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Vesicles with asymmetric membranes from amphiphilic ABC triblock copolymers

R. Stoenescu[a] and W. Meier[ab]

[a] Department of Chemistry, University of Basel, Klingelbergstrasse 80, CH-4056 Basel, Switzerland
[b] School of Engineering and Science, International University of Bremen, Campus Ring 1, D-28759 Bremen, Germany. E-mail: w.meier@iu-bremen.de

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A new amphiphilic ABC triblock copolymer (poly(ethylene oxide)–poly(dimethyl siloxane)–poly(methyl oxazoline)) has been synthesized and demonstrated to form vesicular structures with asymmetric membranes in aqueous media.

During the last decade self-organization of soft materials has shown to be valuable for the creation of a wide variety of nanostructures that could be used for applications in fields ranging from materials science to biology. In this context amphiphilic block copolymers are of particular interest due to their ability to self-assemble in aqueous media, their broad accessibility to different length and time scales and levels of interaction.[1,2] Similar to conventional low molar mass surfactants they may form micelles, vesicles or lyotropic mesophases. These aggregates are significantly more stable than those formed by low molar mass amphiphiles and additionally they can be further stabilized by a subsequent crosslinking polymerisation.[2–4] This makes, *e.g.*, block copolymer vesicles highly interesting as transfection vectors[5] protective shells for sensitive enzymes[6] or as confined reaction vessels that allow to perform (bio-) chemistry even at a single molecular level.[7] The walls of the block copolymer vesicles are formed by membrane-like superstructures that can be regarded as mimetics of biological membranes. In fact, recently we were able to show that despite their enormous thickness and stability, such block copolymer membranes can be used as a matrix for functional reconstitution of membrane proteins.[6,8]

Most membrane proteins are asymmetric and in natural membranes oriented such that they expose an extracellular part to the outside and a cytoplasmic part to the inside of a biological cell. Artificial membranes, however, are symmetric with respect to their midplane if curvature effects are neglected. That holds, of course, also for block copolymer membranes formed by AB or ABA-type block copolymers. Therefore, during reconstitution, membrane proteins are randomly inserted without any preferred direction. Unfortunately many potential technical applications of such reconstituted systems depend on the correct orientation of the protein. This brought us to the idea of using intrinsically asymmetric membranes as a matrix. Here block copolymer chemistry offers a particularly interesting approach. Different water-soluble polymers are inherently incompatible and undergo phase separation in aqueous media. Therefore, membranes formed by ABC triblock copolymers (with water soluble blocks A and C and a hydrophobic middle block B) should be asymmetric: one side predominantly covered by the blocks A and the other by the blocks C. Although the synthesis of ABC triblock copolymers of this type has been reported previously,[9] up to now there is no evidence for a membrane-forming system.

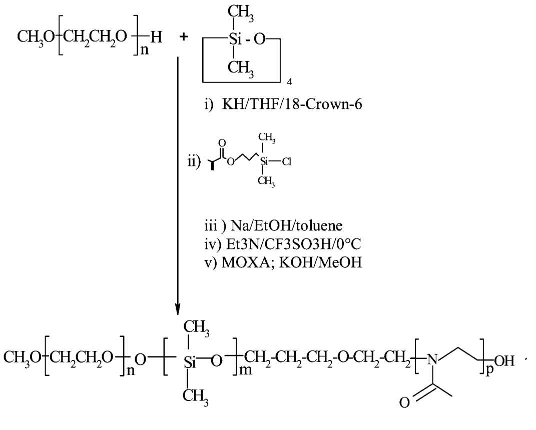
In this paper we describe for the first time amphiphilic poly(ethylene oxide)-block–poly(dimethylsiloxane)-block–poly(2-methyloxazoline) (PEO-PDMS-PMOXA) triblock copolymers that self-assemble in aqueous media into asymmetric, membrane-like superstructures.

The synthesis of a representative PEO-PDMS-PMOXA copolymer is sketched in the following (see also Scheme 1). All steps were carried out under argon atmosphere. A solution of 6.2 g of poly(ethylene oxide) monomethyl ether (M_n = 2 × 10^3 g mol^{-1}, M_n/M_w = 1.03) in dry tetrahydrofuran ($[PEO]_0$ = 125 g L^{-1}) was added to a dispersion of potassium hydride in THF. Then 18-crown-6 (19 mg, 7.11 × 10^{-5} mol) was added and the reaction solution was stirred for 4 h. Subsequently, 1.2 mL of 2,6-dimethylpyridine were added dropwise (50 μL min^{-1}) and the reaction stirred for another 30 min. The resulting alcoholate anion was used as an initiator for the anionic ring opening polymerization of octamethyltetracyclosiloxane (D_4) (18.7 g, 63 mM) The polymerization time was 20 h at a temperature of 55 °C. The chain growth was terminated using methacryloyloxypropyldimethylchlorosilane (2.8 g, 12.6 mM). The resulting PEO-PDMS diblock copolymer was purified by column chromatography (THF/methanol (8:2, v/v)) and ultrafiltration with water. After evaporation of the solvent the pure PEO-PDMS diblock copolymer was obtained as a yellowish oil. Reduction of the ester end-group was accomplished using a modified Bouveault–Blanc method.[10] An excess (40%) of sodium in ethanol was added to a solution of the polymer in ethanol (1:1, v/v) and allowed to react for 16 h at 75 °C. After dissolving the precipitated salt by addition of water, the resulting alcohol was extracted with diethyl ether and distilled under reduced pressure.

The subsequent cationic ring opening polymerization of methyloxazoline followed a procedure described by Hirt *et al.*[11] The purity and the structure of the block copolymers was confirmed by ^1H, ^{29}Si NMR and IR spectroscopy. Their molecular weights were calculated from the molecular weight of the PEO precursor and the chemical composition.

To check whether the block copolymers form vesicles, the copolymers were dispersed in water (typically at 20 mg mL^{-1} PEO-PDMS-PMOXA). Indeed, transmission electron microscopy (TEM) and dynamic light scattering measurements revealed that this led to vesicles with sizes in the range of 60–300 nm. Fig. 1 shows a representative TEM image of spherical ABC block copolymer vesicles with a rather broad size distribution. The polydispersity of the originally formed vesicles could, however, be reduced by subsequent extrusion through filters with 0.1 μm diameter pores.

To get information about the orientation of the triblock copolymers within the vesicular walls we applied an approach



Scheme 1 Synthesis of PEO-PDMS-PMOXA.

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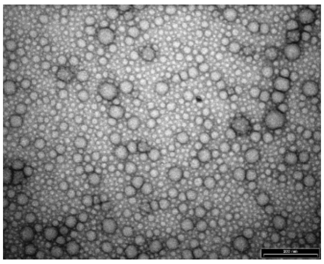

Fig. 1 Transmission electron micrograph of negatively stained aggregates of $A_{45}B_{67}C_{346}$.

similar to that recently used for amphiphilic AB diblock copolymer vesicles.[12] Here we labelled selectively one end of the triblock copolymers with a fluorescent dye (7-methoxycoumarin) and used quenching experiments to determine whether the labelled hydrophilic block was oriented toward the inside or the outside of the vesicles.

For that purpose 7-methoxycoumarinazide (5 mg mL^{-1}) was dissolved in chloroform. A five-fold excess of this solution was added to the $A_xB_yC_z$ triblock copolymer (with A = PEO, B = PDMS, C = PMOXA and x,y,z = number of repeat units of the respective block) and heated to 65 °C for 22 h. Upon heating the azide decomposes and forms an isocyanate that reacts (*in situ*) with the hydroxy groups at the end of the PMOXA blocks. Afterwards the product was separated and purified from unreacted dye by ultrafiltration with ethanol/water (7 : 3, v/v). ^1H NMR spectroscopy indicated a conversion of 30% of the terminal hydroxy groups for $A_{45}B_{67}C_{346}$ and 12% conversion for $A_{45}B_{40}C_{97}$ under these conditions. These rather low conversions are, however, not relevant for the following experiments.

For the quenching experiments we mixed labelled ($A_{45}B_{67}C_{346}$-7-methoxycoumarin and $A_{45}B_{40}C_{97}$-7-methoxycoumarin) with the corresponding non-labelled polymers ($A_{45}B_{67}C_{346}$ and $A_{45}B_{40}C_{97}$, respectively) in molar ratios of 344 : 1 and 10 : 1. From these mixtures we prepared vesicles following the procedure described above. Interestingly light scattering proved that the diameters of the labelled vesicles were similar to those of the non-labelled (*i.e.*, 69 nm for $A_{45}B_{65}C_{346}/A_{45}B_{65}C_{346}$-coumarin *vs.* 80 nm for $A_{45}B_{67}C_{346}$ and 74 nm for $A_{45}B_{40}C_{97}/A_{45}$ $B_{40}C_{97}$-coumarin *vs.* 115 nm for $A_{45}B_{40}C_{97}$). Obviously the presence of the fluorescent dye did not disturb the self-assembly of the polymers.

To get information about the orientation of the labelled PMOXA blocks we added Co^{2+} ions to the external solution. Co^{2+} is known to quench the fluorescence of coumarin. However, due to the thickness of the block copolymer membranes (*i.e.*, about 10 nm) and their impermeability toward Co^{2+} ions only the coumarin located at the outer surface of the vesicles can be quenched.

Fig. 2 shows the results of the quenching experiments for the labelled $A_{45}B_{65}C_{346}$ and the $A_{45}B_{40}C_{97}$ systems together with data for non-labelled block copolymer vesicles. Also the non-labelled vesicles of the control experiment showed a fluorescence emission around 420 nm, however, with a ten times lower intensity than the coumarin-labelled ones. This is presumably due to traces of impurities in the block copolymers. The presence of the Co^{2+} ions influenced this 'polymer' fluorescence, however, only very little. Interestingly the data for the $A_{45}B_{40}C_{97}/A_{45}B_{40}C_{97}$-coumarin vesicles were identical with the control system within the experimental error, *i.e.*, the fluorescence emission remains nearly unaffected by the presence of the quencher molecules (see Fig. 2). In contrast to that for the $A_{45}B_{65}C_{346}/A_{45}B_{65}C_{346}$-coumarin vesicles, with the longer PMOXA block, the data follow a Stern–Volmer relation and the fluorescence is quenched completely by Co^{2+} ions.

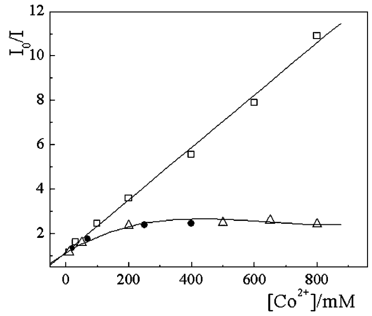

Fig. 2 The variation of steady state fluorescence with concentration of Co^{2+} ions quencher for spherical vesicles containing (I_0: fluorescence intensity in absence of Co^{2+} ions; I: fluorecence intensity in presence of variable concentrations of Co^{2+}: (A) $A_{45}B_{67}C_{346}/A_{45}B_{67}C_{346}$-coumarin (–□–); (B) $A_{45}B_{40}CA_{97}/A_{45}B_{40}C_{97}$-coumarin (–△–); (C) $A_{45}B_{67}C_{346}$ (–●–) (control with non-labelled polymer).

These results indicate that obviously in the $A_{45}B_{65}C_{346}$ system all PMOXA blocks are oriented toward the outside of the vesicles and consequently the PEO blocks toward the interior. For the shorter and less voluminous PMOXA blocks of the $A_{45}B_{40}C_{97}$ system the arrangement is reversed, *i.e.*, PEO points outwards and PMOXA toward the interior. This agrees with geometrical considerations: due to the curvature of the vesicle walls it is favourable when the hydrophilic blocks with lower volume segregate toward the interior.

Although many open questions still remain to be solved, we believe that these first results indicate clearly that the new amphiphilic ABC triblock copolymers are well-suited to prepare asymmetric membranes in aqueous media. We expect that these new artificial membrane systems could be an important breakthrough particularly for biologically inspired technical applications. Further work concerning the influence of molecular parameter of the polymers on their aggregation behavior and studies on the 'directed' reconstitution proteins in ABC-type membranes are in progress.

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