mV s^{-1} starting from the equilibrium potential and covering the −0.6 to +0.6 V potential range, was applied.

2.2.4. Ellipsometric measurements

In ellipsometric measurements, changes in the state of polarization of polarized light due to reflection at a planar surface are detected, and the ellipsometric angles Ψ and Δ are determined. These are related to the ellipsometric ratio ρ, according to:

$$\rho = \tan\Psi \exp(i\Delta) = \frac{r_p}{r_s} \quad (1)$$