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Interaction of poloxamine block copolymers with lipid membranes: Role of copolymer structure and membrane cholesterol content

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ABSTRACT

Interactions of X-shaped poly(ethylene oxide)–poly(propylene oxide) (PEO–PPO) block copolymers with cell membranes were investigated recording the π-A isotherms of monolayer systems of dipalmitoylphosphatidylcholine (DPPC):cholesterol 100:0; 80:20 and 60:40 mol ratio and evaluating the capability of the copolymers to trigger haemolysis or to protect from haemolytic agents. Four varieties of poloxamine (Tetronic 904, 908, 1107 and 1307) were chosen in order to cover a wide range of EO and PO units contents and molecular weights, and compared to a variety of poloxamer (Pluronic P85). The π-A isotherms revealed that the greater the content in cholesterol, the stronger the interaction of the block copolymers with the lipids monolayer. The interactions were particularly relevant at low pressures and low lipid proportions, mimicking the conditions of damaged membranes. Relatively hydrophobic copolymers bearing short PEO blocks (e.g., T904 and P85) intercalated among the lipids expanding the surface area (ΔG_{exc}) but not effectively sealing the pores. These varieties showed haemolytic behavior. Oppositely, highly hydrophilic copolymers bearing long PEO blocks (e.g., T908, T1107 and T1307) caused membrane contraction and outer leaflet sealing due to strong interactions of PEO with cholesterol and diamine core with phospholipids. These later varieties were not haemolytic and exerted a certain protective effect against spontaneous haemolysis for both intact erythrocytes and cholesterol-depleted erythrocytes.

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1. Introduction

Amphiphilic block copolymers are being shown as very versatile materials in the biomedical field not only as constituents of drug nanocarriers, but also as "active" components able to regulate physiological/cellular responses to the drugs and even to exert some therapeutic actions by themselves [1,2]. Compared to common surfactants, most block copolymers exhibit improved capability to self-assemble into micelle-like or more complex structures (vesicles and polymersomes) that can host a variety of drugs, leading to higher apparent solubility, stability and capability to target the payload to specific tissues or cells [3,4]. Suitability for being decorated with a variety of ligands and/or prepared with stimuli-responsive components makes block copolymers-based nanocarriers attractive to face up to a variety of therapeutic demands [5]. In addition to the performance when they are in the aggregate state, some block copolymers as individualized chains (unimers) have been demonstrated useful for inhibition of efflux pumps [1,6], for triggering differentiation of mesenchymal stem cells in a variety of lineages, including osteoblasts and adipocytes [7,8], or for sealing damaged membranes (e.g., after a thermal burn or electrical shock) minimizing the leakage of intracellular contents [9].

All the above referred performances point out amphiphilic block copolymers as multifaceted tools in biomedicine. Nevertheless there is still a paucity of information regarding the mechanisms through which unimers of amphiphilic block copolymers interact with cell membranes, which could explain why some varieties are more efficient than others. Most information refers to linear poly(ethylene oxide)–poly(propylene oxide)–poly(ethylene oxide) copolymers, PEO-b-PPO-b-PEO, known as poloxamers or Pluronic® [10–12]. Studies carried out with model lipid mono- and bi-layers (Langmuir monolayers, fluorescence microscopy and small-angle X-ray scattering) and cell cultures evidenced copolymer insertion into the membranes when the lipid density is lower than that of a

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healthy cell membrane. From a practical point of view, copolymer insertion can be useful for selective sealing of porated or permeabilized regions of cell membranes [13–15]. Once the membrane recovers the lipid ordering, the copolymer unimers are expected to be squeezed out. Pluronic P188 (currently F68; Mw 8400 Da; 2× 76 EO and 29 PO units) has been shown to protect or to help the recovery of cells exposed to heat shock, glutamate toxicity, ionizing radiation or physical shearing [16–18] and recently granted for treatment of sickle cell disease due to its capability to attach to and seal damaged erythrocyte membranes [19]. A similar role has been reported for the poloxamine variety Tetronic® 1107 (Mw 15,000; 4× 60 EO and 4× 20 PO units), which differently to Pluronics consists in a X-shape structure with four arms of PPE-PPO blocks linked to a central ethylenediamine group and exhibits multi-responsiveness [20,21]. For example, Tetronic 1107 has been shown to protect erythrocytes and lymphocytes from ionizing radiation [22]. For membrane sealing applications, the copolymers have been tested at concentrations (0.1–1.0 mM; roughly 1–10 mg/mL) below, but close to, the CMC in order to maximize the concentration of unimers [9]. Regarding structure–activity relationships, poloxamer varieties with short PPO block (e.g., Pluronic F68) have been shown to insert the PPO block in the bilayer as a loop with the two PEO end blocks extending laterally onto the phospholipid heads at the same side of the membrane; namely, the PPO chain length does not allow for membrane spanning, but the PEO chains can effectively seal defects on the membrane. Poloxamers with longer PPO block (e.g., Pluronic P85; Mw 4600 Da, 2× 26 EO and 40 PO units), close to the length of the acyl chains of lipids at the cell bilayer (dimensions of the hydrophobic part of the lipid bilayer estimated to be ca. 20 Å), can fully insert into the cell membrane with the PEO blocks oriented to opposing sides of the bilayer [14]. Thus, distinct varieties exhibit different anchoring to the membrane and different swelling of the lamellar structure [14]. It has also been found that the higher the EO/PO units ratio, the lower the squeeze-out pressure [23]. Nevertheless, the protective effect can be maintained if the copolymer remains adsorbed onto the membrane; hydrophilic Pluronic P188 and Tetronic 1107 have been shown to efficiently protect liposomes from peroxidation [24].

Importantly, block copolymers interact differently with healthy cells than with tumor cells. As mentioned above, the copolymers poorly insert into non-damaged membranes and they are squeezed out when the damaged ones are restored. Oppositely, some block copolymers penetrate better into intact tumor cells probably because their lower content in cholesterol (~20% versus 40% in healthy cells) [25,26]. In fact, hydrophobic Pluronics L10 (Mw 3200 Da, 7 EO and 50 PO units) and L61 (Mw 2000 Da, 4 EO and 31 PO units) have been shown to sensitize tumor cells against hyperthermia treatment and ionizing radiation [27,28].

The aim of this work was to gain further insight into the interaction behavior with cell membranes of four varieties of poloxamine (Tetronic 904, 908, 1107 and 1307) and a variety of poloxamer (Pluronic P85), recording the π-A isotherms in monolayer systems of dipalmitoylphosphatidylcholine (DPPC):cholesterol 100:0; 80:20 and 60:40 mol ratio (as a mimic of the outer leaflet of cell membrane). Poloxamine varieties were chosen to cover a wide range of EO and PO units and molecular weights, also including the variety previously investigated as sealant (T1107) [22] and the one identified as osteogenic (T908) [7]. Pluronic P85 was chosen due to its reported capability to insert into the lipid bilayer and alter diverse cell pathways to inhibit P-glycoprotein efflux pumps [1]. Although poloxamine-lipid monolayer systems have not been evaluated before, it has been pointed out that poloxamines might be more effective as membrane sealant than poloxamers due to their bulkier hydrophobic center [9]. Additionally, the protective effect of the block copolymers on erythrocyte hemolysis was recorded. The information gathered from this study may help to understand how differences in block lengths and copolymer architecture affect to the interaction with cell membrane, and thus to elucidate the role of poloxamines not only as sealant agents, but also as components of drug nanocarriers and modifiers of cell response.

2. Materials and methods

2.1. Materials

Tetronic® varieties 904 (T904; Mw 6700 Da, 4× 15 EO and 4× 17 PO, HLB 12–18), 908 (T908; Mw 25,000 Da, 4× 114 EO and 4× 21 PO, HLB >24), 1107 (T1107; Mw 15,000 Da, 4× 60 EO and 4× 20 PO, HLB 18–23) and 1307 (T1307; Mw 18,000 Da, 4× 72 EO and 4× 23 PO, HLB >24) and Pluronic® P85 (P85; HLB 12–18) were from BASF Corporation (Florham Park, NJ, USA). Cholesterol (purity >99%) and 1,2-dipalmitoyl-rac-glycero-3-phosphocholine (DPPC) were from Sigma–Aldrich (Steinheim, Germany). Chloroform and n-amyl alcohol (1-pentanol) were from Merck (Darmstadt, Germany). Phosphate buffer solution (pH 7.4) was prepared in purified water (resistivity >18 MOhm cm; MilliQ®, Millipore, Spain) obtained by reverse osmosis. All other reagents were of analytical grade.

2.2. π-A isotherms

π-A isotherms were recorded using a single barrier NIMA 611 surface balance (Coventry, UK) and a trough with total area 560 cm^2 (previously cleaned with chloroform, ethanol and water). USP phosphate buffer solution (pH 7.4) was used as subphase and temperature was kept at 30 ± 1 °C. To record the π-A isotherms of the single components, solutions (50–100 μL) of DPPC (0.2 mg/mL) and of each copolymer (0.1 mg/mL) were prepared in chloroform (with a minor addition of amyl alcohol for improving extension), deposited by means of a syringe (Hamilton, USA) at the air–water interface and allowed to stand for at least 10 min in order to ensure the complete evaporation of the solvent. For the mixed monolayers, solutions of the copolymer and of DPPC–cholesterol were prepared in separate in chloroform (0.2 mg/mL), mixed at different proportions (as indicated in Fig. S1 in Supporting Information) and then processed as for the monocomponent monolayers. The compression was then initiated with a barrier speed of 15 cm^2 min^{-1}, and the surface pressure, π, was recorded as a function of the area of the monolayer and referred to the number of molecules. The surface pressure was measured (accuracy 0.1 mN/m) using a Wilhelmy plate made from chromatography paper (Whatman Chr1, UK).

2.3. Erythrocyte binding assay

Binding assay of copolymers to erythrocytes membrane was carried out as previously reported [10] with some modifications. Erythrocytes from healthy donors (Galician Transfusion Center, Spain) were separated by centrifugation and then dispersed in PBS to obtain suspensions of 0.0375%, 0.075%, and 0.15% hematocrit. Separately, dispersions of T904, T908, T1107, T1307 and P85 in PBS were prepared covering a wide range of concentrations (0.0001 M, 0.001 M, 0.005 M and 0.01 M). Aliquots (100 μL) of copolymer solution were placed in triplicate into Eppendorf Lobind tubes, and then 100 μL of each erythrocytes suspension were added. In parallel, erythrocytes suspensions were mixed with 4% Triton X-100 in order to force 100% lysis. After 4 h of incubation, the Eppendorf tubes were centrifuged at 600 × g during 4 min, and the supernatants were transferred to 96-wells plates. Absorbance at 415 nm was measured (BIORAD Model 680 microplate reader, USA). Copolymer solutions without erythrocytes were used as blank.

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