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Interaction of lysozyme with a tear film lipid layer model: A molecular dynamics simulation study

Alicja Wizert[a], D. Robert Iskander[a], Lukasz Cwiklik[b,*]

[a] Department of Biomedical Engineering, Faculty of Fundamental Problems of Technology, Wroclaw University of Science and Technology, Wroclaw, Poland
[b] J. Heyrovský Institute of Physical Chemistry, Czech Academy of Sciences, v.v.i., Prague, Czech Republic

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ABSTRACT

The tear film is a thin multilayered structure covering the cornea. Its outermost layer is a lipid film underneath of which resides on an aqueous layer. This tear film lipid layer (TFLL) is itself a complex structure, formed by both polar and nonpolar lipids. It was recently suggested that due to tear film dynamics, TFLL contains inhomogeneities in the form of polar lipid aggregates. The aqueous phase of tear film contains lachrymal-origin proteins, whereby lysozyme is the most abundant. These proteins can alter TFLL properties, mainly by reducing its surface tension. However, a detailed nature of protein-lipid interactions in tear film is not known. We investigate the interactions of lysozyme with TFLL in molecular details by employing coarse-grained molecular dynamics simulations. We demonstrate that lysozyme, due to lateral restructuring of TFLL, is able to penetrate the tear lipid film embedded in inverse micellar aggregates.

1. Introduction

Tear film is a thin layer of fluid that covers the eye. Tear film is important to the health and optics of the eye and it forms a barrier against the outside environment [1,2]. The molecular composition of tear film is being better and better understood and provides knowledge about tear film dynamics [3], mechanisms of its instabilities [4], interactions of tear film with contact lens material [5,6], and the etiology of the dry eye disease [7]. Still, several issues related to tear film composition are far from being resolved, in particular regarding tear film lipids and their function [8,9]. Human tear film is rich in proteins. In studies of Green-Church et al. [10] and Souza et al. [11], 97 and 491 unique proteins have been identified in the human tear film, respectively. That number has been recently increased to 1543 proteins [12]. From that large number, lipocalin, lysozyme and lactoferrin take up to about 80% of the total protein concentration.

Lipocalin, previously known as prealbumin [13], is a major protein of the tear fluid that is also present in other body fluids and found in other species [14]. It comes from a family of lipid-binding proteins, appears to lower tear surface tension [15] and acts as a scavenger protecting the underlying epithelium [16]. Lysozyme constitutes approximately 20 to 40% of the total tear protein [17]. It is an antibacterial protein with a major role to equip the eye with resistance to infection [18]. A reduction of lysozyme in tear film has been associated with several eye diseases including dry eye [19], where a reduction of tear lipocalin is also observed [20], herpetic eye [21], and observed in patients prescribed with beta blockers [22]. Lactoferrin plays an important role in the maintenance of ocular health, particularly in relation to immunological protection [23]. It has been suggested that quantification of each of those three major tear proteins could serve as a single supplemental biomarker for assessing ocular surface [20,24].

In the classical three-layer model of tear film, tear proteins of lachrymal origin are present in the aqueous layer, which is covered by the outer lipid layer, so-called tear film lipid layer (TFLL) [25,26]. This lipid structure is fundamental for maintaining tear film stability. Its function is typically associated with ability to reduce surface tension of tear film [13]. Moreover, it is assumed that TFLL reduces friction during blinks, promotes tear film re-spreading, and reduces tears evaporation [27]. A typical model of tear film organization assumes that TFLL forms a relatively flat multilayered structure atop the aqueous subphase [25]. As demonstrated in our previous molecular dynamics (MD) simulations [28,29], such a lipid arrangement can be expected to exist under relaxed static conditions. However, lateral compression and decompression of the lipid layer resulting from eye blinks cause tear film to undergo a constant restructuring. This leads to formation of three-dimensional lipid structures [30–32], some of them in a form of polar lipid aggregates, both in the water subphase and in nonpolar layers of TFLL [28]. Those of the aggregates that reside in nonpolar layers typically resemble inverse micellar structures and may serve as polar lipid reservoirs during tear film restructuring.

* Corresponding author.
E-mail address: lukasz.cwiklik@jh-inst.cas.cz (L. Cwiklik).

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Biological lipid layered structures contain numerous and often specific proteins. For instance, plasma membrane which structurally is made of a lipid bilayer as well as the pulmonary surfactant that is basically formed by a lipid monolayer is both rich in proteins [33]. Similarly, it was observed that lipid-protein interactions play a role in TFLL. In particular, experiments show that tear film proteins add to reducing surface tension by adsorbing to, or even penetrating the outer lipid layer [34,35]. However, the mechanisms of such adsorption or penetration and, in general, the issues related to protein-lipid interaction in tear film, are presently not well understood.

The main protein known for altering TFLL properties is lysozyme [35]. One of the recommendations of the International Workshop on Meibomian Gland Dysfunction [7] is to focus future studies on finding interactions between the lysozyme and tear lipids and to ascertain whether structural changes in the lipid layer caused by lower lysozyme concentration, could lead to increased rates of evaporation. Hence, the aim of this study is to evaluate the interactions between the lysozyme molecules and TFLL using the recently developed realistic model of tear film lipid layer [28] in the framework of coarse grain MD simulations.

2. Methodology

The considered model of TFLL consisted of a mixture of polar and non-polar lipids simulated at the water-air interface [28]. Polar lipids were chosen to mimic the experimentally obtained lipidome of the tear film [36]. These polar components included 1-palmitoyl-2-oleoyl-phosphatidylcholine (POPC), 1 palmitoyl-2-oleoyl-phosphatidylethanolamine (POPE), *N*-palmitoyl-D-erythro-sphingosine (Cer), and *N*-palmitoyl-D-erythro-sphingosyl-phosphorylcholine (SM). The composition of the polar subphase (in mol percent: POPC – 68%, POPE – 22%, SM – 5%, Cer – 5%) mimicked the experimentally obtained lipid ratios [36]. The non-polar lipids were modeled by a mixture of glycerine trioleate (TO) and cholesteryl oleate (CO) which are the two most abundant nonpolar lipids as observed experimentally [25]. TO to CO molar ratio of roughly 1:1 was arbitrarily chosen as detailed lipidome data are not available. The number of nonpolar lipids was chosen as to be able to form a relatively thick multilayer at the interface so that the outside (nonpolar lipid-vacuum) interface in not affected by the dynamic fluctuations of the polar monolayer during compression. MD simulations were carried out employing the coarse grain MARTINI force field [37]. It enables simulation length- and timescales required for description of multicomponent lipid films under varying conditions while providing a near-atomistic description of considered molecular species. The MARTINI force field was previously successfully used for the description of lipid films [38–40], including TFLL models [28–30,41,42]. Lipid force field parameters were taken from the MARTINI library while the missing Cer parameterization was obtained by using SM residue with the headgroup beads exchanged to a single bead of polar interaction (P1) type [37]. The molecular structure of lysozyme from the Protein Data Bank (PDBID: 1HEL) was employed [43], and the MARTINI coarse grain representation of the protein (both structure and topology) was created using martnize.py tool. The elastic network approach was employed with the elastic bond force constant set to 500 $kJ{\cdot}mol^{-1}{\cdot}nm^{-2}$ and lower and upper elastic bond cut-off to 0.5 and 0.9 nm, respectively. Finally, the protein structure was minimized, solvated, and simulated for 100 ns for equilibration purposes, with root mean square displacements used to control the equilibration. The ability of the elastic network constraints to maintain the structure of protein during its interaction with lipid film was confirmed (see Fig. S1 in SI).

Simulations of the TFLL model were performed in the periodic box containing a slab of water placed in the middle of the box. The box was elongated in one direction hence forming two independent air-water interfaces. Two lipid films of the same composition were placed at both interfaces. The presence of two interfaces allows for obtaining better statistics as two system replicas are simulated during a single simulation run. Such a simulation box setup is standardly used in lipid film studies. Two systems with different sizes were considered in simulations: a small one with a single lysozyme molecule and a big one with 32 lysozyme molecules in the water phase. The small system contained in total (at two interfaces) 6400 lipid molecules (1456 polar and 4944). The big simulation box was employed to improve the sampling of protein-lipid interactions and contained in total 25,600 lipid molecules (5824 polar and 19,776 nonpolar). The two considered systems contained 86,840 and 630,345 water beads in the slab, respectively. Note that each water bead in the MARTINI model represents four actual water molecules. The composition of the lipid film was chosen in accord with our previously developed model [28]. We employed area per polar lipid (APPL, defined as the number of polar lipids at the interface divided by the interface area) to characterize lateral packing of lipids. The sizes of the boxes were $47 \times 47 \times 104\ nm^3$ and $22 \times 22 \times 104\ nm^3$. These values corresponded to APPL = 67 $Å^2$; in some of simulations the boxes were further laterally compressed to reduce APPL, maximally down to APPL = 45 $Å^2$. These two lateral compressed systems correspond to flat and undulated TFLL model [28] and mimic the tear film under varying surface pressure expected during eye blink. The above-described system setup regarding the lipid film corresponds to the setup that was employed in our previous study [28] but here larger lateral box sizes are considered because of the presence of the protein.

Molecules of lysozyme were introduced in the water phase, approximately in the middle of the slab in order to avoid initial protein-TFLL interactions. Either a single or up to 33 proteins were considered. Chloride counterions (eight per each lysozyme molecule) were introduced to neutralize the protein charge. As we verified, the ions during simulations were not in direct permanent contact with the protein. The systems were shortly equilibrated (tens of nanoseconds) upon introduction of the protein; note that this equilibration timescale is much shorter than the timescale of protein diffusion toward the interface.

MD simulations were performed employing the GROMACS 4.6.5 software suite [44] using a standard protocol advised for MARTINI simulations with this version of GROMACS [45]. Namely, 1.1 nm cut-off was employed for non-bonded interactions using the potential-shift-Verlet method implemented in GROMACS. The reaction-field algorithm was used to account for long-range electrostatics with the relative electrostatic screening parameter equal to 15. Equations of motions were integrated with 10 fs time step. The temperature of 310 K was controlled using the velocity rescale algorithm with 1.0 ps coupling parameter. Simulations with the fixed APPL were performed within the canonical ensemble with the size of the box kept constant. Trajectories of 500 ns were calculated with first 150 ns of each simulation treated as equilibration. Different variants of non-equilibrium simulations of lateral squeezing of the interfacial film were also performed, with lateral compression realized by employing semi-isotropic barostat algorithms (either Berendsen or Parrinello-Rahman) with the pressure between 1 and 3 bars [46,47]. The simulation time in this case varied between 140 and 900 ns. Free-energy profiles of protein penetration into the lipid film were calculated from potential of mean force obtained employing the umbrella sampling MD simulations [48]. The distance between the protein center of mass and the water slab mid-plane was used as a reaction coordinate. Initial windows configurations with the spacing of 0.1 nm were generated by the pulling algorithm employing the force constant of 3000 $kJ{\cdot}mol^{-1}{\cdot}nm^{-2}$ and the pull rate of 0.008 $nm{\cdot}ps^{-1}$. In free energy calculations, surface tension coupling was employed to allow for lateral restructuring of the film during protein penetration, with the surface tension of 727 bar nm (the value estimated from previous NVT simulations) controlled with Berendsen algorithm. Each window was then simulated for 50 ns with the reaction coordinate restrained using harmonic force with 3000 $kJ{\cdot}mol^{-1}{\cdot}nm^{-2}$ force constant. Initial 25 ns were treated as equilibration, therefore, not used in further analysis. Weighted Histogram Analysis Method was then used to