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Hybrid vesicles from lipids and block copolymers: Phase behavior from the micro- to the nano-scale

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ABSTRACT

In recent years, there has been a growing interest in the formation of copolymers-lipids hybrid self-assemblies, which allow combining and improving the main features of pure lipids-based and copolymer-based systems known for their potential applications in the biomedical field. In this contribution we investigate the self-assembly behavior of dipalmitoylphosphatidylcholine (DPPC) mixed with poly(butadiene-b-ethyleneoxide) (PBD-PEO), both at the micro- and at the nano-length scale.

Epifluorescence microscopy and Laser Scanning Confocal microscopy are employed to characterize the morphology of micron-sized hybrid vesicles. The presence of fluid-like inhomogeneities in their membrane has been evidenced in all the investigated range of compositions. Furthermore, a microfluidic set-up characterizes the mechanical properties of the prepared assemblies by measuring their deformation upon flow: hybrids with low lipid content behave like pure polymer vesicles, whereas objects mainly composed of lipids show more variability from one vesicle to the other. Finally, the structure of the nanosized assemblies is characterized through a combination of Dynamic Light Scattering, Small Angle Neutron Scattering and Transmission Electron Microscopy. A vesicles-to-wormlike transition has been evidenced due to the intimate mixing of DPPC and PBD-PEO at the nanoscale. Combining experimental results at the micron and at the nanoscale improves the fundamental understanding on the phase behavior of copolymer-lipid hybrid assemblies, which is a necessary prerequisite to tailor efficient copolymer-lipid hybrid devices.

1. Introduction

Since their first description by Discher and Eisenberg [1], polymersomes have raised the interest of the scientific community because of their mechanical stability and tunable chemical design. Their possible applications span from nanocarriers for drug delivery, medical imaging, advanced nanoreactors or electronics to protocells mimicking cell structure and functions [2]. On the other hand, the structural membrane components, lipids, are biocompatible but their long-term stability is limited. Hybrid lipid/polymer vesicles combine the inherent advantages of their components: the biocompatibility of lipids and the mechanical stability and chemical versatility of copolymers. The research on this topic is still in its infancy, and most of it deals with giant vesicles [3] in the effort to understand their phase diagram, miscibility and stability limits, and tune their morphology and structural features within the bilayer. The few examples about hybrid nanovesicles [4–10] mainly deal with the assessment of their dual nature, possible applications to drug delivery and recently the existence of phase separation at the nanoscale. The parameters influencing their morphology and phase separation are far from being understood. Moreover, studies concerning hybrid systems leading both to nano-objects and giant vesicles are very scarce [6,9]. With respect to giant vesicles, polydimethylsiloxane (PDMS), polybutadiene (PBD) and polyisobutylene (PIB) are the most thoroughly investigated hydrophobic blocks, as they do not crystallize at room or body

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temperature and possess a glass transition temperature below 0 °C, which guarantees a sufficient flexibility of the polymer chains during the commonly used electroformation process (*i.e.* electric field-assisted film hydration). The observed hybrid vesicles can be homogeneous or inhomogeneous with lateral domains. PDMS and PBD-based block copolymers with different molecular weights have been studied in blends with lipids in the liquid (POPC, 2-Oleoyl-1-palmitoyl-sn-glycero-3-phosphocholine) and gel (DPPC, dipalmitoylphosphatidylcholine) phase. The effects of size mismatch of the hydrophobic blocks and lipid fluidity on giant vesicle homogeneity have started to be systematically investigated for PDMS based copolymers. In this case, it was demonstrated that the hydrophobic mismatch is an important parameter for lipids in the fluid phase. Indeed, when the block copolymer molecular weight increases, homogeneous vesicles form in a larger range of compositions in the region of low lipid content [5,11]. At higher lipid content phase-separated vesicles form, and budding is observed; phase-separated vesicles are not stable and budding degenerates in vesicle fission for copolymers of higher molecular weights. In this case, a high percentage of pure lipid or polymer vesicles is always observed, resulting from fission phenomena. This also explains the experimental results on PBD-based block copolymers in the case of high hydrophobic mismatch. In this case, homogeneous hybrid vesicles form in the polymer-rich domain; and in the lipid-rich domain homogeneous hybrid vesicles form together with vesicles of the pure components. POPC gives rise to homogeneous giant vesicles in a restricted range of compositions [12] for PBD based copolymers, while for PDMS based triblock copolymers, vesicles always form. On the other hand, DPPC always form inhomogeneous systems with domains independently of the hydrophobic mismatch in the case of PDMS copolymers [11]. Homogeneous GUVs (Giant Unilamellar Vesicles) are also observed for PIB block copolymers in the lipid-rich and polymer-rich regions (below 18 mol% and above 30 mol% DPPC), while phase separation occurs only for intermediate compositions [13]. For DPPC hybrid systems, electroformation is conducted above lipid melting temperature and phase separation occurs after cooling down to room temperature. The cooling rate affects domains size and shape, fast cooling producing round domains whereas slow cooling produces flower-like or irregular shape domain. For higher mismatches, round domains formed, irrespectively of the cooling rate. In the case of PIB and PDMS based block copolymers, hybrid vesicles form in all the range of composition, for PBD-based copolymers only one composition has been reported. As mentioned above, small unilamellar vesicles were also investigated for a few systems, composed of lipids both in the gel and liquid phase. The vesicles' hydrodynamic radius [6–8] and permeability together with changes in the thermal properties of the bilayer [7,8] are used as an indication of the hybrid nature of the vesicles. Hybrid vesicles are more stable than liposomes and the effect was related to literature results on the higher stability of pegylated liposomes [7]. Vesicles permeability always decreases in the presence of the block copolymers. On the other hand, the effect on the hydrodynamic size is difficult to rationalize due to the lack of systematic studies using copolymer with different molecular weight and above all it is not always in agreement with the effects on the bilayer properties. In the case of PDMS-based triblock copolymers [7], for example, the copolymer with the highest mismatch induced the largest effect on permeability and melting temperature of hybrid DPPC-polymer vesicles, but less impact on the vesicles' size. Very recently Dao et al. [11] underlined the need for more detailed investigations on the morphology of the nanostructures, the distributions of the components in the bilayers in order to understand the important parameters (*i.e.* chemical nature, curvature and hydrophobic mismatch) involved. This could also shed some light on the correlation between behaviors at the micro and nano scales if it exists.

Here we report a study on vesicles, both at the micro- and nanoscales, formed by PBD_{43}-PEO_{20} and DPPC. Although PBD *per se* is not biodegradable, the conjugation with PEO makes PBD-PEO block copolymer biocompatible [14]. In addition, it is well established that PEO improves the pharmacokinetic properties of nanodrugs and drug delivery nanodevices, due a stealth effect hindering the recognition by the mononuclear phagocyte system (MPS) [15,16] Thus, PBD-PEO based systems have been recently proposed for biomedical applications as drug carriers, and investigated both *in vitro* and *in vivo* [17,18]. Concerning the lipid building block DPPC, though generally not present in biological membranes, is fully biocompatible and thus of general interest for biomedical applications. In particular, due to the fully saturated nature of the hydrophobic chain, pure DPPC assemblies are characterized by a relatively high melting temperature (41 °C, [19]), of particular relevance for the design and development of smart temperature responsive drug delivery systems [20]. We have established the phase diagram in all the range of compositions by microscopy techniques (epifluorescence and scanning confocal microscopy) in the case of GUV obtained by electroformation, and by scattering techniques in the case of nanovesicles obtained by film rehydration and extrusion.

2. Materials and methods

2.1. Materials

PBD-PEO (PBD(2300)PEO(900) ((polybutadiene (Mw 2300)-b-polyethyleneoxide (Mw 900))) was purchased from Polymer Source Inc. (Dorval Montréal, Canada) and characterized by ^{1}H NMR and Size Exclusion Chromatography. DPPC (dipalmitoylphosphatidylcholine) and β-Bodipy 2-(4,4-diuoro-5,7-dimethyl-4-bora-3a,4adiaza- sindacene- 3-pentanoyl)-1 hexadecanoyl-sn-glycero-3-phosphocholine were purchased from Avanti Polar Lipids, Inc. (Alabaster, AL). DiI-C20 (1,1′-dieicosanyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate) was purchased from Molecular Targeting Technologies (Westchester, PA), OG-DHPE (Oregon Green™ 488 1,2-Dihexadecanoyl-sn-Glycero-3-Phosphoethanolamine) was purchased from Invitrogen Life Technologies (Saint Aubin, France). D_2O, MeOH, $CHCl_3$, sucrose were provided by Sigma-Aldrich (St. Louis, MO).

2.2. Preparation of Giant Unilamellar Vesicles

Giant Unilamellar Vesicles (GUVs) were prepared on a *Vesicles prep pro* instrument produced by Nanion. It is based on the well-known electroformation method described for the first time by Angelova et al. [21] for lipid-based GUVs. Briefly, 5 μL of a 1 mg/mL solution of the polymer in chloroform, lipid or their blend was deposited (approximately 1 cm^2) on an ITO-coated glass slide and dried under vacuum. Then an electroformation chamber was built-up by surrounding the dry film with a 1 mm o-ring and it was subsequently filled with 250 μL of a 240 mM sucrose solution. The chamber was closed with another ITO-coated glass slide and connected to an alternate current generator through electrodes connected with the ITO-coated glass slides. A peak-to-peak voltage of 3 V and 10 Hz was then applied at 50 °C for one hour to form the GUVs. The temperature and the voltage were then slowly decreased and the vesicles were collected with a pipette.

2.3. Preparation of small unilamellar vesicles

Nano-sized vesicles were prepared by the method of film rehydration. Briefly, 1 mg/mL solutions of pure lipid, pure polymer or lipid/polymer blend were prepared in chloroform. A dry film from

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