Artifical Membrane Pores

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Polymer-Induced Transient Pores in Lipid Membranes**

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amphiphiles · lipid bilayer membranes · polyelectrolytes · polymers · pore formation

Closed lipid bilayer membranes in the form of vesicles or liposomes have been widely used as mimics for cell membranes,[1] for the delivery of small molecules, such as ions and pharmaceuticals,[2] and larger molecules, such as DNA, mostly for transfection purposes.[3] The release of enclosed components from the interior of a vesicle requires controlled breakup mechanisms, which enable a controlled porosity of the membrane for freeing the molecule of interest. In contrast to the uncontrolled destruction of the vesicular membrane,[4] the triggered generation of pores of defined size within the lipid bilayer membrane has become a major topic of research, in particular through the use of synthetic membrane channels or pores.[5] Inspired by biological counterparts, such as gramicidine A, amphotericine, or valinomycine, a large variety of supramolecular highly organized molecules with molecular weights M_n below 2000 Da have been tested, generating pores within lipid membranes (for recent reviews, see references [5–11]). Recurring structural motives within these molecules often include the formation of barrel-shaped structures within the lipid membrane, with supramolecular motives consisting of helices, liquid crystalline stacks,[7] barrel-hoop pores[12] or barrel-rosette pores.[11] An important structural feature for most pore-forming molecules is the necessity to accommodate the hydrophobic lipid bilayer structure inside the membrane, but to still keep sufficient hydrophilicity on the outside of the membrane to allow the open state of the final pore.[13] Thus, simple lipids clustering within a membrane, such as C_2 and C_{16} ceramides, can also generate large stable pores within a membrane.[14]

Apart from these structurally highly defined organic molecules, unfolded synthetic macromolecules have shown similar pore-formation abilities upon contact with lipid bilayer membranes, although their final structure is less predictable.[15] In contrast to small- and medium-sized organic molecules, the structure, conformation, and phase-separation abilities of polymers is more complex, which hinders the prediction of exact conformations, shapes, or their status of ionization within a lipid bilayer membrane. When an amphiphilic polymer molecule approaches a membrane, as shown in Figure 1, the interaction of polymeric molecules with lipid membranes can lead to a large variety of structural phenomena within the lipid bilayer membrane.[16–18] Apart from a total disruption of the membrane (not shown),[4] nonionic amphiphilic block copolymers, such as PEO-PPO-PEO triblock copolymers, can insert into membranes to form pores by the "barrel-stove"-mechanism, generating a supramolecular aggregate of several molecules, and thus a hole for transport across the membrane (Figure 1a). Hydrophilic polymers with grafted hydrophobic side chains can interact either by pure membrane insertion (Figure 1b), or by formation of a transient pore by the "carpet" mechanism (Figure 1c). This mechanism relies on inducing the pore by a strong change of the membrane's curvature. Alternatively, polymers with ionizable groups (polyelectrolytes) can generate pores by

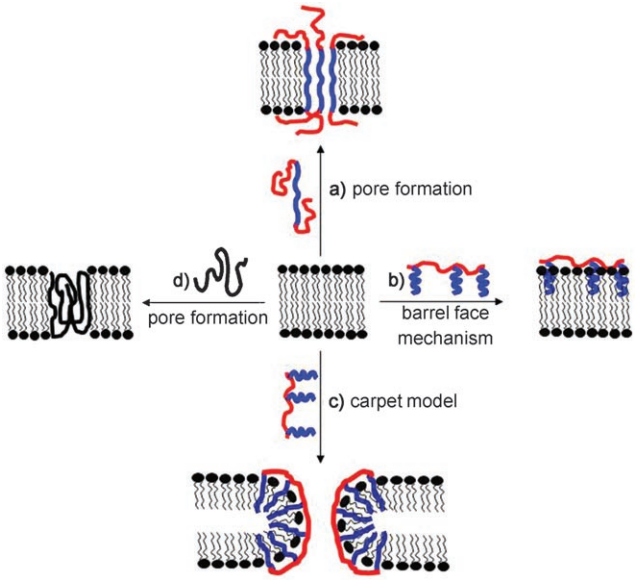

Figure 1. Models of polymer-induced pore formation into lipid bilayer membranes. a) Pore formation by amphiphilic triblock copolymers; b) barrel-face mechanism, by insertion of hydrophobic chains (blue) of graft polymers; c) carpet model, by insertion of amphiphilic (charged) graft polymers; and d) pore formation by full insertion of polyelectrolytes.

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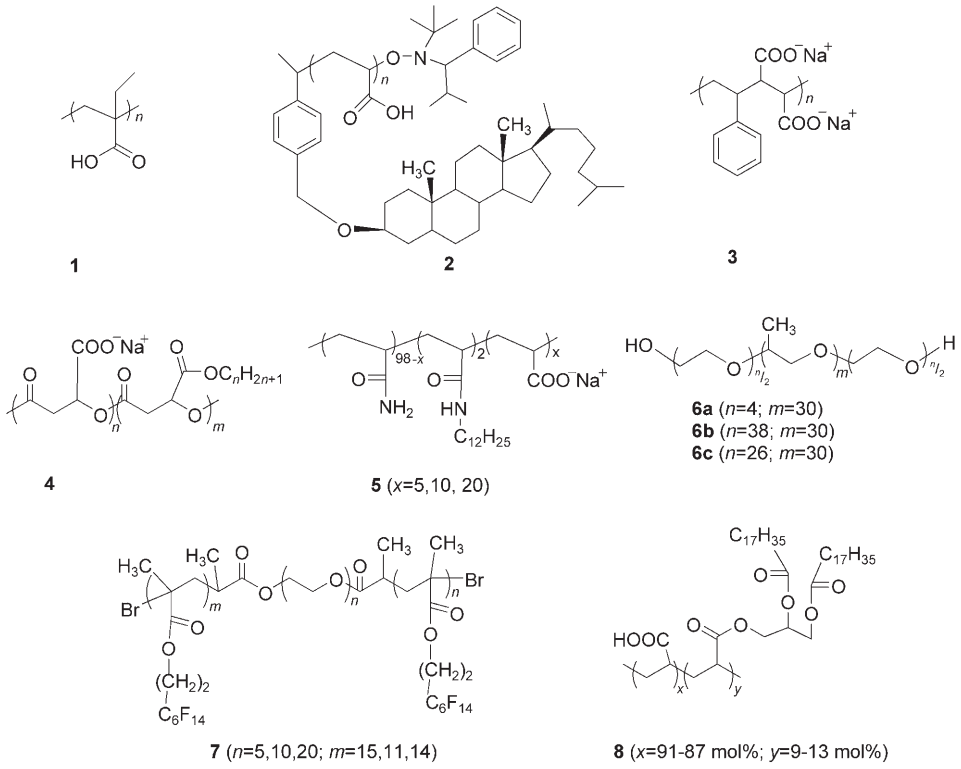
clustering in the nonionized state (Figure 1d), leading to pores with modulative properties which depend on the conformation of the polymer. The latter principles in particular are closely related to low-molecular weight surfactants acting to induce transient pores either by membrane-curving effects,[19] or transient micelle-formation within the lipid bilayer membrane.

One of the oldest but still most efficient methods that uses homopolymers to generate transient pores within a lipid bilayer membrane is with a polyelectrolyte, such as poly(2-ethyl acrylic acid) **1** (PEAA; see Scheme 1).[20] At pH values of around 7.0, PEAA changes its conformation from an expanded, hydrophilic coil at higher pH into a compact, globular structure at lower pH with amphiphilic properties.[21] Transient pore formation can be observed with polymer concentrations of 10 mol% relative to the neutral lipid DOPC or DPPC because of membrane adsorption and subsequent insertion by its amphiphilic properties.[22] The observed PEAA channels are cation selective, with a ratio $[Na^+]/[Cl^-]$ ranging from 2–11.[23] Pore formation is not observed with either anionically charged lipids or purely acrylic acid polymers,[24] whereas the more hydrophobic poly(2-propyl-acrylic acid) induces a strongly lytic effect on the membrane.[25] The pH-induced coil-to-globule conformational change of an acrylic acid polymer was used recently by incorporating the cholesterol-terminated poly(acrylic acid) **2** into liposomes, generating polymer-caged liposomes.[26] The liposomes underwent a triggered calcein release arising from changing the pH from 7.4 to 7.5 by the formation of pores by the coil-to-globule conformational change.

Much stronger effects are observed when polyelectrolyte copolymers or graft polymers, often with hydrophobic side chains, are used for a more efficient anchoring of the polyelectrolyte chain into the lipid bilayer structure, thus enhancing the formation of pores within the lipid bilayer membrane. A large variety of hydrophobic graft copolymers can be used to generate transient pores within the membrane, such as PS-*alt*-MAA **3** (poly(styrene-*alt*-methacrylic acid),[27] poly(maleic acid) copolymers **4**,[28] octyl-modified poly(acrylic acid),[29,30] octadecyl-modified dextrane,[31] or various acrylamides.[32]) Usually, only a small fraction of hydrophobic moieties (a few mol%) is needed within the polymer to effect an efficient pore formation, whereas a higher content of the polyelectrolyte within the copolymer leads to a more efficient leakage of the membrane, as recently studied by Wang et al.[33] by using copolymers of acrylamide and poly(acrylic acid) **5**. Most investigations point towards a primary insertion of the hydrophobic moieties of the copolymers into the lipid bilayer membrane according to Figure 1b, followed by pore formation according to the carpet model (Figure 1c).

A long-standing question is whether regular pores are really present within the membrane, rather than just random, undefined pores or leakage sites. A recent study by Tribet et al.[29] has claimed to verify the presence of defined pores by studying the interaction of so-called amphipols, or polydisperse copolymers (consisting of acrylic acid/*N*-octylacrylamide/*N*-naphtylacrylamide in mole ratios of 0.71:0.25:0.04) with black lipid membranes by measuring the current by patch-clamp techniques. The occurrence of discrete currents with increasing polymer concentration was observed which correlate with the pore size. The pore size ranged from 0.3 nm (at very low polymer concentration) to a constant radius at higher polymer concentration of roughly 4 nm. Whereas the initial state corresponds to only one individual polymer molecule inserted into the membrane, the presence of a distinct, 4-nm-sized pore is a strong indication of the carpet model indicated in Figure 1c.

Recently, nonionic block copolymers have been shown to induce the formation of pores in lipid bilayer and lipid monolayer membranes. The main focus has been directed



Scheme 1. Chemical structures of pore-forming homo- and copolymers.

towards ABA triblock copolymers containing poly(propyleneoxide) (PPO) or poly(ethyleneoxide) (PEO) as the central B-block. Experiments were conducted by Krylova and Pohl[34] on the Pluronic L61 **6** (see Scheme 1). A distinct pore-formation effect of this polymer on black lipid membranes has been demonstrated by patch-clamp techniques, proposing an assembly of only few polymers across the membrane, but also the presence of frequent larger aggregates (carrier mode, as shown in Figure 1 a). The same group has proposed increased flip-flop movements of the individual lipid molecules upon interaction of Pluronics of the type **6** and PEO-PPO amphiphiles with dendritic headgroups to explain the disturbance of the membrane and the induction of transporting abilities.[35] Interactions of such amphiphilic macromolecules were further investigated with Langmuir films using different Pluronics **6b**, **6c**[36,37] and the triblockcopolymer **7**, bearing perfluorinated endblocks as the hydrophobic part.[38] Distinct incorporation of the polymers into the membrane could be detected by surface-pressure/area isotherms, in-situ small-angle neutron scattering measurements,[37] and infrared reflection spectroscopy. After the hydrophobic blocks were incorporated, a hydrophilic PEO loop formed outside, towards the hydrophilic solvent, thus forming pores into the membrane. Additionally, the interaction of Pluronic L64 (**6c**) was investigsted by studying ion transport using a perforated vessel at different concentrations.[37] As in the case of the charged amphipols, the formation of distinct pores at low and medium concentration of the Pluronic L64 **6c** was observed with electrical measurements. The proposed polymers show great potential as gene delivery agents,[3] as they avoid the membrane disruption or the classical lipoplex formation that occurs using cationic polymers, such as poly(ethyleneimine) (PEI).[39]

As lipid membranes are limited in their stability, the question arises as to whether polymeric membranes (polymersomes) can exhibit similar pore formation, possibly triggered by an external stimulus, such as changes in pH or temperature. Complete destruction of polymersomes by an external stimulus are well known,[40] whereas polymersomes with distinct pores made from synthetic polymers are not. However, recent work by Chiu et al.[41] demonstrates this point by using the graft copolymer **8**, consisting of a low percentage (9–13 mol %) of stearyl side chains and polyacrylic acid moieties (PAA) as the main component (Figure 2). Using a double-stage emulsion process, polymersomes were generated with a membrane consisting of the copolymer. The PAA chains form domains within the membrane, and their conformation shows the pH-dependent coil-to-globule transition described above. Thus, a change in pH from 5.0 to 8.0 induces the transition, thus opening the pore to release the component from the interior of the polymersome.

Polymers, like their more highly organized pore-forming molecules in supramolecular chemistry, are also able to form pores and channels within lipid bilayer membranes. Various polymerization methods are available to form polymers with tailored and defined structures, which is of great significance to the targeted incorporation into membranes. The supramolecular arrangement of the polymers within or at the phase boundary of structured lipid double-layer membranes is largely uninvestigated, and will without a doubt be the subject of future studies.

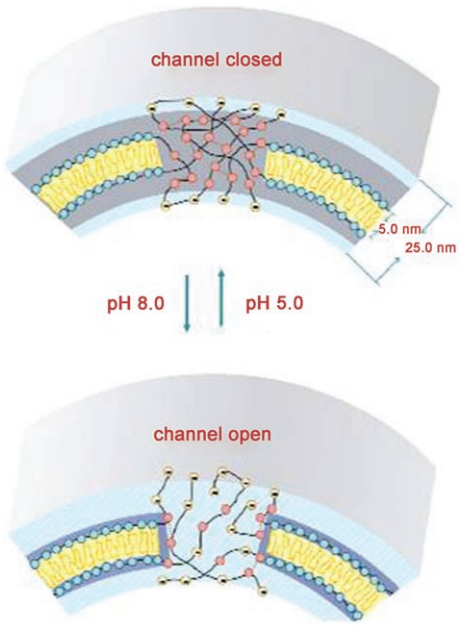


Figure 2. Pore formation in polymersomes, formed from graft copolymer **8**, consisting of a low percentage (9–13 mol %) of stearyl-side chains and polyacrylic acid moieties (PAA). The pores can be reversibly switched between an open and closed state by changing the pH between 5.0 and 8.0. (Reproduced from reference [41] with permission).

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