The Influence of Ions in the Interaction of Methylene Blue with DPPC Membranes

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***Abstract*—Methylene blue (MB) and its derivatives have applications as photosensitizers in photodynamic therapy. However, little is known about their interactions with phospholipid membranes at the molecular level. We employed molecular dynamics simulations to model the binding between MB and a dipalmitoyl phosphatidylcholine (DPPC) bilayer. This was done in the presence of molecular oxygen within the membrane. The abitily of MB to induce photodamage was inferred based on its immersion depth and degree of exposition to a higher oxygen concentration inside the membrane. In addition, the effect of the presence of ions in the solvent was explicitly considered.**

Keywords–methylene blue, lipid bilayer, molecular dynamics simulation

I. INTRODUCTION

Photosensitization is the basis of photodynamic therapy (PDT), a technique that has been used to treat various solid tumors in the skin, breast, lung, bladder, and esophagus [1]. The method relies on the administration of a sensitizer molecule that is able to induce the formation of reactive oxygen species (ROS), for example, singlet oxygen ($^1O_2$), when exposed to light of the appropriate wavelength [2]. ROS-mediated reactions in the cell membrane eventually lead to severe tissue damage since these species can act as powerful oxidizing agents, able to induce irreversible photodamage, necrosis or apoptosis, in tumor tissues [1].

The study of biological membranes involves the understanding of the influence of many different organelles and proteins. Model membranes, such as phospholipid bilayers, are often used in PDT studies. In particular, DPPC is the most abundant phospholipid in lung surfactant, primarily responsible for the reduction of surface tension to near 0 mN/m during expiration [3]. DPPC is zwitteronic having a negative charge on the phosphate group and a positive charge on the amine.

Methylene blue is a phenothiazinium dye with the following properties: strong absorbance in the range of 550–700 nm, significant quantum yield ($\phi_\Delta = 0.52$) [2], triplet with long intrinsic lifetime, low fluorescence quantum yield and lifetime, and low reduction potential [4]. Because of these characteristics, it has been used in a variety of photochemical applications including solar energy conversion [4] and PDT [5].

Localization of the sensitizer is a key issue in the understanding and determination of the PDT efficiency [1]. To facilitate drug development, it is often necessary to identify a target. A systematic study of structure-activity relationships can then aid in improving the therapeutic procedure. Our aim in this work was to explore the interaction between a model membrane (DPPC) and a photosensitizer (MB). In addition, the effect of the presence of ions in the solvent was investigated, since they might play a crucial role in determining the structural and electrostatic properties of model bilayers [6]. This allowed us to access the role of solvent ions in the interaction of MB with DPPC.

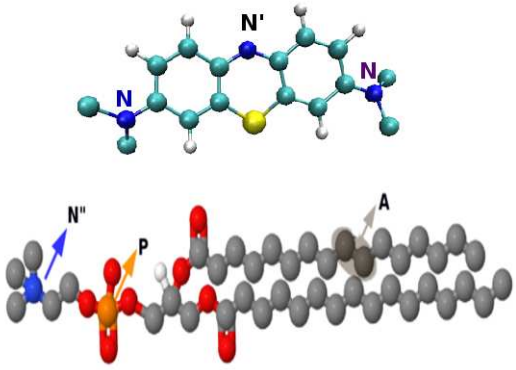

Figure 1. Schematic representation of methylene blue (MB) and DPPC indicating relevant regions and atoms.

This manuscript is organized as follows. In Section II, we briefly describe our theoretical approach. In Section III, our results for the interaction of MB and model DPPC membrane are discussed and compared to other works whenever posssible. Finally, a summary is presented in Section IV.

II. THEORETICAL MODELING

Molecular dynamics simulations were performed using the GROMACS 4.5.1 simulation package [7][8]. Molecular motions were computed by numerical integration of Newton's equations with a time step of 2 fs. Fully hydrated lipid bilayers made of DPPC were represented using the force field developed by Kukol [9]. The interaction parameters were based on the GROMOS53A6 force field [10], in which aliphatic carbon atoms and their adjacent hydrogens are treated as united atoms. Figure 1 shows the structure of methylene blue (MB) and DPPC indicating relevant atoms (N, N', N", and P) and regions (A). To simulate fully hydrated lipid bilayers, the SPC model [11] was used for water. A single oxygen molecule was added to the aqueous phase. The oxygen molecule dissolved

 ISBN: 978-1-61208-380-3

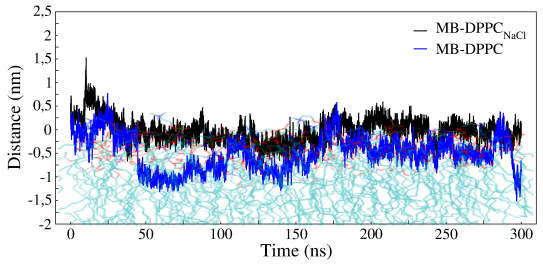

Figure 2. Temporal evolution of the drug-membrane binding process for MB. The top half of the lipid bilayer is also represented in the background.

in the membrane was described with parameters taken from the literature [12]. For compatibility, the methylene blue was assembled using the standard functional groups in the GROMOS53A6 force field [10]. The MB partial charges were taken from the density functional calculations performed using the Gaussian package [13].

Starting configurations for molecular dynamics were obtained from a pre-equilibrated membrane patch with 128 lipid molecules. Methylene blue was initially placed at the aqueous phase at a distance of ca. 3 nm from the bilayer surface. Only one MB molecule was added. A Cl^- ion was added to neutralize the system. In order to simulate systems at polar ionic strength, 27 Na^+ and 27 Cl^- ions are added to the aqueous phase. Overall, each simulated system had lateral dimensions of ca. 6.2 nm parallel to the membrane surface (xy-plane) and ca. 8.5 nm along the bilayer normal (z-axis). Periodic boundary conditions were applied in all Cartesian directions. The simulation protocol started with an equilibration run for 5.5 ns, during which the position of MB was kept restrained. The molecule was then released and molecular trajectories were recorded for 300 ns under controlled temperature (310 K) and pressure (1 atm).

The drug-membrane binding process was followed in time by recording both the position and the orientation of the different photosensitizers with respect to the bilayer. Density distributions of the membrane building blocks and the photosensitizer were calculated along the z axis. The degree of overlap between the distributions of photosensitizers and molecular oxygen was taken as an indicator of the expected $^1O_2$ generation efficiency. Further details of our theoretical modeling can be found in [14].

III. Results and Discussion

In the first step of our study, we have investigated the influence of ions in the MB-membrane binding process. Figure 2 shows the temporal evolution of the MB-membrane binding process. The immersion depth is defined as the distance between the center of mass of the photosensitizer and the water/bilayer interface. The top half of the lipid bilayer is also represented in the background. In both cases, MB started at the aqueous phase and reached the membrane surface within the first 2 or 3 ns, driven by favorable drug-membrane electrostatic interactions. As clearly seen in Figure 2, MB reaches a largest immersion depth, ∼0.5 to 0.8 nm, when ions were not considered, compared to ∼0.2 nm in the case they were included.

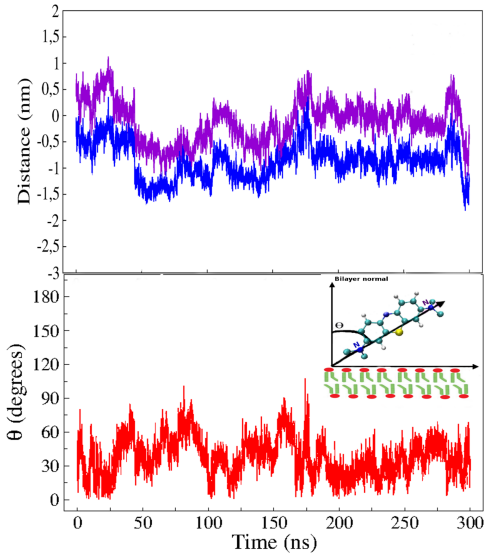

Figure 3. Upper panel: temporal evolution of the positions of the nitrogen atoms with respect to the bilayer/water interface, when ions are not considered. Lower panel: temporal evolution of the angle, θ, between the nitrogen atoms vector with respect to the membrane-water interface.

In order to access the orientation of MB when immersed on the DPPC bilayer, we analyze the relative positions of the the nitrogen atoms, indicated by N in Figure 1. In the upper panel of Figure 3 the temporal evolution of the positions of the nitrogen atoms with respect to the bilayer/water interface (when ions are not considered) is presented. These relative positions allowed us to extract the angle, θ, of the nitrogen atoms vector with respect to the membrane-water interface. The temporal evolution θ is shown in the lower panel of Figure 3. MB immersion occurs with the molecule forming an average angle close to 40° with the membrane-water interface, although a large fluctuation is observed. A similar average angle, ∼35°, is observed when ions are added to the water solution, as shown in Figure 4. However, in this case θ's fluctuation is much smaller. Together, Figures 2, 3, and 4 clearly show that, in the absence of ions, MB presents a higher mobility.

Next, we analyze the density profiles of different atoms or functional groups of MB and DDPC and the oxygen molecule, as shown in Figure 5, when ions are considered, and Figure 6, when ions are not taken into consideration. These density profiles give the spatial probability to find different molecules or functional groups along the bilayer normal. The density profiles for the phosphatidylcholine atoms N" and P and the functional group A are represented by dashed lines. In the insets, the black dashed lines represent the DPPC membranes in both cases.

For both systems, the nitrogen atoms of the AM molecule (N–N_{MB}) present a bimodal distribution (violet solid lines). The membrane surfaces were heuristically defined at the peaks of the phosphate group distribution, since the water density dropped from its bulk value to zero within the region occupied by this group. It is clear from Figure 5 that, in the presence of ions, the methylene blue molecule immersion is in such a way that the one of the symmetric nitrogen atom is in the region

 ISBN: 978-1-61208-380-3

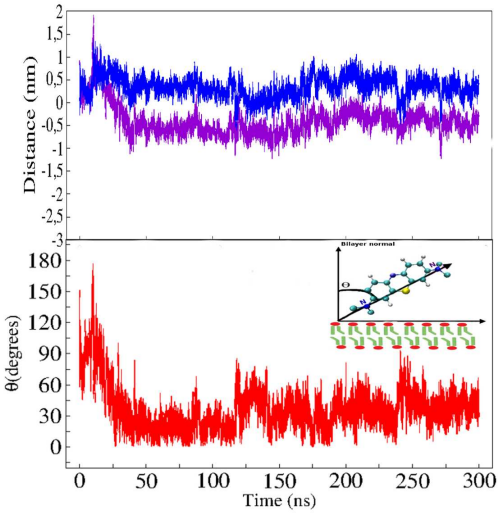

Figure 4. Upper panel: temporal evolution of the positions of the nitrogen atoms with respect to the bilayer/water interface, when ions are considered. Lower panel: temporal evolution of the angle, θ, between the nitrogen atoms vector with respect to the membrane-water interface.

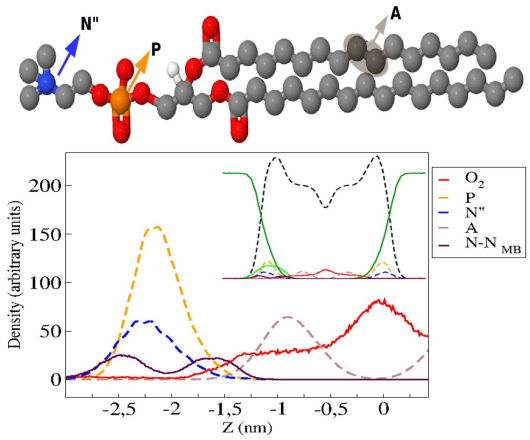

Figure 5. Density profiles, i.e. spatial probability to find different molecules or functional groups along the bilayer normal when ions are considered. Density profiles were arbitrary scaled in order to allow a better visualization.

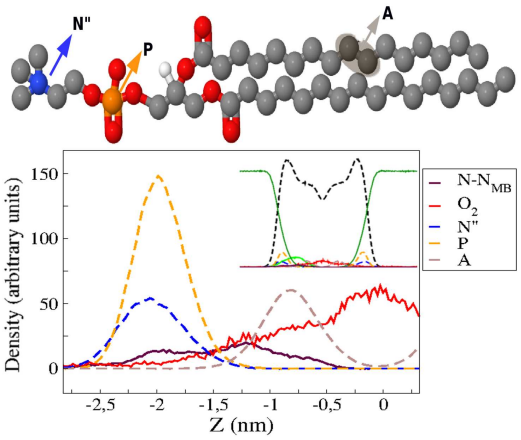

Figure 6. Density profiles, i.e. spatial probability to find different molecules or functional groups along the bilayer normal when ions are not considered. Density profiles were arbitrary scaled in order to allow a better visualization.

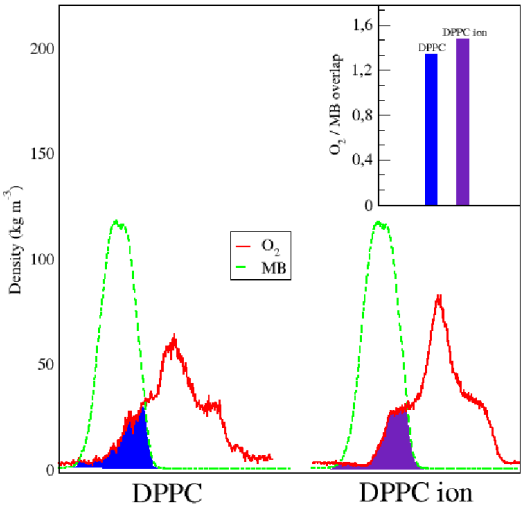

Figure 7. MB and the oxygen molecule distribution along the z axis when ions are explicitly considered in the solvent (left) or not (right). In the inset, the integral overlap between the density profiles of MB (ρ_{MB}) and of molecular oxygen (ρ_{O_2}) along the z axis for both systems are compared.

of the phospholipid head group, indicated by P. When ions are not included, as depicted in Figure 6, the MB molecule immersion is such that the nitrogen atom is closer to the A region, around the region where C=O bonds can be found.

The density profiles in Figures 5 and 6 predict an increased oxygen concentration in the membrane interior as compared with the aqueous phase. Figure 7 shows how MB and the oxygen molecules are distributed along the bilayer normal when ions are present in the solvent (right) or not (left). The probability of electron/energy transfer via type I mechanism can be estimated, in a simple but effective way [14], in terms of the integral overlap between the density profiles of MB (ρ_{MB}) and of molecular oxygen (ρ_{O_2}) along the z axis. This probability can be obtained as

$$P_{O_2/MB} \propto \int dz \rho_{MB}(z) \bullet \rho_{O_2}(z) \qquad (1)$$

where the integral runs over the entire system length along the z axis. A direct correlation can not be assumed between photodynamic efficiency and the probability of ROS generation since the membrane binding might be influenced by the presence of ions in the solvent. The integral overlap O_2/MB is indicated in the inset of Figure 7. Since the calculated values for both cases are very close, we believe that the relative photodynamic efficiency is probably defined by differences in membrane binding.

In Photodynamic Therapy (PDT), an excited photosensitizer can undergo type I (electron transfer) and/or type II (energy transfer) reactions to produce highly reactive oxygen species (ROS), resulting in necrosis and/or apoptosis of exposed cells [1]. Type I reactions generate radical and radical anion species (e.g., O_2^-, HO^-), while type II reactions produce singlet oxygen ($^1O_2$) by energy transfer between a photosensitizer in its excited state and an oxygen molecule.

 ISBN: 978-1-61208-380-3

In this later case, membrane damage results from the tendency of $^1O_2$ to add to hydrocarbon double bonds, forming hydroperoxides. Since DPPC is a saturated lipid, the formation of hydroperoxides via a Type I mechanism is not possible. However, it is already well stablished [15] that eukaryotic cell membranes contain mixtures of glycerolipids (in mammalina cells, all phospholipids except sphingomyelin), sphyngolipids, and sterols. As a consequence, DPPC can be found in cell membranes with different unsaturated lipids. Photodynamic damage is believed to occur in the close vicinity of the sensitizer because teh main cytotoxic product, singlet oxygen, has a short life time. Therefore, it is fair to say that when MB is incorporated in a DPPC domain, the photodamage in vicinal unsaturated lipids might occur. Following this argument, it is possible to infer that the photodamage might occur in the head group region, when ions are included, or close to the hydrophobic region, when ions are not considered.

By unveiling the interaction of DPPC with AM at a molecular level, we are contributing for a better knowledge of structure-activity relationships of such systems. We believe that this will help in improving the the use of AM as a PDT agent.

IV. Conclusions

In this work, molecular dynamics simulations were employed to examine the interactions between MB and DPPC bilayer at a molecular level when ions are present in the solvent or not. We found that the immersion depth of MB is higher in the absence of ions, but the molecule orientation is the same in both cases: MB is slightly tilted. Our data also suggests that MB mobility is also higher when ions are not present. With ions in the solvent, the methylene blue molecule immersion is in such a way that it is placed between the phospholipid head group and the C=O bond region. When ions are not considered, the MB molecule immersion is higher in such way that electron/energy transfer mechanisms might also take place in the hydrophobic region of the DPPC bilayer. In addition, the probability of energy/electron transfer is estimated and found to be very similar whith or without the presence of ions.

Acknowledgements

The authors acknowledge financial support from CNPq and FAPESP (project 2012/50680-5).

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 ISBN: 978-1-61208-380-3