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Research paper

Binding free energies of small-molecules in phospholipid membranes: Aminoacids, serotonin and melatonin

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HIGHLIGHTS

- A competition between small-molecule binding to water and to DPPC has been observed, with stable structures including simultaneous binding to both DPPC and water.
- Histidine is solvated by water most cases, with very low affinity to the membrane.
- Serotonin shows huge affinity for the phospholipid interface, where most stable binding sites are those of glycerol and phosphate oxygens.
- All molecules stay at the water-membrane interface, so that crossing the membrane by diffusion is highly uncommon in the time scale of our simulations.

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ABSTRACT

Free energy barriers associated to the binding of small-molecules at phospholipid zwitterionic membranes have been computed at 323 K for a variety of species: tryptophan, histidine, tyrosine, serotonin and melatonin bound to a model membrane formed by di-palmitoyl-phosphatidyl-choline lipids inside aqueous sodium chloride solution. We have computed the radial distribution functions of all species for a variety of membrane and water-related sites and extracted potentials of mean force through the reversible work theorem. In all cases but histidine, the molecular probes are able to either be fully solvated by water or be embedded into the interface of the membrane. Our results indicate that binding of all species to water corresponds to free energy barriers of heights between 0.2 and 1.75 kcal/mol. Free energy barriers of association of small-molecules to lipid chains range between 0.6 and 3.1 kcal/mol and show different characteristics: all species but histidine are most likely bound to oxygens belonging to the phosphate and to the glycerol groups. Histidine shows a clear preference to be fully solvated by water whereas the aqueous solvation of serotonin is the less likely case of them all. No free permeation through the membrane of any small-molecule has been observed during the time span of the simulation experiments.

1. Introduction

The principal components of human cellular membranes are phospholipids, cholesterol and proteins, all of them embedded in a salty water solution. Phospholipid membranes provide the framework to biomembranes and they consist of two leaflets of amphiphilic lipids which are molecules with a hydrophilic head and one or two hydrophobic tails [1]. The fluidity of the membrane is mainly regulated by the amount of cholesterol, in such a way that membranes with high cholesterol contents are stiffer than those with low amounts but keeping the appropriate fluidity for allowing normal membrane functions.

In this letter we have focussed our efforts in the study of zwitterionic phospholipid membranes that can help understand basic biological membrane functions and its interaction with the environment. As an example of a prototype membrane, the one formed by di-palmitoyl-phosphatidyl-choline (DPPC) is one of most relevant of all, being a major constituent (about 40%) of pulmonary lungs [2]. In addition, human lungs are coated with a lattice-like structure formed by protein and lipid mixture called lung surfactant, preventing the lungs from collapsing and protecting us from bacterial and viral infections. A large number of simulations have already been performed on DPPC, often including the influence of cholesterol in water environments [3]. On the other hand, the role of proteins and drugs and their interactions with the membrane structure is undoubtedly a relevant field of research. In this work we have considered the introduction into the lipid

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bilayer structure of small biological probes of different kinds: three aminoacids, namely tryptophan [4] (TRP), histidine-E (HIS) and tyrosine (TYR); the neurotransmitter serotonin [5] (SRO) and the hormone melatonin [6] (MEL).

Aminoacids are organic compounds containing amine (-NH2) and carboxyl (-COOH) functional groups, along with a side chain (R group) specific to each aminoacid. It is well known that aminoacids can either be *essential*, i.e. indispensable or *non-essential*. An essential aminoacid cannot be synthesized *de novo* by the organism and it would be necessarily supplied by the diet. The nine aminoacids humans cannot synthesize are: phenylalanine, valine, threonine, tryptophan, methionine, leucine, isoleucine, lysine, and histidine.

Tryptophan is able to act as a building block in protein biosynthesis, while proteins are fundamentals required to sustain life. In addition, it helps in the regulation of human sleep. In turn, histidine is an alpha-aminoacid that is also used in the biosynthesis of proteins. It is positively charged at physiological pH. Initially thought essential only for infants, longer-term studies have shown it is essential for adults also. Differently, tyrosine is another of the 20 standard aminoacids that are used by cells to synthesize proteins, but it is non-essential. Tyrosine is required for the synthesis of the neurotransmitter dopamine. We selected these three particular aminoacids to explore whether or not their interactions with zwitterionic cell membranes are related to their essential or non-essential characteristics.

Serotonin is a neurotransmitter biochemically derived from tryptophan and it is primarily found in the gastrointestinal tract, blood platelets and at the central nervous system of animals, including humans. It is thought to be a contributor to the regulation of human mood and happiness. Serotonin can be converted to melatonin (a neurohormone), that may help humans to the regulation of biological rhythms, to induce sleep, to work as a strong antioxidant and also contribute to the protection of the organism from carcinogenesis and neurodegenerative disorders such as Alzheimer's disease [7].

In summary, given the importance of aminoacids, neurotransmitters and hormones for the correct function of the body, we have explored their interactions with the prototypical cell membrane formed by DPPC and water in sodium chloride solution using all-atom molecular dynamics (MD) simulations, analyzing its local structure through free energy profiles based on the reversible work theorem. Some previous studies indicated the strong interaction of serotonin with di-myristoil-phospahtidyl-choline (DMPC) and di-oleoyl-phosphatidyl-choline (DOPC) membranes [8] or of some neurotransmitters with DPPC [9].

We provide the details of the simulations in Section 2 and explain the main results of the work in Section 3, focusing our attention especially on the free energy barriers of the adsorption of small-molecule species. Finally, some concluding remarks are outlined in Section 4.

2. Methods

2.1. Preparation of simulations

A model of a zwitterionic lipid bilayer membrane in aqueous sodium chloride solution has been build by means of the CHARMM-GUI tool [10,11]. The membrane was composed by: 204 lipids, distributed in two leaflets of 102 flexible DPPC ($C_{40}H_{80}NO_8P$) molecules, surrounded by TIP3P [12] water (W) molecules (enough to ensure full hydration in all cases), with 17 sodium and 17 chlorine ions, corresponding to physiological concentration, plus one small-molecule. In order to compare several probes of different chemical structure and able to performing a variety of biological functions, we considered five species. Three aminoacids: tryptophan ($C_{11}H_{12}N_2O_2$); histidine-E ($C_6H_9N_3O_2$) and tyrosine ($C_9H_{11}NO_3$), a neurotransmitter, serotonin ($C_{10}H_{12}N_2O$) and a hormone, melatonin ($C_{13}H_{16}N_2O_2$).

Sketches of the backbone structure of the small-molecules and DPPC are represented in Fig. 1. Each molecule was described with atomic resolution. MD simulations were performed with the NAMD2 simulation package [13] at a fixed temperature of 323.15 K and at the averaged pressure of 1 atm. At this temperature, the DPPC membrane is fully at the liquid crystalline state (see for instance Refs. [14,15]). The temperature was controlled by a Langevin thermostat [16] with a damping coefficient of 1 ps^{-1}.

Initially, we employed the CHARMM-GUI tool to generate a full set-up consisting of the small-molecule embedded in the DPPC bilayer membrane inside the aqueous ionic solution. This was performed online (see http://www.charmm-gui.org/?doc=input/membrane) and involved a series of steps indicated by the owner of the software, which produced a package including input files for energy minimization and thermal equilibration of the system. The final output was that the small molecule was initially placed at the center of the system ($z \sim 0$) and it slowly evolved towards its equilibrium position, normally at the bilayer interface.

We considered all systems at the isobaric-isothermal ensemble. i.e. at constant number of particles (N), pressure (P) and temperature (T) conditions, with equilibration periods for all simulations of more than 40 ns. After equilibration, we recorded statistically meaningful trajectories of more than 80 ns. A typical size of the system was of 80 Å × 80 Å × 81 Å, regardless of the probe considered, since the biggest part of the membrane was made of the same components, i.e. DPPC, water and ions in exactly the same concentrations. The simulation time step was set to 2 fs in all cases. We considered the CHARMM36 force field [17,18], which is able to reproduce the area per lipid in excellent agreement with experimental data. All bonds involving hydrogens were fixed to constant length, allowing fluctuations of bond distances and all sorts of angles for the remaining atoms. Van der Waals interactions were cut off at 12 Å with a smooth switching function starting at 10 Å. Long ranged electrostatic forces were taken into account by means of the particle mesh Ewald method [19], with a grid space of about 1 Å. Electrostatic interactions were updated every time step. Finally, periodic boundary conditions were applied in the three directions of space.

As a general fact, we did not observe any natural permeation of a small-molecule across the DPPC membrane (from one interface to the other) at the time scale of our simulations, in agreement with the findings of Wood et al. for serotonin and tryptophan adsorbed at a 1-palmitoyl-2-oleoyl-phosphatidyl-choline (POPC) membrane [20]. This is in good qualitative agreement with the reported work of Kell et al. [21] on pharmaceutical drug permeation, who stated that diffusion of a small-molecule or drug through a cell membrane can only happen by means of the help of some mediating-carrier. Nevertheless, other authors such as Di et al. [22] reported evidence of pure diffusion of small drugs across membranes, like in the case of brain-blood barrier permeation of lipophilic small-molecules [23]. From our findings we cannot support any of these results, essentially due to the limited length of our calculations in the range of 100 ns, given that some diffusion processes may occur at longer time scales.

2.2. Calculation of free energy differences

A common way to analyze the microscopic forces relevant for the binding process is obtaining the Helmholtz free energy by means of the so-called potential of mean force (PMF) between particles 1 and 2, namely $W_{12}(r)$, that can be readily obtained from the pair (atom-atom) radial distribution function $g_{12}(r)$ given by:

$$g_{12}(r) = \frac{V \langle n_2(r) \rangle}{4 N_2 \pi r^2 \Delta r}, \quad (1)$$

where $n_2(r)$ is the number of atoms of species 2 surrounding a given atom of species 1 inside a spherical shell of width Δr. V stands for the total volume and N_2 is the total number of particles of species 2. $W_{12}(r)$ is the reversible work required to move two tagged particles from infinite separation to a relative separation r (see for instance Ref. [24], chapter 7):