Reactive and Functional Polymers 94 (2015) 9–16

Contents lists available at ScienceDirect

Reactive and Functional Polymers

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

Polymer–lipid interactions: Biomimetic self-assembly behaviour and surface properties of poly(styrene-alt-maleic acid) with diacylphosphatidylcholines

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ARTICLE INFO

Article history:
Received 19 March 2015
Received in revised form 24 June 2015
Accepted 26 June 2015
Available online 3 July 2015

Keywords:
Biomimetic
Phosphorylcholine
Surface properties
Self-assembly
Amphiphiles

ABSTRACT

Various lubricating body fluids at tissue interfaces are composed mainly of combinations of phospholipids and amphipathic apoproteins. The challenge in producing synthetic replacements for them is not replacing the phospholipid, which is readily available in synthetic form, but replacing the apoprotein component, more specifically, its unique biophysical properties rather than its chemistry. The potential of amphiphilic reactive hypercoiling behaviour of poly(styrene-alt-maleic acid) (PSMA) was studied in combination with two diacylphosphatidylcholines (PC) of different chain lengths in aqueous solution. The surface properties of the mixtures were characterized by conventional Langmuir–Wilhelmy balance (surface pressure under compression) and the du Noüy tensiometer (surface tension of the non-compressed mixtures). Surface tension values and ^{31}P NMR demonstrated that self-assembly of polymer–phospholipid mixtures were pH and concentration-dependent. Finally, the particle size and zeta potential measurements of this self-assembly showed that it can form negatively charged nanosized structures that might find use as drug or lipids release systems on interfaces such as the tear film or lung interfacial layers. The structural reorganization was sensitive to the alkyl chain length of the PC.

1. Introduction

There is an increasing interest in mimicking the molecular behaviour of natural systems. One important target is the spreading and lubrication behaviour of biological fluids such as tears, pulmonary surfactants and synovial fluid. These fluids exhibit some remarkable similarities in composition in that they all contain a phospholipid, dipalmitoyl phosphatidylcholine (DPPC), in combination with amphipathic apoproteins, such as SP-B and SP-C. The challenge in producing replacements for these fluids in treating lubricity deficiency diseases such as dry eye, respiratory distress syndrome (RDS) and arthritis is not replacing the phospholipid, which is readily available in synthetic form, but in replacing the apoprotein component. In this paper we investigate the self-assembly behaviour and surface properties of the poly(styrene-alt-maleic acid)–diacylphosphatidylcholine system that was initially identified in these laboratories as a potential reactive functional substitute [1,2].

The native lung surfactant consisting mainly of DPPC and the apoproteins SP-B and SP-C has been shown to greatly accelerate the kinetics of adsorption, decrease the surface tension upon compression to nearly zero, and induce good respreadability [3,4]. However, due to the relative scarcity, as well as hydrophobic and surface active nature of these proteins, it is difficult and costly to isolate them from natural sources in high purity [5]. For example, the complex structure of SP-B makes it challenging to synthesize chemically and then obtain a properly folded form. For that reason, analogues have been developed to mimic its biophysical properties instead of its chemistry. An effective lung surfactant must have at least three fundamental biophysical properties: (1) rapid adsorption to the air–water interface, (2) the ability to reach near-zero surface tension upon film compression, and (3) the ability to re-spread upon multiple compressions and expansions of surface area with minimal loss of surfactant into the subphase [6]. Non-natural analogues of SP-B such as amphiphilic peptides and peptoids have been previously studied [7,8]. However, since peptides are relatively expensive to synthesize and purify, there is great interest in developing synthetic analogues as protein mimics for therapeutic purposes [9,10].

A similar interfacial role has been identified in the tear film, although there the physiological role of the lipoidal component differs from that in the lung. The tear film has a coating of phospholipids, which are necessary for the formation of a stable tear film. In this regard, they must be clear and colourless, unlike conventional aqueous preparations of phospholipids that may be opaque.

Responsive hydrophobically associating polymers or hypercoiling polymers, can in many ways be considered to be analogous to apoproteins in their ability to form compact molecules with a defined secondary structure, and hence, functionality. These molecules are characterized by the presence of alternating charged and hydrophobic

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http://dx.doi.org/10.1016/j.reactfunctpolym.2015.06.015
1381-5148/

groups. The balance between charge repulsion and hydrophobic interactions is sensitive to environmental pH and therefore changes in pH above or below their pKa, produce controllable conformational changes and increased functionality. The change from a charged extended chain to a collapsed uncharged coil structure is sometimes referred to as "hypercoiling" behaviour and enables the polymer to act as a simple switch between an 'on' and 'off' state [2] in a reactive way. Copolymers of maleic acid and styrene have this behaviour. The potential of PSMA as a polymeric drug and its conjugation with several drugs has already been exploited with clinical success to treat several types of cancer [11,12], to inhibit human immunodeficiency virus type 1 (HIV-1) [13,14], and it has also been reported to be strong inhibitor of spermatozoa motility [15], among others. It represents a promising raw material due to its low cost and toxicity.

Phosphatidylcholines (PC) are considered as zwitterionic surfactant molecules due to the existence of two charged groups with different sizes [16]. Phosphatidylcholines can spontaneously organize into bilayers due to their cylindrical shapes [17]. Due to their amphiphilic nature, they are also called as membrane-forming lipids with the polar head group aligned towards the aqueous phase and the hydrophobic tail groups forming the hydrophobic core. The so-called hydrophobic effect is believed to be the main driving force in self-association [18]. They way in which they form the bilayers, their solubilities and their stability, depend strongly on their chain length and self-assembly properties [19,20].

PC-containing polymers have been widely used in various biomedical applications including contact lenses coatings, drug delivery systems, biochip-based diagnosis systems, blood-contacting medical devices, and tissue engineering devices [21–24]. When functional hypercoiling polymers are combined with film-forming lipids they associate to produce lipid–polymer nanostructures analogous to naturally occurring lipoprotein assemblies in the form of flattened disk-like molecular assemblies. The reactive pH-dependent complexation of anionic polyelectrolytes with phospholipid vesicles has been studied before [25]. Such behaviour was explained in terms of hydrogen bonding between the charged carboxylic acid pendant groups of the polymer and the phosphodiester head groups of the phospholipid. Lowering of the pH caused a loss of charge and predominant hydrophobic interactions. Alternating copolymers of styrene and maleic acid (i.e. hydrolysed styrene/maleic anhydride polymers) have a pKa value in the region of 3.75–4.0 [26], the pKa for the individual acid functions being approximately 1.97 and 6.24. In this case, the amphipathic segments surround the phospholipid bilayer in a 'doughnut' arrangement [2,27]. As previously mentioned, PSMA-phospholipid complexes could be used in several applications related to medical conditions affecting mucosal surfaces. In addition, they could be used as biomimetic analogues for the tears, due to their transparency once they are formed. Preparation of clear solutions requires a lowering of the pH to 4–5. Upon decreasing the pH, the hydrophobic microdomains of PSMA disrupt the vesicle membranes by associating with the lipoidal core of the bilayer, resulting in a membrane reorganization and formation of small discoidal complexes. This results in the formation of optically clear, aqueous suspensions.

Although these complexes can be formed with DPPC it is necessary to maintain the mixture at an elevated temperature above the main phase transition temperature (melting point, Tm) of DPPC of around 42 °C. e.g. at about 50 °C, during the process. For the wider range of applications envisaged here, it is logical to examine systems that can be formed at room temperature which logically leads to the exploration of DMPC (1,2-dimyristoyl-sn-glycero-3-phosphocholine) and DLPC complexes (1,2-dilauroyl-sn-glycero-3-phosphocholine), with 14 and 12 carbon atoms (Tm 23 °C and −2 °C), respectively, so that they are in a disordered bilayer or liquid crystalline phase at room temperature.

The use of different analytical techniques to explore the association between anionic polyelectrolytes and lipid vesicles has long been investigated by several researchers using different approaches (e.g. fluorescence spectroscopy, differential scanning calorimetry, neutron scattering and FTIR [27,28]). In this study the Langmuir–Wilhelmy balance combined with the du Noüy tensiometer can analyse monolayers as simplified models since various parameters like density, packing, nature of lipids as well as the subphase composition, pH and temperature can be varied in a well-defined manner. For this reason, these techniques may provide information regarding conformational transitions that play a crucial role in the development of vesicular biomimetic systems [29–31]. Finally, the ability to form stable polymer–phospholipid complex nanoparticles, were analysed by means of sizes distribution, surface charges and morphologies.

2. Materials and physico-chemical characterization

2.1. Materials

Dilauroyl phosphorylcholine (DLPC, ≥99%, Mw 622 g/mol), was purchased from Sigma-Aldrich (Gillingham, UK), and dimyristoylphosphorylcholine (DMPC, ≥99%, Mw 678 g/mol) from Avanti Polar Lipids Inc. (Alabaster, Alabama, USA). Styrene-maleic anhydride copolymer (50:50, 1600 MW), was purchased from MP-monomer polymer & Dajac Labs. All the solvents (HPLC grade) and the phthalate buffer tablets were from Fisher Scientific (Loughborough, UK). First, styrene-maleic acid copolymer was obtained as alternative copolymer of styrene and maleic anhydride by hydrolysis in water at basic pH adjusted by 1 M NaOH and was kept at 80 °C for 6 h. After hydrolysis, PSMA became soluble in weak acid.

2.2. Physico-chemical characterization

The Langmuir–Wilhelmy balance (NIMA Tech. Ltd., Coventry, UK) had a subphase area of 30 × 20 cm and a maximum monolayer containment area of 535 cm^2. It was equipped with an IU4 computer interface unit and operating software version 7.8. A stock solution of styrene maleic acid 1.9 mM (30 mg/ml) was prepared and stored at room temperature (20 °C). Stock solutions of the phospholipids were prepared in chloroform in the concentration of 2 mM (1.2–1.5 mg/ml), and were sealed and stored at room temperature.

The Langmuir–Wilhelmy balance, which was kept inside a cabinet, was equipped with two barriers that moved simultaneously towards the centre of the trough, which allowed for symmetric compression of the film. It was fabricated in poly(tetrafluoroethylene) (PTFE), a material that avoids contamination of the surface. The surface pressure (π), varied as the area between the barriers was changed, and was measured by means of the Wilhelmy plate method where continuous surface pressure measurements are obtained by a transducer interfaced with the software. The performed experimental procedure consisted basically of the following steps: (1) Cleaning the trough and barriers with HPLC grade chloroform and HPLC ultra-pure water. (2) Addition of the subphase (HPLC ultra-pure water), the subphase temperature was kept constant and equal to 20 °C by means of an external temperature controller unit. (3) Attaching the Wilhelmy plate (strip of Whatman's Chromatography paper) connected to an electrobalance. (4) Spreading, drop by drop, of the phospholipid solution. (5) Evaporating the solvent for 10 min. (6) Compressing the film at 100 cm^2/min and recording the π versus area data. PSMA studies followed an equivalent procedure but, the PSMA solution was spread over the water subphase. In the experiments examining the effect of PSMA on the molecular packing of phospholipids monolayers, the PSMA solution was injected beneath the pre-forming phospholipid monolayer. Spreading solutions were deposited onto the aqueous subphase with a Hamilton microsyringe. The pH-dependent self-association of the polymer and pH-dependent complexation of PSMA with phospholipids were studied with phthalate buffer of pH 4 as subphase.

To measure the surface tension of the polymer–lipid complexes the du Noüy tensiometer with Pt–Ir ring by the ring detachment technique