Journal of the Taiwan Institute of Chemical Engineers 000 (2017) 1–7

Contents lists available at ScienceDirect

Journal of the Taiwan Institute of Chemical Engineers

journal homepage: www.elsevier.com/locate/jtice

Effect of number of hydroxyl groups of fullerenol $C_{60}(OH)_n$ on its interaction with cell membrane

Hideya Nakamura*, Yusuke Nozaki, Yuhei Koizumi, Satoru Watano

Department of Chemical Engineering, Osaka Prefecture University, 1-1 Gakuen-cho, Naka-ku, Sakai, Osaka 599-8531, Japan

ARTICLE INFO

Article history:
Received 7 September 2017
Revised 8 November 2017
Accepted 10 November 2017
Available online xxx

Keywords:
Fullerene
Cell membrane
Hydrophilicity
Hydrophobicity
Molecular dynamics

ABSTRACT

Fullerenol $C_{60}(OH)_n$, where multiple hydroxyl groups are chemically bound to surface of a fullerene C_{60}, is a highly potential nanomaterial for biomedical and pharmaceutical applications. To realize such biomedical and pharmaceutical applications, the $C_{60}(OH)_n$ is required to be transported on the cell surface and/or to be transported into the cell interior. However, molecular mechanism underlying the interaction of $C_{60}(OH)_n$ with the cell membrane is still far from being understood. This study presents an atomistic molecular dynamics (MD) simulation study on the interaction of $C_{60}(OH)_n$ with the cell membrane. Here we focused on effect of number of the hydroxyl groups of $C_{60}(OH)_n$ on its adhesion on, penetration into, and permeation across the cell membrane. The MD simulation results suggested that number of the hydroxyl groups of $C_{60}(OH)_n$ can be a critical factor to control the interaction with a cell membrane; *i.e.*, $C_{60}(OH)_n$ with less number of hydroxyl groups ($n \leqq 2$) can penetrate into hydrophobic core of a lipid bilayer, while $C_{60}(OH)_n$ with higher number of hydroxyl groups ($n \geqq 8$) can adhere onto surface of a cell membrane. Our simulation results also revealed that $C_{60}(OH)_n$ with intermediate number of hydroxyl groups ($n = 6$) can permeate across whole lipid bilayer and reach to inside of the cell.

1. Introduction

The biomedical and pharmaceutical applications of nanomaterials have rapidly emerged in recent years. Among various nanomaterials, C_{60} fullerene is a highly potential candidate. By taking advantages of its small size, highly tunable surface properties, and unique structural and electronic properties, many promising applications have been proposed, including drug- and gene-deliveries, therapeutic agents, and diagnostic [1–3]. Meanwhile, it has been reported that C_{60} can cause adverse effects [4], eliciting concerns about their toxicity. To realize the biomedical and pharmaceutical applications, C_{60} is required to be transported on the cell surface and/or transported into the cell interior. In this case, the cell membrane is the most critical barrier. Therefore, it is critical to understand interaction of C_{60} with the cell membrane.

It is well recognized that the pristine C_{60} is extremely hydrophobic and insoluble in water and biological medium, hampering usage in the biomedical and pharmaceutical applications. Thus, chemical modifications of C_{60} to increase hydrophilicity and water solubility are necessary. A variety of C_{60} derivatives modified with various polar functional groups have been developed [5,6]. One extensively studied C_{60} derivative is fullerenol (or fullerol) $C_{60}(OH)_n$, where multiple hydroxyl groups are chemically bound to surface of the C_{60}, leading to enhanced water solubility. Number of the hydroxyl groups can be varied by adjusting synthesis conditions [7]. The surface modification with hydroxyl groups can also provide beneficial biological impacts. $C_{60}(OH)_n$ has been shown to enhance the lifespan of organisms [8] and reduce cytotoxicity [9] as compared to the pristine C_{60}. The unique photothermal and photoacoustic properties of $C_{60}(OH)_n$ can be applied for cancer theranostics [10,11]. However, it has also been shown to cause cell membrane damage [12] and to exhibit cytotoxicity [13,14]. One critical factor affecting the biological impacts of $C_{60}(OH)_n$ is its hydrophilicity/hydrophobicity, *i.e.*, number of the hydroxyl groups. Thus, understanding the effect of number of the hydroxyl groups of $C_{60}(OH)_n$ on its interaction with the cell membrane is a key issue.

However, molecular mechanism underlying the fullerene-cell membrane interactions is still far from being understood. Although some experimental studies on the fullerene-cell membrane interactions have been reported, it is still difficult to investigate the molecular mechanism by means of current experimental techniques. One powerful approach to investigate the fullerene-cell membrane interactions is a computational modeling. In particular, molecular dynamics (MD) simulations have been utilized [15–26]. In the MD simulations, dynamic evolution of a system consisting of a fullerene, phospholipid bilayer (model cell membrane), and

* Corresponding author.
E-mail address: hnakamura@chemeng.osakafu-u.ac.jp (H. Nakamura).

https://doi.org/10.1016/j.jtice.2017.11.016
1876-1070/

Please cite this article as: H. Nakamura et al., Effect of number of hydroxyl groups of fullerenol $C_{60}(OH)_n$ on its interaction with cell membrane, Journal of the Taiwan Institute of Chemical Engineers (2017), https://doi.org/10.1016/j.jtice.2017.11.016

solvents can be simulated at the molecular scale. Most of the previous studies have investigated the interaction of the pristine C_{60} with a lipid bilayer [15,17,18,21,22,24,25], and only few studies have focused on $C_{60}(OH)_n$ [16,19,23,26]. Qiao et al. [16] first reported a MD simulation of interaction of fullerenol with a lipid bilayer. However, they solely investigated $C_{60}(OH)_{20}$, and no systematic investigation on the influence of number of the hydroxyl groups was reported. A few simulation studies have investigated $C_{60}(OH)_n$ with different number of the hydroxyl groups using a coarse-grained MD simulation [19,23,26]. However, in these simulation studies spatial configuration of the hydroxyl groups on C_{60} was arbitrarily modeled due to the coarse-grained model, where a group of atoms are represented by a large single interaction site. Quantum chemical computations [27,28] revealed that the hydroxyl groups are not randomly distributed on C_{60}, but covalently bonded with specific carbon atoms, resulting in unique spatial configurations of the hydroxyl groups. Thus, a MD simulation with more realistic molecular configurations of $C_{60}(OH)_n$ is necessary.

This study presents an atomistic MD simulation study on the interaction of fullerenol $C_{60}(OH)_n$ with a model cell membrane. $C_{60}(OH)_n$ with 2, 6, 8, and 24 hydroxyl groups and a pristine C_{60} were used. The spatial configurations of the hydroxyl groups on the C_{60} were explicitly modeled so that $C_{60}(OH)_n$ with realistic molecular configurations were examined. Firstly, dynamics of the $C_{60}(OH)_n$ including their adhesion on and penetration into the model cell membrane were investigated. Secondly, a potential of mean force (PMF) associated with a migration of $C_{60}(OH)_n$ across the cell membrane was calculated, and free energy change for adhesion/penetration of $C_{60}(OH)_n$ on/into the cell membrane was investigated. Finally, probability of finding $C_{60}(OH)_n$ along the membrane normal at the equilibrium state was calculated using a one-dimensional stochastic modeling. Through these investigations, effect of number of the hydroxyl groups of $C_{60}(OH)_n$ on the permeability across the cell membrane was discussed.

2. Methods

2.1. Molecular dynamics simulation

Fig. 1a shows an initial configuration of the simulation system used in this study. The simulation system was composed of a phospholipid bilayer, water molecules, and a $C_{60}(OH)_n$. As the phospholipid molecule, DMPC (1,2-dimyristoylphospatidylcholine) was used. The simulation system contained 200 DMPC molecules, 9000 water molecules, and a single $C_{60}(OH)_n$. The MD simulations were conducted using a classical atomistic MD simulation. The DMPC molecule was modeled by a united atom force field proposed by Berger et al. [29]. The water molecules were modeled by the extended simple point charge (SPC/E) model [30]. The van der Waals interaction between carbon atoms of C_{60} was calculated according to a force field proposed by Girifalco [31]. For the van der Waals interaction between C_{60} and water, a force field proposed by Werder et al. [32–34] was used. Geometric combining rules were employed for the van der Waals interaction between C_{60} and DMPC. The C_{60} core was modeled as a rigid body with C–C bond lengths of 0.140 and 0.145 nm. A pristine C_{60} and four types of $C_{60}(OH)_n$ with 2, 6, 8, and 24 hydroxyl groups were used in this simulation (Fig. 1b). In modeling $C_{60}(OH)_n$, location of the carbon atoms covalently bonding with the hydroxyl groups were determined in accordance with results from a quantum chemical computation [27]. This allowed to model $C_{60}(OH)_n$ with much more realistic molecular configurations as compared to coarse-grained MD simulations [19,23,26]. The C–O and O–H bond lengths of the hydroxyl group were 0.14 and 0.095 nm, respectively [16,29]. The C–O–H bond angle was 106.3° [16,29]. A force field proposed by Chiu et al. [35] was used for the van der Waals interaction parameters of the hydroxyl groups. The partial charges on the pristine C_{60} were set as zero. In $C_{60}(OH)_n$, partial charges of –0.8*e* and +0.3*e* were assigned to the oxygen and hydrogen atoms, respectively [16]. A partial charge of +0.5*e* was also assigned to the carbon atoms bonding a hydroxyl group [16].

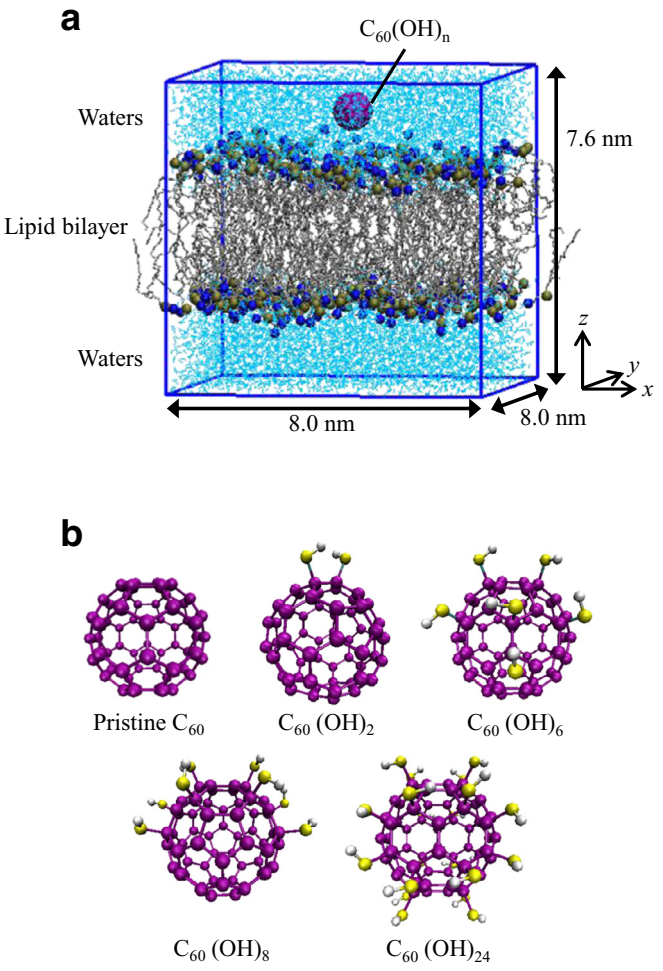

Fig. 1. (a) Initial configuration of simulation system. (b) $C_{60}(OH)_n$ used in this study. Color code: C_{60} (purple), Water (cyan), N (blue), P (tan), carbons of lipid bilayer (gray), O (yellow), H (white).

The MD simulations were performed under the conditions of constant temperature and constant pressure (the NPT ensemble). The temperature and pressure were maintained at 310 K and 1 bar using the Nose–Hoover thermostat [36,37] with a time constant of 0.2 ps and the Parrinello–Rahman pressure coupling method [38] with a time constant of 1.5 ps, respectively. The Lennard–Jones potentials for van der Waals interactions were calculated within a cut off length of 1.8 nm. The electrostatic interactions were calculated using the particle mesh Ewald (PME) method [39,40] with a real space cut off length of 1.0 nm and a fast Fourier-transform grid spacing of 0.15 nm. Lengths of the covalent bonds were constrained using SETTLE algorithm [41] for water and using LINCS algorithm [42] for the other molecules. Periodic boundary conditions were applied in all directions. The leap-frog Verlet algorithm was used as numerical time integration scheme with the time step of 2 fs. For setting the initial molecular configuration, a $C_{60}(OH)_n$ was placed at 1.5 nm above the upper surface of the lipid bilayer. This configuration was preliminarily equilibrated by a MD simulation for 20 ns with constraining the $C_{60}(OH)_n$. After this equilibration run, dynamics of the $C_{60}(OH)_n$ and the other molecules were simulated without constraining the molecules (*i.e.*, unconstrained

Please cite this article as: H. Nakamura et al., Effect of number of hydroxyl groups of fullerenol $C_{60}(OH)_n$ on its interaction with cell membrane, Journal of the Taiwan Institute of Chemical Engineers (2017), https://doi.org/10.1016/j.jtice.2017.11.016