BBA - General Subjects 1861 (2017) 2872–2882

Contents lists available at ScienceDirect

BBA - General Subjects

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

Effects of fullerene on lipid bilayers displaying different liquid ordering: a coarse-grained molecular dynamics study

Judit Sastre[a], Ilaria Mannelli[b], Ramon Reigada[a,c,*]

[a] Departament de Ciència dels Materials i Química Física, Universitat de Barcelona, c/Marti i Franqués 1, Pta 4, 08028 Barcelona, Spain
[b] ICFO-Institut de Ciències Fotòniques, The Barcelona Institute of Science and Technology, 08860 Castelldefels, Barcelona, Spain
[c] Institut de Química Teòrica i Computacional (IQTCUB), Universitat de Barcelona, c/Marti i Franqués 1, Pta 4, 08028 Barcelona, Spain

ARTICLE INFO

Keywords:
Fullerene
Environmental impact
Lipid membrane
Cholesterol
Lipid raft
Coarse-grained molecular dynamics

ABSTRACT

Background: The toxic effects and environmental impact of nanomaterials, and in particular of Fullerene particles, are matters of serious concern. It has been reported that fullerene molecules enter the cell membrane and occupy its hydrophobic region. Understanding the effects of carbon-based nanoparticles on biological membranes is therefore of critical importance to determine their exposure risks.

Methods: We report on a systematic coarse-grained molecular dynamics study of the interaction of fullerene molecules with simple model cell membranes. We have analyzed bilayers consisting of lipid species with different degrees of unsaturation and a variety of cholesterol fractions. Addition of fullerene particles to phase-segregated ternary membranes is also investigated in the context of the lipid raft model for the organization of the cell membrane.

Results: Fullerene addition to lipid membranes modifies their structural properties like thickness, area and internal ordering of the lipid species, as well as dynamical aspects such as molecular diffusion and cholesterol flip-flop. Interestingly, we show that phase-segregating ternary lipid membranes accumulate fullerene molecules preferentially in the liquid-disordered domains promoting phase-segregation and domain alignment across the membrane.

Conclusions: Lipid membrane internal ordering determines the behavior and distribution of fullerene particle, and this, in turn, determines the influence of fullerene on the membrane. Lipid membranes are good solvents of fullerene molecules, and in particular those with low internal ordering.

General significance: Preference of fullerene molecules to be dissolved in the more disordered hydrophobic regions of a lipid bilayer and the consequent alteration of its phase behavior may have important consequences on the activity of biological cell membranes and on the bioconcentration of fullerene in living organisms.

1. Introduction

Carbon nanomaterials are of exceptional importance in nanoscience given their unique electrical, thermal, chemical and mechanical properties [1]. Their range of applications is very wide [1]: composite materials, energy storage and conversion, sensors, drug delivery, field emission devices and nanoscale electronic components. Although the production of nanomaterials is rapidly growing [2] their toxicology and environmental impact are still matters of serious concern [3]. Nanomaterials could possibly enter human cell [4] and, for instance, harmful effects of inhaled nanoparticles on lungs, brain and olfactory bulb have been reported [5–7]. A better understanding of their interaction with biological systems is then required to minimize their adverse effects on living beings.

Since the discovery of fullerene (C_{60}) in 1985, it has become one of the most widespread carbon-based nanomaterials and it has attracted attention in many research fields due to their exclusive properties. Applications of C_{60} and its derivatives range from material science [8] to nanomedicine, in this latter context acting such as X-ray contrast agents [9], antioxidant drugs for neurodegenerative diseases [10], inhibitors of the allergic response [11] and targets for bone tissue [12]. Despite the large variety of uses of fullerene and its increasing worldwide production [13,14] little is known about possible biological negative effects. In fact, strong evidences that fullerenes are cytotoxic have been reported [15–17], and it has been demonstrated that fullerene aggregates can penetrate cells and cross the blood brain barrier [3,6]. It is necessary then to develop empirical and modeling tools to assess the effects of this compound when released to the environment.

* Corresponding author at: Departament de Ciència dels Materials i Química Física, Universitat de Barcelona, c/Marti i Franqués 1, Pta 4, 08028 Barcelona, Spain.
E-mail address: reigada@ub.edu (R. Reigada).

http://dx.doi.org/10.1016/j.bbagen.2017.08.004
Received 10 April 2017; Received in revised form 27 July 2017; Accepted 1 August 2017
Available online 02 August 2017

Since the cell membrane is the first obstacle to overcome when interacting with living beings, a better understanding of the mechanism of lipid membrane penetration and membrane alterations due to fullerene interaction is needed. Simple and reproducible systems, such as model lipid membranes, are appropriate to elucidate the physical principles governing these complex interactions. A few experimental studies have been performed on the distribution of fullerene between aqueous solutions and simple solid supported lipid membranes [18,19]. Global descriptors such as the water/lipid partition coefficient clearly evidence the ability of fullerene to be preferentially placed in the lipid phase [18,19], thus confirming its potential bioaccumulation in biological cell membranes. The use of small lipid vesicles as biocompatible platforms for the dispersion of fullerene particles has been also attempted [20,21]. By encapsulating aqueous solutions containing fullerene clusters within vesicles, fullerenes may be transferred to the hydrophobic region of the vesicle bilayer [22] and dispersed as molecular entities or small nano-aggregates [20]. These lipid/fullerene assemblies have received considerable attention due to their potential biochemical applications [21].

The consequences of fullerene accumulation on cell membranes or its interaction with lipid bilayers, which are often used as model biological membranes, require a deep understanding of the produced effects at the molecular detail. In which parts of the lipid bilayer matrix are the fullerene molecules partitioned once inside the membrane? What are the resulting local and global effects on the lipid bilayer structure as well as on its molecular dynamical properties? How these effects depend on the lipid composition and organization of the membrane lipid matrix? These and other issues have to be analyzed at the molecular detail in order to understand the consequences of fullerene accumulation on the functionality of cell membranes. Molecular dynamics (MD) simulations provide an excellent approach to unveil the ultimate molecular mechanisms regulating the behavior of interacting systems and they have been successfully used to obtain direct insights into many lipid membrane processes. MD simulations using a full atomistic description of the interacting molecules have been already used to study the effects of fullerene molecules on simple lipid bilayers [23,24]. Atomistic MD simulations, however, are limited to short time (a few hundred of ns) and length scales (10-20 nm), whereas coarse-grained (CG) MD covers much longer scales still preserving the main molecular characteristics of the simulated moieties. Recently, a CG model for fullerene has been developed and used for single-lipid bilayer simulations [25–27], unveiling interesting details of the fullerene translocation process through the bilayer and its consequences on some basic membrane properties such as thickness, lipid order, diffusivity and elastic and compressibility moduli [25]. Moreover, CG simulations also show that fullerene clusters can be absorbed from an aqueous solution and disaggregate once inside the bilayer [25,27]. A detailed analysis provides the molecular clues to explain why lipid bilayers are better solvents for fullerene molecules than similarly apolar alkane moieties [27].

All previous computational studies focused on single-lipid bilayers as model membranes. However, cell membranes of different organisms display different lipid compositions, and even within a single organism, cell lipid composition can vary [28]. Lipid composition determines many membrane properties such as thickness, internal ordering, lipid diffusivity, etc. To take into account this diversity, in this Paper we analyze the effects of fullerene in lipid membranes formed by phospholipid moieties with different degrees of saturation, and containing different fractions of cholesterol -an important component of eukaryotic cell membranes-. Fullerene translocation through the membrane and its partitioning inside the bilayer are analyzed depending on the bilayer characteristics while modification of structural and dynamical membrane properties due to the presence of fullerene molecules are as well studied. Lipid cell membrane composition is also particularly relevant in relation to one of the emergent issues in biophysics: the raft concept for cell membranes [29]. The raft hypothesis is based on the idea that lipids in plasma membranes are distributed inhomogeneously, forming small liquid-ordered (*lo*) domains rich in cholesterol and saturated lipids that are embedded in a liquid-disordered (*ld*) medium preferentially containing unsaturated lipids. Although some aspects of the raft phenomenology remain currently controversial [30], it is well accepted that such structures are implicated in many biological processes [31]. Therefore, fullerene effects on biological cell membranes should consider lipid heterogeneity and *lo/ld* coexistence. Consequently, we have also investigated the behavior of fullerene molecules on phase-segregating membranes consisting of a saturated lipid, an unsaturated lipid and cholesterol.

2. Methods

2.1. Coarse-grained description and simulated membranes

The coarse-grained model proposed by the Martini force field is used here to describe the simulated molecules. This model is based on a 4-to-1 mapping where on average four heavy atoms are represented by a single interactive bead, except for ring-like molecules that are mapped with higher resolution (for instance, cholesterol is described by a 3-to-1 resolution) [32]. The Martini model has been successfully applied to study a large variety of lipid membrane phenomena [33–35]. The numerical simulations are carried out for membranes consisting of three phosphatidylcholines (PC) displaying different levels of unsaturation in their acyl chains: DUPC (with two double-unsaturated 16:2 tails), POPC (with an unsaturated oleoyl 18:1 tail and a saturated palmitoyl 16:0 tail) and DPPC (with two saturated palmitoyl 16:0 tails). Each PC is described by a positively charged choline group, a negatively charged phosphate, two neutral glycerols, and two tails with four (for 16 carbon length tails) or five (for the oleoyl tails) apolar particle beads (see Fig. 1). Cholesterol (Chol) is also added to some of the simulated membranes. Chol molecules are described by eight particles: a polar bead for the hydroxyl group, five representing the ring sterol system and two for the short alkyl tail (Fig. 1) [32]. Fullerene molecules are

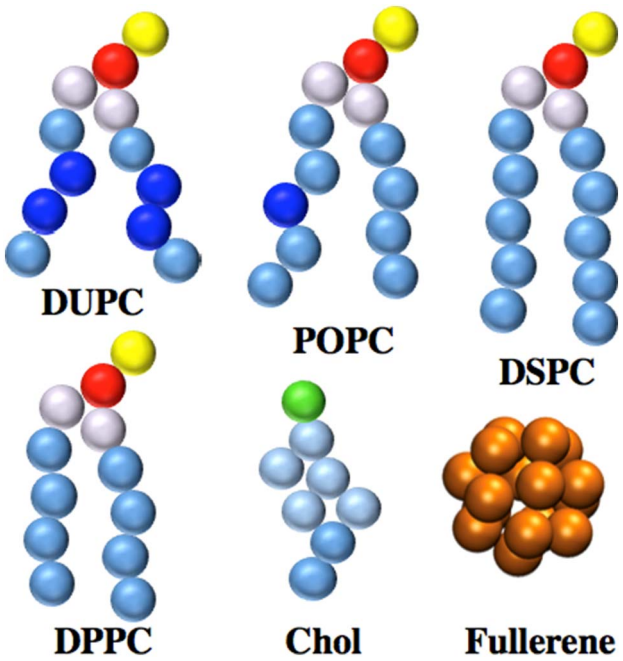

Fig. 1. Schematic representation of the simulated molecules according to their CG Martini description. Blue beads correspond to apolar particles forming the PC tails (dark: unsaturated segments, normal: saturated segments) and Chol structure (light: ring structure, normal: saturated ending tail). Yellow, red, white and green beads stand for choline, phosphate, glycerol and hydroxyl groups, respectively. Fullerene is formed by 16 apolar beads (in orange).