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Small molecule interaction with lipid bilayers: A molecular dynamics study of chlorhexidine

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ABSTRACT

Chlorhexidine (CHX) is an effective anti-bacterial agent whose mode of action is thought to be the disruption of the cell membrane. We tested the capability of the Slipids all atom force fields using data from neutron scattering and NMR experiments on the drug chlorhexidine in a 1,2-dimyrisoyl-3-*sn*-phosphatidylcholine (DMPC) membrane. Since it is not known what the charge of the CHX molecule is inside an apolar environment, a neutral, as well as a +1 and +2 charge model for the molecule were created and tested at several concentrations. This study shows that the location of CHX is minorly dependent on concentration, and dominantly reliant on the charge. The effect of adding CHX to DMPC is a thinning of the membrane, thus increasing the area per lipid.

1. Introduction

Molecular dynamics (MD) simulations, have become one of the powerful techniques to analyze the dynamic motion and gross structure of bio-membranes. MD simulations can reproduce the experimental environment of molecules in a computer and provide atomic-level information not detected in experiments. Over the last two years, more advanced force fields specific for lipid molecules have been developed, with experimental results in mind. Mainly, simulations are verified if they can properly reproduce NMR S_{CD} order parameters and form factors from neutron and X-ray scattering experiments [1–3].

Atomistic molecular dynamic simulations have tremendous utility for providing quantitative thermodynamic and mechanistic analysis of small molecule–lipid interactions. However, the typical time scale of such large simulations are below 200 ns, somewhat insufficient to detect larger molecule displacements or to obtain complete conformational sampling. Such simulations are made all the more difficult when looking for the effects of high concentration of the molecule, which only lengthens the computational time. This can preclude answering the typical key question of a membrane-active compound; where does a given solute reside within a bilayer and what factors govern its membrane binding or partitioning at equilibrium?

Coarse grained force fields have the advantage of having a simplified version of the modelled system which gives it the leverage of needing less computational time, leading to larger and longer simulations. However, coarse grain models have faired much worse in reproducing the thermodynamics of charged molecule–membrane interactions as a function of its protonation state, such as the case of the amino acid arginine important for ion channel modelling [4]. Although it is rare for a particular force field to be able to reproduce every known system, it is important to understand their limitations.

Biguanides are an important class of compounds, which resemble arginine, having multiple tautomers and protonation states. Their particular structure have shown to have extensive medical applications. Proguanil (an antimalarial agent) and Metformin (an antidiabetic compound) are biguanide derivatives, which are available as drugs [5,6]. Other important compounds in this series are phenformin, buformin, chlorophenylbiguanide, and chlorhexidine. Apart from the well-established antidiabetic and antimalarial effects, biguanide derivatives have been shown to exhibit antimicrobial, antiviral, and antiplaque effects and also have been known to influence gastric acid secretion [7–9].

We wanted to test the capability of one new lipid force field using data from neutron scattering to predict the partition location of the biguanide derivative drug chlorhexidine (CHX), shown in Fig. 1. This elongated flexible molecule presents an

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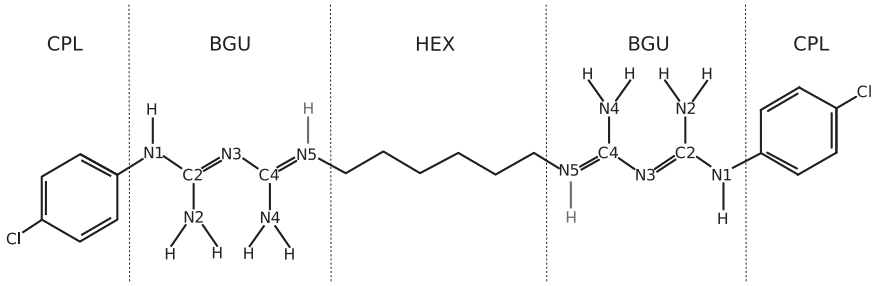

Fig. 1. Chlorhexidine representation with three groups; chlorophenol (CPL), biguanide (BGU) and hexane (HEX). Additional hydrogen atoms needed to create a CHX models with +1 and +2 charge are shown on the biguanide N5.

interesting biophysical modelling challenge; symmetrically composed of a (hydrophobic) hexane linker joining two (polar, hydrophilic) biguanides and (lipophilic) chlorophenol rings. We expect the free-energy competition between these subunits to determine the location of the CHX within a bilayer. We have previously experimentally established the location of the hexane of CHX in DMPC, at two high molar concentrations, using neutron diffraction in a manner that yields the time-averaged distribution within the bilayer.

The p*K*a in water of the similar compound, poly(hexamethylene-biguanide hydrochloride) (PHMB), was estimated to be around 13.5, meaning in aqueous solution at pH 7, PHMB will most likely exist in its +1 ionized form. Modelling studies have further showed that the most stable form of PHMB is one that has the +1 charge delocalized over the whole biguanide section, as would occur in the conjugated tautomer [10]. For CHX, the p*K*a of the singly ionized form was estimated to be 10.15, and doubly ionized at 9.55 [11]. The lower p*K*a indicates the biguanide in CHX is easier to deprotonate, but harder to protonate, than PHMB, becuase the chlorophenyl is less electron donating than an alkyl group [11].

However, the p*K*a of CHX or any biguanide has not been estimated when in an apolar environment. Only recently has it has been shown that the amino acid arginine may remain at least fractionally protonated even when in a low dielectric lipid hydrocarbon environment, although without some form of stabilization from additional negative amino acids or lipid molecules, there is significant disruption to the membrane structure as macroscopic quantities of water associate with the charge inside the normally hydrophobic region [12,13,4]. Since our system does not include such a stabilizing negative charge, nor do we see evidence of bulk water in the membrane, we began with the assumption that CHX is neutral, however we tested the +1 and +2 charge forms as well.

We wish to answer the following questions; can a CHX molecule find an equilibrium location in the bilayer (without prior knowledge) within a reasonable simulation time? What are the effects of adding additional charge to the CHX molecule? What are the effects of high concentration of CHX on the limited-sized simulation, and can those perturbations reproduce the experimental results?

2. Computational methods

We constructed an all-atom force field of chlorhexidine from the CHARMM36 force field [14–16] using well established parameters of certain amino acids [17]. Breaking the molecule into three sections as shown in Fig. 1; the chlorophenol ring, biguanide and hexane, for which we selected the bond lengths, angles and torsions from Tyr, Arg, and Lys respectively.

Partial charges were calculated usung GAUSSIAN software [18] and methods used for refining all-atom force feilds [1]. The partial charges taken from the amino acids Tyr, Arg and Lys to create a first approximation model of chlorhexidine. This model was simulated in a box of methanol to produce a dielectric constant similar to that of the headgroup region of lipids where the molecule has been experimentally observed to reside [19]. The simulation was run for 1 ns, where 500 different snapshots of the molecule were taken 2 ps apart. For each conformation, atomic charges were computed with the DFT method using B3LYP exchange-correlation functional with the cc-pVTZ basis set. The partial charges of each atom were then averaged over the 500 snapshots to achieve a partial charge that takes into account the distribution of conformations during a simulation in a lipid system. The newly obtained charges were then used in the structure of chlorhexidine for further simulations.

Additional information is required for an accurate representation of the bond structure and chemistry of the biguanide sections. Amide bond resonance has been a topic of much study, including theoretical studies of guanides and biguanides. [20–22]. The general consensus from these quantum chemistry calculations is that the configuration of the most stable neutral tautomer is characterized by conjugative interaction of the π-bonds, leading to alternating single and double bonds along the backbone, and therefore no hydrogen atom on the central nitrogen atom N3 (See Fig. 1). This means two hydrogen attached to each of the N2 and N4 nitrogen, and the final hydrogen then rests on either N1 or N5. Placing it on N1 fixes the double bonds to be between C2-N3 and C4-N5. The alternative tautomer would likely be entirely equivalent. A +1 charged CHX involves placing an additional hydrogen on the N5 of just one of the ends of the molecule, and a +2 charge would place a hydrogen on the N5 of both biguanide sections.

An initial 3D structure of the neutral, +1, and +2 charged chemical schematics shown in Fig. 1 was constructed using the OpenBabel [23,24] from the appropriate SMILES representation, and then refined by hand in the software Avogadro [25]. The structure was then optimized using self-consistent field theory with closed-shell restricted Hartree–Fock (RHF) wavefunctions to the level of 6-31G* with the software NWChem [26]. The final bond lengths did not differ by more than 0.05 nm from those of the CHARMM36 force field. The final topology of CHX can be found in the supplementary information.

The topology of 1,2-dimyristoyl-3-*sn*-phosphatidylcholine (DMPC) was obtained from the Stockholm lipids website [1,2]. Initial coordinates of a 128-lipid DMPC bilayer in an $8 \times 8 \times 2$ configuration were obtained from CHARMM-GUI [27] with 30 TIP3P water molecules per lipid. The system was checked for correctness by running a pure bilayer simulation for 100 ns at 303 K and 323 K. Such lipid force fields are continually being validated and refined, and reproduce the essential characteristics of pure lipid bilayers accurately [28,29].

All simulations were performed using the GROMACS simulation software (version 4.6.1) [30] for 100 ns at 323 K followed by another 100 ns at 309 K, under periodic boundary conditions in a simple orthorhombic box. Systems containing charged CHX models were neutralized by adding Cl ions into the water in order to achieve a system with zero net charge. The temperature of the