Colloids and Surfaces B: Biointerfaces 111 (2013) 626–635

Contents lists available at SciVerse ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

Insights about α-tocopherol and Trolox interaction with phosphatidylcholine monolayers under peroxidation conditions through Brewster angle microscopy

Carla M. Castro[a], Marina Pinheiro[a], Marlene Lúcio[a], Juan J. Giner-Casares[b], Luis Camacho[b], José L.F.C. Lima[a], Salette Reis[a,*], Marcela A. Segundo[a]

[a] REQUIMTE, Departamento de Ciências Químicas, Faculdade de Farmácia, Universidade do Porto, R. Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal
[b] Departamento de Física Química y Termodinámica, Universidad de Cordoba, Campus de Rabanales, Edificio Marie Curie, Córdoba E-14014, Spain

ARTICLE INFO

Article history:
Received 11 March 2013
Received in revised form 29 May 2013
Accepted 25 June 2013
Available online 12 July 2013

Keywords:
Antioxidant
Brewster angle microscopy
Langmuir monolayer
Peroxidation
Tocopherol
Trolox

ABSTRACT

Membranes are major targets to oxidative damage, particularly due to lipid oxidation, which has been associated to aging. The role, efficacy and membrane interaction of antioxidants is still unclear, requiring further understanding of molecular interaction. Hence, the objective of this work was to evaluate the interaction between antioxidants (α-tocopherol and its aqueous soluble analog Trolox) and the monolayer formed by phosphatidylcholine molecules at air/liquid interface upon peroxidation conditions, promoted by peroxyl radicals from thermal decomposition of 2,2′-azobis(2-methylpropionamidine) (AAPH). The interaction with three different monolayers, containing (i) 1,2-dihexadecanoyl-*sn*-glycero-3-phosphocholine (DPPC), (ii) DDPC + α-linolenic acid, or (iii) egg yolk L-α-phosphatidylcholine (EPC), was ascertain by surface pressure (π)–molecular area (A) isotherms and by monitoring monolayer features through Brewster angle microscopy (BAM). The interaction of antioxidants with DPPC monolayers was confirmed by modifications on DPPC domain shape for α-tocopherol and through the maintenance of typical multilobed domain shape during an extended surface pressure interval for Trolox. Under peroxidation conditions, BAM images showed a clear interaction between components of AAPH subphase with the monolayer through changes on DPPC domain shape and appearance of white dots, located mainly at the frontier between the condensed and expanded liquid phases. White branched structures were also observed whenever both α-linolenic acid and α-tocopherol were present, indicating the segregation of these components within the monolayer, which is highly significant in biological systems. For EPC monolayers, no information from BAM was obtained but π–A isotherms confirmed the existence of the same interactions observed within the other two monolayers.

1. Introduction

Biological membranes are not only a barrier to keep the cell intact, but they also allow exchange of substances required for or produced from cell metabolism [1]. Membranes are centrally involved in control and execution of a large variety of cellular processes, thus requiring the maintenance of their proper structure and function [2,3]. Hence, any disruption of the bilayer structure may lead to modifications of membrane properties and, consequently, changes on its physiological role. Phospholipids are the main components of the biological membranes, liable to free radical attack that induces lipid peroxidation. Thus, uncontrolled oxidative reactions generate cytotoxic compounds and disrupt several important structural and protective functions associated with biomembranes. Oxidative phenomena have also been implicated in the etiology of many diseases, including cancer, cardiovascular and neurological diseases, and other oxidative stress mediated dysfunctions [4].

In fact, several biophysical changes and associated kinetic features have been identified on membrane models under peroxidation conditions by either experimental or theoretical approaches. For instance, peroxidation of liposomes was shown to be faster when the oxidizable lipids reside in the relatively rigid gel phase bilayers than in the less tightly packed liquid crystalline bilayers [5–7]. Moreover, exposure of liposomes comprising dilinoleoylphosphatidylcholine (DLPC) and cholesterol to conditions of spontaneous oxidation caused the formation of moderate levels of hydroperoxides and a concentration-dependent formation of discrete immiscible cholesterol-rich domains, as observed by small angle X-ray scattering (SAXS) [8]. This technique also allowed the observation of a marked reduction of the width of the hydrocarbon core, a similar decrease of the overall width of the membrane, and a

* Corresponding author. Tel.: +351 220428672; fax: +351 226093483.
E-mail address: shreis@ff.up.pt (S. Reis).

0927-7765/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.colsurfb.2013.06.043

decrease of the molecular volume of the bilayers as a consequence of iron(II)/ascorbate-induced peroxidation of liposomal DLPC [9]. Theoretical molecular dynamics simulations led to similar conclusions concerning changes featured on organized lipid structures [10–13].

The deleterious consequences of membranes peroxidation have stimulated numerous studies on the efficacy and mechanisms of action of biologically relevant antioxidants [14–18]. Lipidic biomimetic models, namely large unilamellar vesicles (LUVs), have been applied to assessment of antioxidant activity of compounds upon peroxidation conditions [19–22]. Langmuir monolayers of phospholipids have been successfully used as a model for studying a number of biologically relevant chemical reactions at the lipid/solution interface, namely the lysis of lipids by the phospholipase A_2 [23].

Langmuir monolayers formed by phosphatidylcholines bearing different acyl chains, with and without unsaturation, have also been applied for studying of rosmarinic acid interaction with this biomimetic model, showing that cholesterol decreases the penetration of rosmarinic acid [24]. More recently, by using Brewster angle microscopy (BAM) and Langmuir monolayer compression isotherms, the presence of an oxidatively modified phosphatidylcholine in monolayers was shown to efficiently oppose the miscibility transition and to stabilize micron-sized domain separation at lipid lateral packing densities at equilibrium lateral pressure that is suggested to prevail in bilayer membranes [25]. Despite these examples, information about mechanistic details, especially those concerning the role of interactions between antioxidant compounds and membranes, are still to be unveiled.

Hence, the objective of the present work is to evaluate the interaction between antioxidants and the organized monolayer formed by phosphatidylcholine molecules at air/liquid interface upon peroxidation conditions. For this, a biophysical approach will be implemented, by profiling the changes of surface pressure (π) and molecular area (A) upon monolayer compression and by monitoring monolayer features and domain formation through BAM. Peroxidation conditions will be fostered by the presence of peroxyl radicals generated by previous thermal decomposition of 2,2′-azobis(2-methylpropionamidine) dihydrochloride (AAPH) in the aqueous buffered subphase. The role of α-tocopherol, a relevant natural antioxidant, and of its water soluble analog Trolox (Supplementary information, Fig. S1) will be assessed through modifications in the π–A isotherms profile and changes on formation of lipid domains obtained by BAM.

2. Experimental

2.1. Reagents and solutions

Egg yolk L-α-phosphatidylcholine (EPC, purity ≥99%), potassium phosphate monobasic, 2,2′-azobis(2-methylpropionamidine) dihydrochloride (AAPH), α-linolenic acid (LA), and α-tocopherol were purchased from Sigma–Aldrich (St. Louis, MO). 1,2-Dihexadecanoyl-*sn*-glycero-3-phosphocholine (DPPC) was purchased from Avanti Polar Lipids Inc. 6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) was obtained from Fluka (Buchs, Switzerland). Chloroform from Landilab (Porto, Portugal) was used as co-spreading solvent. The subphase used, 75 mM hydrogen phosphate/phosphate buffer (pH 7.4), was prepared using Milli-Q water (resistivity >18.2 MΩ cm, Millipore, Billerica, MA).

Lipid solutions were prepared in chloroform and they contained DPPC (0.734 mg mL^{-1}), DPPC + LA (9:1, w/w; 0.642 mg mL^{-1} of DPPC) or EPC (0.751 mg mL^{-1}). Trolox was added to the subphase buffer at 20 μM. For generation of peroxyl radicals, AAPH (12 mM) was dissolved in hydrogen phosphate buffer at 37 °C and kept at this temperature during 10 min. Whenever necessary, Trolox was dissolved in this solution immediately before it was poured into the trough. α-Tocopherol (20 μM) was incorporated onto lipid solutions prior to monolayer spreading (DPPC:α-tocopherol molar ratio of 50).

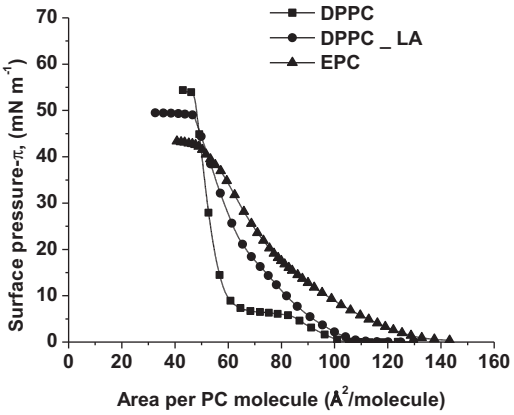

Fig. 1. Surface pressure–area isotherms of monolayers formed by DPPC (■), DPPC + LA (●) and EPC (▲) upon a phosphate buffer (pH 7.4) subphase.

2.2. Surface pressure–area measurements

Spreading solutions were deposited onto the aqueous subphase with a Hamilton microsyringe, precise to 2.0 μL. After spreading, the monolayers were left to equilibrate for ca. 10 min for solvent evaporation prior to film compression (barrier speed of 20 cm^2 min^{-1}). π–A isotherms, expressed for the absolute molecular area (Å^2 per molecule) of DPPC or of EPC, were recorded with a NIMA 601 (Nima Technology, Coventry, UK) Langmuir trough (total area = 600 cm^2; subphase volume ca. 400 mL). Surface pressure was measured with the accuracy of ±0.1 mN m^{-1} using a Wilhelmy type dynamometric system with a strip of filter paper. All measurements were performed at 21 °C (controlled room temperature). Before each measurement, the trough was cleaned thoroughly with chloroform and double deionized water. The parameters pertaining to each isotherm (elastic modulus, C_s^{-1}; collapse pressure, $\pi_{collapse}$, molecular area at collapse, $A_{collapse}$, minimum area per lipid molecule, A_{min}) were assessed as described elsewhere [26].

2.3. Brewster angle microscopy

Brewster angle microscopy images were obtained from a I-Elli 2000 apparatus (supplied by Nanofilm Technologies, Göttingen, Germany) using a Nd:YAG diode laser, which were recorded with a lateral resolution of 2 μm. The image processing procedure included a geometrical correction of the image, as well as a filtering operation to reduce interference fringes and noise. Furthermore, the brightness of each image was scaled to improve contrast. The size of the images is 430 μm in width. The microscope and film balance were located on a table with vibration isolation (antivibration system MOD-2 S, Halcyonics, Göttingen, Germany) in a large class 100 clean room.

3. Results

3.1. π–A measurements

The isotherm obtained for DPPC spread on aqueous phosphate buffer pH 7.4 (Fig. 1) presents the characteristic shape reported before [27] for other subphases, with a marked plateau between