Biochimica et Biophysica Acta xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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

Biophysical investigations of the structure and function of the tear fluid lipid layers and the effect of ectoine. Part B: Artificial lipid films

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ARTICLE INFO

Article history:
Received 21 January 2014
Received in revised form 2 May 2014
Accepted 5 May 2014
Available online xxxx

Keywords:
Tear fluid lipid layer
Langmuir film balance
Epi-fluorescence microscopy
Atomic force microscopy
Ectoine
Dry eye syndrome

ABSTRACT

The tear fluid lipid layer is present at the outermost part of the tear film which lines the ocular surface and functions to maintain the corneal surface moist by retarding evaporation. Instability in the structure of the tear fluid lipid layer can cause an increased rate of evaporation and thus dry eye syndrome. Ectoine has been previously shown to fluidize lipid monolayers and alter the phase behavior. In the current study we have investigated the effect of ectoine on the artificial tear fluid lipid layer composed of binary and ternary lipid mixtures of dipalmitoyl phosphatidylcholine (DPPC), cholesteryl esters and tri-acyl-glycerols. The focus of our study was mainly the structural and the biophysical aspects of the artificial tear fluid lipid layer using surface activity studies and topology analysis. The presence of ectoine consistently causes an expansion of the pressure–area isotherm indicating increased intermolecular spacing. The topology studies showed the formation of droplet-like structures due to the addition of ectoine only when tri-acyl-glycerol is present in the mixture of DPPC and chol-palmitate, similar to the natural meibomian lipids. Consequently, the hypothesis of an exclusion of tri/di-acyl-glycerol from the meibomian lipid film in the presence of ectoine in the subphase is confirmed. A model describing the effect of ectoine on meibomian lipid films is further presented which may have an application for the use of ectoines in eye drops as a treatment for the dry eye syndrome.

1. Introduction

Tear fluid lipid layer is the outermost layer of the tear film that forms the outer lining of the ocular surface [1]. It reduces the rate of evaporation of the tear fluid thus preventing drying of the corneal epithelium [2]. Further, it is also involved in maintaining a clear optical surface [3] and acting as a protective barrier against the microbes and organic matter such as dust and pollen. As suggested by Holly [1] in 1973, the tear film is depicted as a two-layered structure: polar lipids forming the lower sublayer and the nonpolar lipids forming the upper sublayer that is in contact with the air. This proposition was further elaborated by Shine and McCulley [4]. Each sublayer is assigned a specific role in maintaining the structural integrity of the tear fluid lipid layer. The lower layer comprising the polar lipids acts as a surfactant and the spreading of the tear fluid lipid layer becomes thermodynamically favorable. The composition of the lower lipid layer was proposed to include polar lipids like the phosphatidylcholines, phosphatidylethanolamine, sphingomyelin (SM), ceramides and cerebrosides [5]. Recently, another group of amphiphiles called (O-acyl)-ω-hydroxy fatty acids has been found to play a major role in the polar lipid sublayer [6]. The upper layer consists of the hydrophobic lipids which form a coating and seal the underlying aqueous portion of the tear fluid. This layer hence plays a major role in preventing the evaporation of the tear fluid as lipid films have low water vapor transmissivity, depending on the thickness and the compositions. The tear fluid lipid layer is believed to consist majorly of lipids secreted by the meibomian gland. The most commonly found classes of lipids in the meibomian glands have been reported to be ubiquitous WEs and cholesteryl esters (CEs) [6–8]. However, the composition of the whole tear lipids has been found to be different than the secretions of the meibomian gland. Primarily the differences involve the higher molar ratio of the low molecular weight wax esters (WE) — type species in the human meibum [7–10]. Further, it has been reported that the meibomian lipids have a lower acyl chain ordering thus leading to a lower melting temperature than the whole tear lipids [11]. However, the whole tear samples showed the spectroscopic signal of organic phosphate ester groups as found in phosphatidylcholines and sphingomyelin [12,13]. Wollensak et al. characterized the whole tear lipids and showed the WEs and the CEs accounting for 45% of the total lipid weight. The free fatty acids (FFAs) and the tri-acyl-

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http://dx.doi.org/10.1016/j.bbamem.2014.05.007
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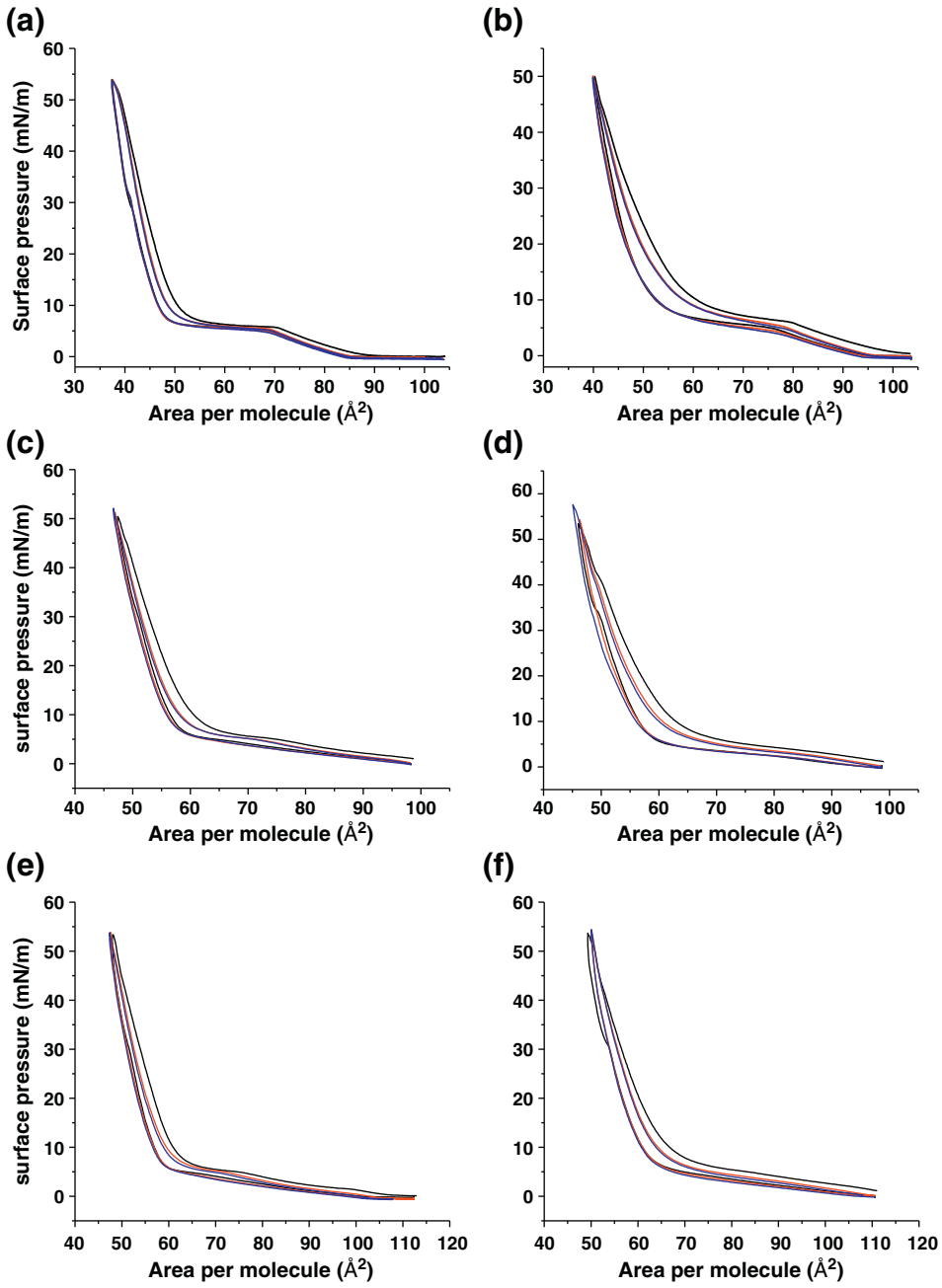

Fig. 1. Cyclic compression–expansion isotherms for (a) DPPC/CP (9:1) on PBS subphase, (b) DPPC/CP(9:1) on 100 mM Ectoine (c) DPPC/DPOG (9:1) on PBS subphase (d) DPPC/DPOG(9:1) on 100 mM Ectoine (e) DPPC/CP/DPOG (8:1:1) on PBS subphase (f) DPPC/CP/DPOG (8:1:1) on 100 mM Ectoine. *Black curve-first cycle*; *red curve-second cycle*; *blue curve-third cycle*. All measurements were performed at 20 °C.

glycerides (TAG) account for less than 15% each and the polar lipids were reported to comprise 15% of the total weight [14]. Further, the presence of polar lipids in the human tears has been shown by Fourier transform infrared spectroscopy [13]. The composition of the tear fluid lipid layer has been established to play an essential role in maintaining the structural integrity of the lipid layer and any alteration can lead to several ocular disorders.

Dry eye syndrome or DES is the most commonly occurring ocular disorder which is accompanied by drying of the ocular surface and the inflammation of the corneal epithelium. It mainly causes premature rupture of the tear film thus leading to increased rate of evaporation of the tear fluid. An altered composition of the tear fluid lipid layer can lead to a structurally unstable film thus leading to a dry eye syndrome. McCulley et al. demonstrated that various forms of dry eye can be caused by meibomian gland dysfunction (MGD) [15] whereby a decreased lipid production or production of lipids with higher melt temperature can cause inefficient formation of the lipid layer and increased rate of evaporation. The alteration in the lipid composition in dry eye patients and healthy volunteers has been determined using various techniques. Recently, nuclear magnetic resonance studies have shown the compositional difference between the MGD patients and the normal individuals [16]. It was shown that the meibum obtained from MGD patients had higher lipid order than the normal individual meibum. A higher lipid order and phase transition temperature has also been evidenced from IR studies with the samples of the meibum from donors with meibomian gland dysfunction [17,18]. Additionally,

Please cite this article as: M. Dwivedi, et al., Biophysical investigations of the structure and function of the tear fluid lipid layers and the effect of ectoine. Part B: Artificial lipid films, Biochim. Biophys. Acta (2014), http://dx.doi.org/10.1016/j.bbamem.2014.05.007