In-silico optimized synergy of selected osmo-protectants (proline-betaine and glycosyl-glycerol) against UV- and surfactant-induced irritation

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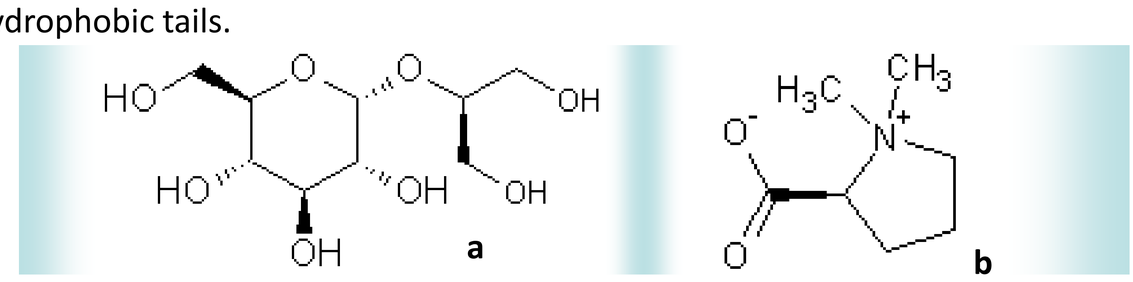
Introduction

Plants and animals living in extreme environments survive severe conditions such as high temperature, pressure or salinity. One of their strategies consists in the accumulation in cellular fluids of small, highly water-soluble molecules, able to reduce the osmotic stress of cells, known as osmo-protectants. These are small organic molecules, typically amino acids or polyols that also stabilize the native state of proteins against denaturation and protect phospholipid bilayer structures from thermal destabilization [1]. Most studies on the mechanism of osmo-protectors has been so far focused on thermal and chemical protein denaturation [2,3,4]. Comparatively little attention has been devoted to the osmo-protectors action on cell membranes. These are very complex systems, consisting of a phospholipids mosaic, of intercalated cholesterol molecules and membrane proteins or glycoproteins. Describing such system with atomistic models is not possible, but simplified models can be built to shed light on general aspects of membrane through computer simulations. A common feature is the phospholipid bilayer, formed in prevalence by neutral phospholipids, with polar heads and hydrophobic tails.

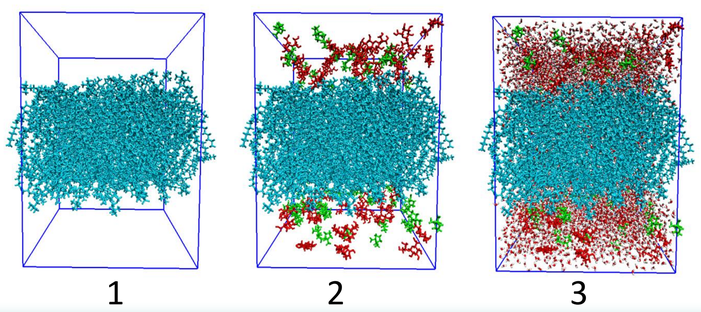


This simplified model allows to understand some basic aspects of membranes without the minute details, as well as to describe the most general aspects of interactions between the membrane and co-solvents, as the molecules of osmo-protectors (those considered here are **proline-betaine** and **glycosyl-glycerol** (Fig. 1a,b). The way in which different osmo-protectors in solution are arranged with respect to the phospholipid membrane has been studied, and how this arrangement is affected by different proportions of two osmo-protectors. In-silico modeling brought to forecast protective and lenitive effects of associated osmotic protectants. Indeed, osmo-protectants could set a specially organized arrangement in water, able to protect cells membrane by-layers from environmental harms. This might enhance the immune defenses from bacterial, physical and allergic stress. This hypothesis was successively confirmed by in-vivo physical and chemical challenge and supported the efficacy of proline-betaine and glycosyl-glycerol association.

Materials and methods

In-silico study **-** This model consists of a double layer of 128 molecules of dipalmitoylphosphatidylcholine (DPPC). In the simulation box, a different number of osmo-protectors molecules of each type has been added to reproduce the different simulated osmolytes concentrations. The total concentration used in all cases is 1 M. When two osmolytes were simultaneously present, their concentrations sum amounted to 1 M. DPPC bilayer initial structure was obtained from Tielmann et al. [5]. Molecular dynamic simulations where performed using Gromacs 4.5.3 program.

In-vivo tests **-** Two in-vivo tests (20 volunteers, 18 - 60 y, I-III Fitzpatrick's photo-type) were carried out with a gel containing a combination of osmotic-protectants in soothing-repairing the chemically/physically damaged skin barrier. The active gel (Aqua, Hydroxyethylcellulose, Proline-Betaine 1.00%, Glyceryl-Glucoside 1.00%, Preservative) was compared to the untreated area (volar forearm, randomly assigned). Two different damages: occlusive patch with 2% aq SLS or 2MED UV irradiation [6]. In the SLS test, we measured TEWL, while erythema was evaluated in the UV test. The data at different times (24h, 48h, 72h of application, twice a day) were statistically compared by means of ANOVA Friedman and Kendall's Coefficient of Concordance for non-parametric and dependent data. The variations were compared by means of Wilcoxon Test for non-parametric and dependent data.

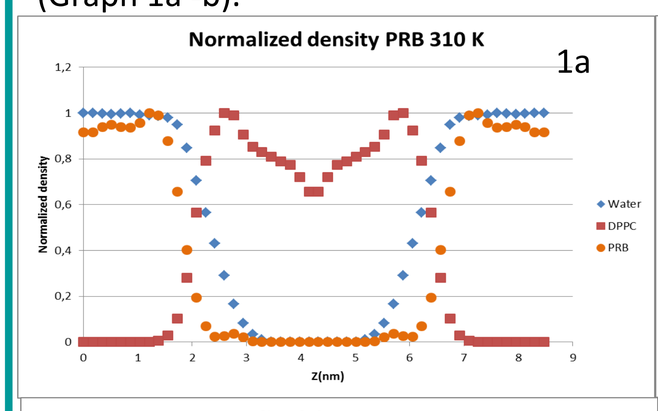


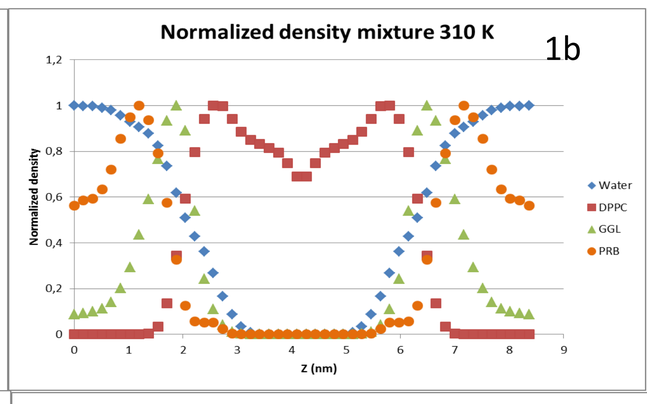
System preparation is schematically drawn: 1)the membrane is inserted into a virtual simulation box 2) osmoprotectors are added 3) the whole system is solvated with water.

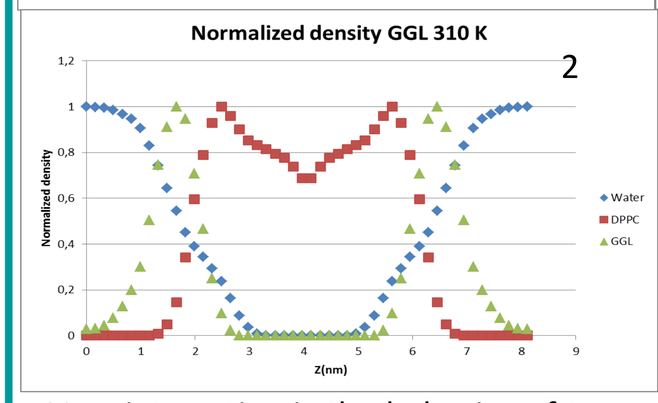
Results and discussion

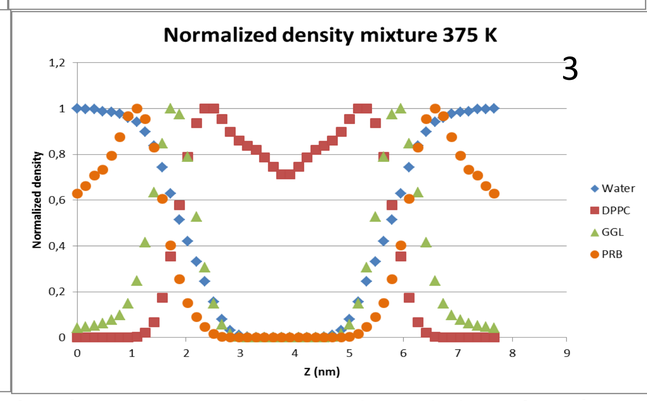
In-silico study **-** To evaluate the distribution of osmoprotectors, 20 ns long molecular dynamics simulations were performed. The density, a function of the coordinates along the vertical axis (Z in graphs) of the solvation box, of the different components was calculated, once reached the equilibrium. The density of DPPC as function of the selected coordinate is zero for Z values corresponding to the box edges. Glycosyl-glycerol (GGL) and proline-betaine (PRB) had radically different behavior.

The trend of the normalized density of proline-betaine is similar to that of water, while glycosyl-glycerol crowds in the vicinity of the double layer, deeply penetrating among the polar heads of phospholipids and interacting with them (Graph 1a -b).

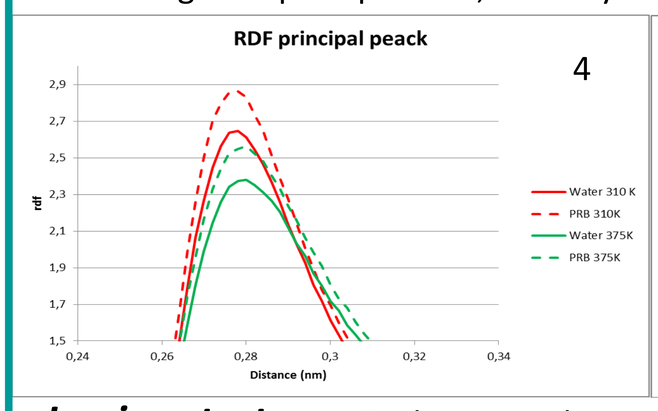


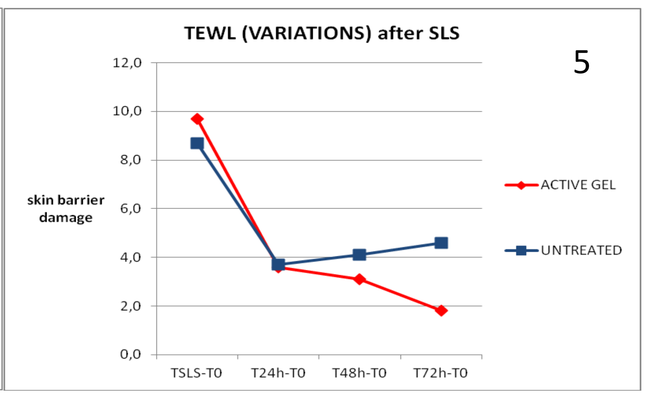






Very interesting is the behavior of two mixed osmo-protectors. At a 1:1 ratio, the density of both osmolytes shows a double peaked distribution (Graph 2). This suggests that the two mixed osmoprotectors exert their protective action on the membrane with different modalities. Glycosyl-glycerol interacts directly with the membrane and Proline-betaine forms a second layer adjacent to the first, stabilizing the locally ordered structure of water. At high T (375°K), in heat stress conditions, a slight compaction of the phospholipid bilayer, due to strengthening of hydrophobic interactions, was visible from the higher density in the aliphatic tails region of the phospholipid bilayer, with respect to 310°K simulations (Graph 3). The behavior of individual osmo-protectors is similar to that observed at 310 K. To test the hypothesis that proline-betaine help protecting the phospholipid membrane, two simulations of 1 M solutions of proline-betaine at 310 and 375°K respectively were performed. The radial distribution function between the oxygen atoms of water has been obtained (rdf_{o-o} henceforth). Its trend informs on the water microscopic structure and its level of order. In particular, for each simulation the rdf_{o-o} must be compared with that of pure water at the same T. The presence of osmo-protector influences the network of hydrogen bonds in the microscopic structure of the solvent. In Graph 4 we see how, at the same T, the rdf_{o-o} peak is more marked in presence of proline-betaine, which therefore stabilizes the local structure of water. It can also be noticed that the presence of osmoprotector does not change the peak position, but only its height.

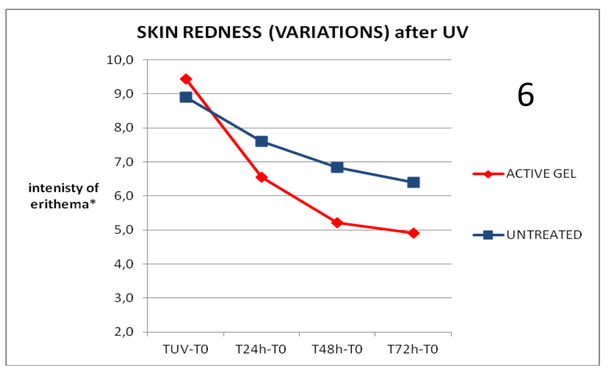




In-vivo tests **–** Both tests showed statistically significant improvements ($p<0.05$) of TEWL values and skin redness in the area treated with the gel containing the osmo-protectants (Graph 5 & 6). For the SLS test, the comparison between treated and untreated area showed a statistically significant difference 72 h after SLS solution removal. In the UV test, the product application on an UV damaged area induced a statistically significant decrease of skin redness values (lenitive effect) already 24 h after UV exposure.

Conclusions

Once more, we can confirm the high forecast potential of the in-silico modeling approach that allows to understand and predict the skin protective actions of hydro-soluble small molecules via their specific interactions with biomolecules in different simulated environments.



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