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Interaction of a double hydrophilic block copolymer with lipid monolayers at the air–water interface

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ABSTRACT

The interaction of poly(*N*,*N*-dimethylacryl-amide)-*b*-poly(*N*,*N*-diethylacrylamide) ($PDMA_{207}$-*b*-$PDEA_{177}$), (DHBC), with 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylcholine (DPPC), 1,2-dipalmitoyl-*sn*-glycero-3-phospho-(1′-*rac*-glycerol) (sodium salt) (DPPG) and stearic acid (SA), was investigated at the air–water interface. The $\pi - A$ isotherms of DHBC/DPPC and DHBC/DPPG binary mixtures are nearly the sum of the contribution of components weighted by the molar composition, — the additive rule. The $\pi - A$ curves of the mixtures show a plateau at ~7 mN m^{-1}, owing to the immersion of the hydrophilic block PDMA, and other at ~27 mN m^{-1} attributed to the immersion of the less hydrophilic PDEA block. Differently, the binary mixtures of DHBC/SA and PDEA homopolymer/SA do not follow the additive rule of the components showing an expanding effect at low surface pressures. Brewster angle microscopy images show that both DHBC and PDEA polymers interact with lipid monolayers, strongly disturbing their morphology.

1. Introduction

Water soluble polymers are promising drug carriers with potential to increase drug circulation time, improve drug solubility, and reduce drug toxicity [1–5]. Particularly interesting are the double hydrophilic block polymers (DHBC) combining two blocks with different hydrophilicities, whose balance can be tuned by temperature. These thermoresponsive polymers can form aggregates, micelles, and polymersomes in aqueous media by changing the temperature above the lower critical solution temperature (LCST) of the thermoresponsive block [6,7]. These vectors can replace the conventional liposomes composed of phospholipids and cholesterol that have the drawback of rapid clearance from the systemic circulation [8]. The interaction of polymer based drug carriers with the cell membrane can still enhance the translocation from the inside to the outside leaflet of the bilayer (flip-flop) [9]. Indeed, amphiphilic non-ionic polymers increase the interleaflet lipid exchange by embedding their hydrophobic moiety in the membrane with the consequent increase of membrane fluidity. The relevance of thermoresponsive DHBC in biomedical applications comes from the versatility of tailoring their structure and composition, that allows the incorporation of a thermoresponsive block in the copolymer with a LCST close to the physiological temperature [7,10]. By changing the temperature above the LCST of the thermoresponsive block induces the formation of nanoaggregates with a core-shell architecture that can provide the safe targeting delivery of low water solubility and toxic drugs to the cells.

Aiming the use of DHBC in biomedical applications, the study of their interaction with model lipid membranes is a fundamental requisite. Examples of simple models for the natural complex systems are liposomes, supported lipid bilayers and Langmuir monolayers [11]. In these simple models the lipid organization mimics the arrangement of lipids in natural cell membranes. In particular, the Langmuir technique allows following *in situ* the formation of the two-dimensional states and studying the processes that occur at the interface [12–14]. Therefore, monolayers of phospholipids at the air–water interface are simple models for the lipid–aqueous interface of biomembranes to investigate the effects of the interaction of drugs or drug delivery systems on lipids [15]. This model allows to study interactions between lipids and different components adsorbed from the subphase [16–18] or incorporated in the monolayers by co-spreading [19,20]. The phase behavior diagrams at the air–water interface can be inferred from the thermodynamic analysis of the $\pi - A$ isotherms and the changes in the lipid morphology can be followed by Brewster angle microscopy (BAM). Additionally, the transference of the monolayers onto a solid substrate by the Langmuir–Blodgett (LB) or Langmuir–Schaeffer techniques allows the surface characterization by several spectroscopic and microscopic techniques.

It was found that poly(ethylene oxide)-*b*-poly(propylene oxide)-*b*-poly(ethylene oxide block copolymers (pluronics) in

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the presence of phospholipid monolayers induce the organization of mesoscopic to nanoscopic clusters of lipid molecules surrounded by a network of block copolymers known as 'lipid corralling' [21,22]. Other studies on pluronics have shown that they penetrate into monolayers of dipalmitoylphosphatidylcholine (DPPC) and dipalmitoylphosphatidylglycerol (DPPG) influencing their membrane-sealing capability [23–26]. The sealing effect of damaged cell membranes by some pluronics has been known for almost three decades and more recently also by other non-ionic block copolymers [18].

A thermo-responsive double hydrophilic block copolymer composed of a PDEA block covalently linked to poly(*N*,*N*-dimethylacrylamide) (PDMA) block ($PDMA_{207}$-*b*-$PDEA_{177}$) was synthesized. The thermo-responsiveness in aqueous medium is assured by the PDEA block [27–32] while the PDMA hydrophilic block provides the water solubility. The thermo-responsiveness of PDEA was investigated at the air–water interface and confirmed by atomic force microscopy (AFM) of LB films deposited on mica [33]. The monolayers of the thermo-responsive double hydrophilic block copolymer $PDMA_{207}$-*b*-$PDEA_{177}$ were studied in the range of 10–40 °C at the air–water interface [34]. Laser scanning confocal fluorescence microscopy (LSCFM) of Langmuir–Blodgett (LB) monolayers deposited on glass substrates, showed a core–shell inversion triggered by temperature increase, and the AFM images confirmed the thermal collapse above the LCST of PDEA [35].

The interaction of $PDMA_{207}$-*b*-$PDEA_{177}$ with the anionic phospholipid DPPG, as a model lipid membrane, was investigated in mixed monolayers using three imaging techniques with different spatial resolution [36]. Phase separation and morphologic changes were followed *in situ* by BAM (meso-scale) and in LB mixed monolayers by LSCFM (micro-scale). These two combined techniques indicated that DHBC incorporates in the expanded phase of DPPG, being excluded from the condensed domains. The inner structure of phase-separated domains was further imaged by AFM of mica supported LB monolayers (nano-scale).

In this work we compare the effect of the interaction of DHBC with three different lipid monolayers followed *in situ* by BAM at constant temperature. The phase behavior of $PDMA_{207}$-*b*-$PDEA_{177}$ in mixed monolayers with the zwitterionic phospholipid (DPPC), the anionic phospholipid (DPPG) and stearic acid (SA), in order to access the role of molecular architecture (double or single alkyl chained) and the charge distribution (zwitterionic or anionic) of the lipids. At low surface pressures, DHBC polymer endows microsegregation (or lipid corralling). At high surface pressures, the polymer immerses underneath the lipid condensed layer forming a double layer structure that influences the collapse surface pressure of the lipidic layer. Despite the similarities found in the global behavior of these polymer–lipid monolayers, some distinctive features were observed due to differences in the molecular structure and chemical composition of lipids (Chart 1).

2. Experimental

2.1. Materials

Stearic acid, SA, ($C_{18}H_{36}O_2$) and DPPC (1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylcholine) 99% pure, were purchased from Sigma and from Aldrich Chemical Co., respectively, and used as received. DPPG (1,2-dipalmitoyl-*sn*-glycero-3-phospho-(1′-*rac*-glycerol) (sodium salt)) was purchased in powder form from Avanti Polar Lipids, Inc. (Alabaster, AL). The homopolymer Poly(*N*,*N*-diethylacrylamide) (PDEA) ($Mn = 33\,800$ g mol^{-1} $Mw/Mn = 1.01$) and the copolymer Poly(N,N-

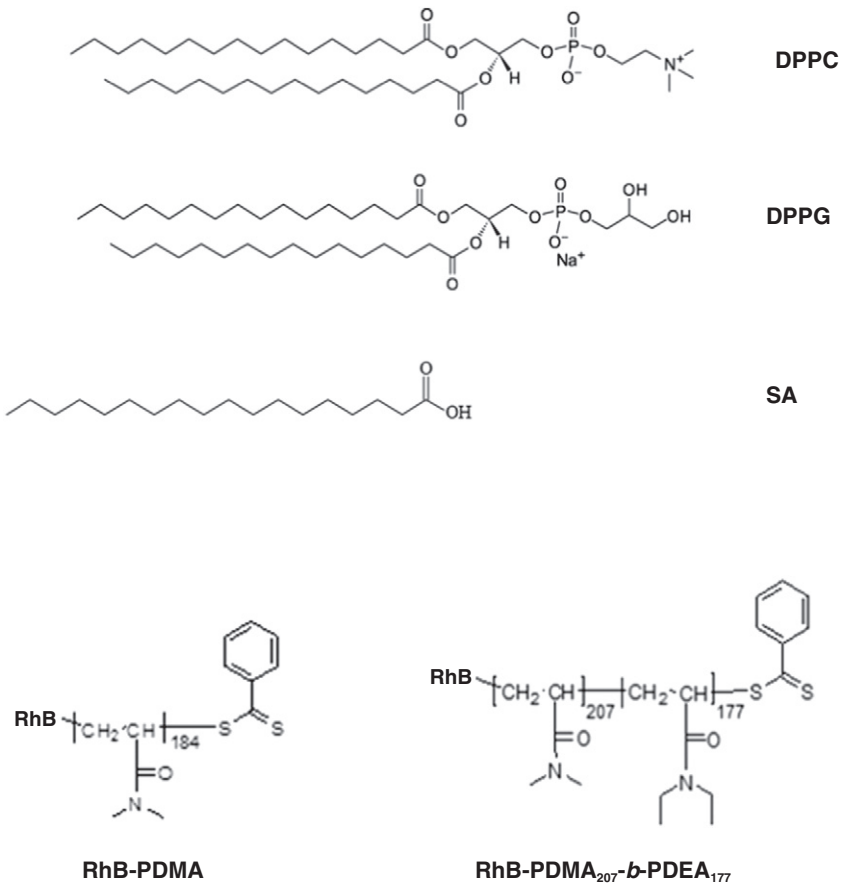

Chart 1. The molecular structures of lipids and polymers.