Biochimica et Biophysica Acta 1848 (2015) 1203–1211

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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

Reduced graphene oxide directed self-assembly of phospholipid monolayers in liquid and gel phases

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ARTICLE INFO

Article history:
Received 8 December 2014
Received in revised form 10 February 2015
Accepted 16 February 2015
Available online 24 February 2015

Keywords:
Reduced graphene oxide
Self-assembly
Phospholipid monolayer

ABSTRACT

The response of cell membranes to the local physical environment significantly determines many biological processes and the practical applications of biomaterials. A better understanding of the dynamic assembly and environmental response of lipid membranes can help understand these processes and design novel nanomaterials for biomedical applications. The present work demonstrates the directed assembly of lipid monolayers, in both liquid and gel phases, on the surface of a monolayered reduced graphene oxide (rGO). The results from atomic force microscopy indicate that the hydrophobic aromatic plane and the defect holes due to reduction of GO sheets, along with the phase state and planar surface pressure of lipids, corporately determine the morphology and lateral structure of the assembled lipid monolayers. The DOPC molecules, in liquid phase, probably spread over the rGO surface with their tails associating closely with the hydrophobic aromatic plane, and accumulate to form circles of high area surrounding the defect holes on rGO sheets. However, the DPPC molecules, in gel phase, prefer to form a layer of continuous membrane covering the whole rGO sheet including defect holes. The strong association between rGO sheets and lipid tails further influences the melting behavior of lipids. This work reveals a dramatic effect of the local structure and surface property of rGO sheets on the substrate-directed assembly and subsequent phase behavior of the supported lipid membranes.

1. Introduction

With the striking progress in nanotechnology, extensive attention has been focused on the applications of nanomaterials in biomedical fields. Biological responses of biosystems to the implant of biomaterials significantly determine the effect and even safety/cytotoxicity of them. A large number of researches have reported that the physical and surface chemical properties of biomaterials, such as their hydrophobic/hydrophilic property and surficial topographies, would significantly influence the cellular behaviors including cellular uptake and the consequent intracellular location and translocation of nanomaterials [1–4]. In addition, cell functioning is, to a large extent, dependent on the extracellular matrix (ECM) environment. It has been established that local physical environment modulates many cell responses, including cell migration, proliferation, and cytoarchitecture [5–7]. However, these modulations are probably cell-type dependent. On the other hand, cell membranes, composed of a basic lipid bilayer platform with incorporated functional proteins, are primarily involved in regulation of these biological processes including regulation of response to the extracellular matrix. Therefore, a fundamental understanding of the interactions between lipid membranes and biomaterials at a molecular level can contribute to the understanding of these processes and development of biomedical applications of nanomaterials [8–10]. Previous studies have shown that subtle differences at the membrane interface with the substrate translate into dramatic differences in lateral fluidities and phase separation in supported lipid membranes [11]. Despite this, a better understanding of the dynamic assembly and environmental response of lipid membranes, which are composed of two asymmetric leaflets of lipid monolayers, is still needed.

In recent years, carbon-based materials, including carbon nanotubes and graphene (or reduced graphene oxide), have been considered attractive candidates for various biomedical applications such as scaffolds in tissue engineering, near-infrared biomedical imaging [12], substrates for stem cell differentiation [13], and surficial coverage of implant

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http://dx.doi.org/10.1016/j.bbamem.2015.02.018
0005-2736/

devices [14]. Recently, many researches have been worked on the cellular responses to carbon-based nanomaterials [15–17]. For example, fibroblasts and HeLa cells were reported to proliferate well on substrates coated with graphene, graphene oxide or carbon-nanotube, which suggests that such carbon-coated surface might be a suitable substrate for cell culturing applications [16]. However, another work found that the self-spreading of supported lipid bilayer on a SiO_2 surface was blocked by graphene oxide pieces [18]. In this context, a fundamental and systematic investigation on interfacial interactions between phospholipid and graphene on a molecular level is needed, which might promise faster and smarter exploitation of the distinct characteristics of graphene for biological applications. Furthermore, incorporation of lipids with graphene provides an important way for preparing multifunctional hybrid materials for biosensing [19,20], drug delivery [21], and photochemical catalysis [22].

Atomic force microscopy (AFM) has been an essential tool to investigate the topology of supported lipid mono/bilayers due to its high sensitivity and localization [23]. In this work, we take advantage of AFM technique to investigate the directed self-assembly of phospholipid molecules, in both liquid and gel phases, in response to the surface features of monolayered rGO sheets that are supported on a mica surface. Results show that subtle structural details in rGO substrate are amplified into dramatic differences in morphology and lateral fluidity in supported lipid monolayers. To the best of our knowledge, this work reports the first experimental evidence highlighting the effect of local topography of rGO surface and lipid phase on the molecular assembly of lipid monolayers. It provides a new framework for understanding the cell membrane responses to rGO and help design novel rGO/lipid nanocomposite biomaterials.

2. Materials and methods

2.1. Chemicals

1,2-Dioleoyl-*sn*-glycero-3-phosphocholine (DOPC), 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), and 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine-N-(lissamine rhodamine B sulfonyl) (Rh-PE) were purchased from Avanti Polar Lipids and used as received. Graphene oxide (2 mg mL^{-1}, dispersion in water) and 1-Oleoyl-rac-glycerol (MO) were purchased from Sigma-Aldrich. Sheet mica and chloroform (99.7%) were purchased from Shanghai Chemical Reagents Company.

2.2. Preparation of rGO sheets on mica

Supported graphene oxide (GO) flakes on mica were first prepared. A volume of 10 μL graphene oxide dispersion, with a concentration of 0.004 mg mL^{-1}, was dropped onto a freshly cleaved mica surface (8 × 8 mm) with a pipette and the solvent was allowed to evaporate slowly in a chamber. After complete evaporation, supported GO flakes were obtained. The sample was then annealed in Ar/H_2 at 450 °C for 30 min for reduction of GO to rGO, which was used for the following characterizations and lipid depositions [24].

2.3. Lipid monolayer deposition through Langmuir–Blodgett technique

A KSV NIMA Langmuir–Blodgett Deposition Trough (KN2002), with a useful surface area of 273 cm^2, was used to prepare lipid films. The lipids were first dissolved in chloroform to 0.2 mg mL^{-1} (1.0 mg mL^{-1} for MO). A volume of 8 μL lipid solution was spread onto the water/air interface in the trough. After evaporation of chloroform, the lipid layer was transferred by lifting the substrate vertically from the aqueous subphase, at a speed of 3 mm min^{-1}, while maintaining the surface pressure constant at the desired setpoint. A surface pressure of 32 mN m^{-1} was selected for the conventional lipid monolayer deposition (unless otherwise stated) as this value is low enough to prevent the monolayer to collapse, and high enough for a condensed state of the monolayer [25]. A much lower surface pressure of 15 mN m^{-1} was also used for control experiments. For the fluorescence experiments, 0.5 mol.% Rh-PE was added to the lipid solution in advance.

2.4. Characterizations

AFM images were collected with an Asylum Research MFP-3D-SA atomic force microscope (Santa Barbara, CA) setup in tapping mode in air. In the heating experiment, the temperature of the system was modulated and maintained at 60 °C with the original temperature control components from AR. The fluorescent image was taken on a Zeiss LSM 710 inverted confocal fluorescence microscope. All the experiments were carried out at room temperature of 22 °C.

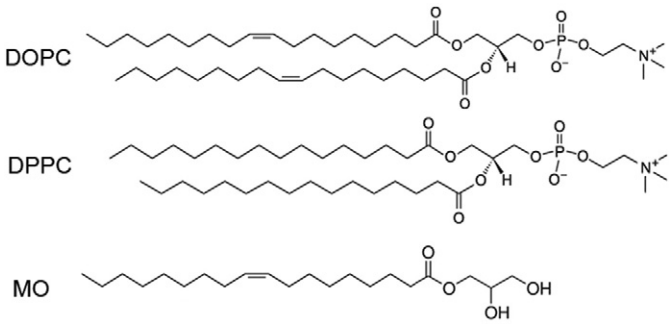
3. Results and discussion

3.1. Fabrication of supported rGO sheets

Scheme 1 presents the molecular structure of the three types of lipids used in this work. In brief, DOPC consists of two unsaturated hydrocarbon tails in addition to the phosphor-nitrogen headgroup, while DPPC has two saturated hydrocarbon tails. Therefore, the melting temperature of DOPC (−20 °C) is much lower than that of DPPC (41 °C) [26]. At room temperature, they exist in liquid crystalline and gel phases, respectively. MO has a similar amphiphilic structure, being composed of a single unsaturated hydrocarbon chain bonded to a glycerol head by an ester bond [27].

For reference, we firstly prepared DOPC, DPPC and MO monolayers on neat mica surfaces by LB technique. The obtained lipid films are defect free and looked quite flat as shown in Figure S1, except that little cracks occurred on DPPC film due to gel phase [28]. To confirm the presence of the lipid films on the mica surfaces, 0.5 mol.% fluorescent Rh-PE lipid was pre-mixed with all the three lipids. Under confocal fluorescence microscopy, homogeneous distribution of fluorescence was found over surfaces of all the three samples (cf. Figure S2a). Due to the super-hydrophilic feature of mica surface (cf. Figure S3), the lipid molecules are supposed to assemble into a monolayer with hydrophilic headgroups fronting on the mica surface while the hydrophobic tails orienting towards the air.

On the other hand, we fabricated GO sheets supported on mica surface by the conventional drop-coating method. Under AFM characterizations as shown in Fig. 1a, the GO sheets were found spread on the mica surface, probably in a monolayer, with a size of a few micrometers. The surficial coverage of the GO sheets on mica can be modulated from below 20%, mostly monolayered, to more than 90%, mainly overlapped, by controlling the volume of GO dispersion dropped on mica. A single GO sheet has a thickness of 0.95 ± 0.05 nm as shown in the height profile in Fig. 1d. This agrees with the previous reports [29,30]. Meanwhile,



Scheme 1. Molecular structures of DOPC, DPPC and MO.