Nanoreactors based on (polymerized) ABA-triblock copolymer vesicles

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A new kind of nanoreactor has been prepared by the incorporation of a channel protein into the shell of (polymerized) vesicles formed from an amphiphilic ABA-triblock copolymer.

A major goal in material science is to miniaturize processes down to the nanometer level. One typical example is the formulation of hollow nanoparticles of reproducible size. For such nanocontainers a widespread range of applications have been suggested. For example, one can think about chemistry in a confined volume under controlled conditions, or protection of guest molecules like enzymes against an hostile outside environment (*e.g.* protease). Such an environment requires a stable shell with a selective permeability.[1] In recent years considerable progress has been made in developing synthetic approaches to control the size and morphology of such particles.[2] Recently we reported a mild procedure for the preparation of stable nanocapsules from an amphiphilic poly(2-methyloxazoline)-block–poly(dimethylsiloxane)-block–poly(2-methyloxazoline) triblock copolymer (PMOXA-PDMS-PMOXA) which carried polymerizable groups at both ends.[3]

This polymer aggregates spontaneously in dilute aqueous solution into vesicular structures, the sizes of which can be controlled in the range 50 to 500 nm.[3] These aggregates can be considerably stabilized by a subsequent crosslinking polymerization of the reactive end groups of the underlying triblock copolymers. In the resulting nanocapsules all the individual block copolymers are interconnected *via* covalent bonds to a giant 'supermacromolecule'. Fig. 1 shows a characteristic cryogenic transmission electron micrograph (Cryo-TEM) of polymerized triblock copolymer vesicles. The thickness of the triblock copolymer shells is estimated to be about 10 nm, in good agreement with previous findings.[3,4]

Prior to any use of these polymer shells as nanosized reactors one had to find ways to control their permeability. Here we suggest the encapsulation of an enzyme. The enzyme should stay trapped in the interior of the particles while the substrates should be able to diffuse into, and the products out of, the nanoreactors. However, owing to their higher hydrophobic thickness, the triblock copolymer shells are even less permeable to small hydrophilic solutes than conventional phospholipid bilayers.[4–7]

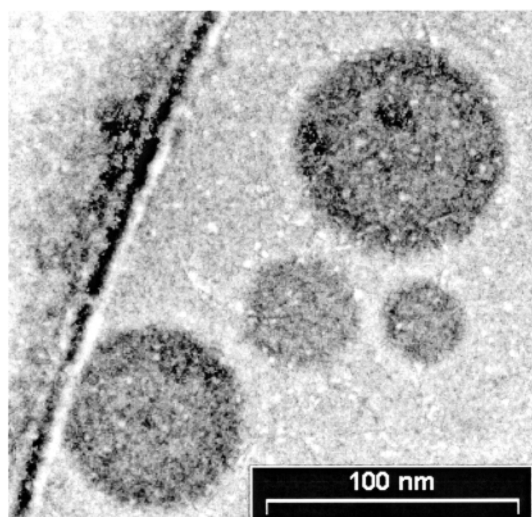

Fig. 1 Cryo-transmission electron micrograph of polymerized triblock copolymer vesicles.

Nature provides a great variety of passive or specific channels which allow the permeation of specific substrates across biological membranes. The lipid bilayer serves as a matrix for such channel proteins. However, many of the membrane proteins require a specific lipid composition, hydrophobic thickness or change distribution to be fully functional. The PMOXA-PDMS-PMOXA triblock copolymer behaves in aqueous solution in many respects like a higher molecular weight analogue of conventional lipids.[3,4] Therefore, in order to control the permeation we wanted to make use of such natural membrane proteins and tried to reconstitute them into our polymeric nanocapsules. (See Fig. 2 for a schematic representation of the resulting nanoreactor).

The block copolymer membranes are considerably thicker than conventional lipid bilayers ($d \approx 5$ nm)[3–7] due to the larger size of the underlying block copolymer molecules. The hydrophobic–hydrophilic pattern of membrane proteins are naturally optimized with respect to the thinner biological membranes and the hydrophobic part of channel forming proteins may therefore be too small to fit through the polymer membranes. This raises the question of whether the proteins can preserve their activity within a block copolymer membrane. However, the high flexibility and the conformational freedom of the polymer molecules may allow a block copolymer membrane to adapt to the specific geometric and dynamic requirements of membrane proteins without considerable loss of free energy. In such a case it is expected that the protein will remain functional.

To control the permeability of the nanocapsules we used a well-characterized nonspecific membrane channel, the bacterial porin OmpF.[8,9] Porins are transmembrane proteins which form trimeric channels in the outer membrane of Gram-negative bacteria. These water-filled channels allow passive diffusion of small solutes like ions, nutrients or antibiotics across the

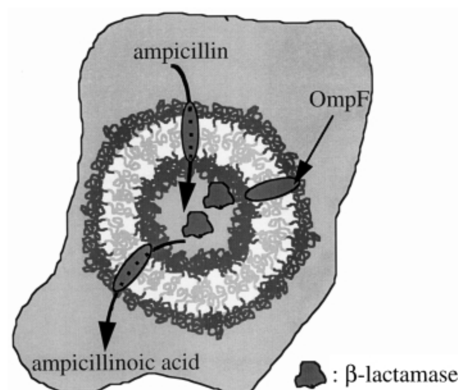

Fig. 2 Schematic representation of a β-lactamase-containing nanoreactor prepared from an amphiphilic triblock copolymer and the porin OmpF.

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membrane. Molecules with a molecular weight above 400 Da are sterically excluded.

The procedure used to prepare the nanoreactors was analogous to that of the (polymerized) triblock copolymer vesicles described previously.[3] Although the porin could also be incorporated into performed vesicles the following procedure turned out to be more convenient. A stock solution of the porin (13.3 mg mL^{-1} in 1 wt% octylpolyoxyethylene, 100 nM NaCl and 10 mM HEPES,† pH 7.4) was mixed with a 17 wt% solution of the triblock copolymer (M_n = 9000 Da) in ethanol to a final molar ratio of 1:1000 (protein:polymer). For encapsulation of the enzyme in the interior of the porin-containing vesicles, the clear and homogeneous solution was slowly added to an aqueous β-lactamase solution (0.024 mg mL^{-1} in 10 mM HEPES, 100 mM NaCl, pH 7.4) to a final triblock copolymer concentration of 1 wt%. Then the resulting dispersion was repeatedly extruded through filters of a defined pore size (Nucleopore filters (Millipore); pore width 200 nm). This gave rather monodisperse triblock copolymer vesicles with an average diameter of 250 nm.[3] Non-encapsulated protein was removed chromatographically (Sephadex G-200) from the porin-containing vesicles. If desired, a crosslinking polymerization of the methacrylate end-groups of the underlying triblock copolymers could subsequently be initiated by irradiating the vesicles dispersion for 2 min with UV-light. Previous investigation had shown that under these conditions the conversion of the methacrylate end-groups is $>90\%$.[3]

It is well-known that the enzyme β-lactamase is able to hydrolyze β-lactam antibiotics like ampicillin. This reaction can be used to assay, *via* a secondary reaction, the activity of the enzyme.[10,11] In contrast to ampicillin, the product of the hydrolysis (the ampicillinoic acid) can reduce iodine to iodide. This can readily be monitored by micro-iodometry, *i.e. via* the decolorization of a starch–iodine complex.[10,11]

In the present system the enzyme is immobilized in the aqueous core domain of the nanocapsules. Consequently, prior to hydrolysis the ampicillin has to enter the interior of the polymer particles. To check the functionality of the system we added 10 μL of a 1 mM ampicillin solution (in 10 mM HEPES, 100 mM NaCl, pH 7.4) to the nanoreactor dispersion and incubated the resulting mixture for 30 min. Starch-iodine reagent was prepared by mixing 5 mL of a 8 mM iodine, 320 mM potassium iodide solution with 20 mL 1 M sodium wolframate in 2 M acid acetic and then adding 5 mL of 2 wt% soluble starch which had been dissolved in 1 M acetic acid by gentle boiling for 3 min. Subsequently 0.5 mL of the starch–iodine reagent was added to the reaction mixture and the absorbance of the starch–iodine complex at 623 nm was measured as a function of time.[10,11] The results are shown in Fig. 3 together with the control experiments for the free, non-encapsulated enzyme and nanocapsules without OmpF channels. It is important to note that within the experimental error the results before and after polymerization of the triblock copolymer shells were always the same and subsequently discussed together.

For the nanocapsules made in the absence of porin the absorbance remained constant over the time course of the experiment. As expected, the ampicillin is not able to diffuse across the thick triblock copolymer shells. It is not therefore hydrolyzed by the enzyme and the iodine is not reduced. In contrast to that for the OmpF-containing nanoreactors the absorbance of the complex decreases slowly with time due to the reduction of the iodine. Obviously the channel protein remains functional despite the extreme thickness of the triblock copolymer shells and even the crosslinking polymerization of the reactive triblock copolymers does not affect its conformation. This is in agreement with systematic investigations on membrane proteins reconstituted into planar triblock copolymer membranes.[4]

Inspection of Fig. 3 shows that the reaction rate of encapsulated enzyme is lower than in the control experiment using free enzyme. This is due to the slow diffusion of the ampicillin and the ampicillinoic acid through the rather limited number of narrow OmpF channels in the shells of the reactors.

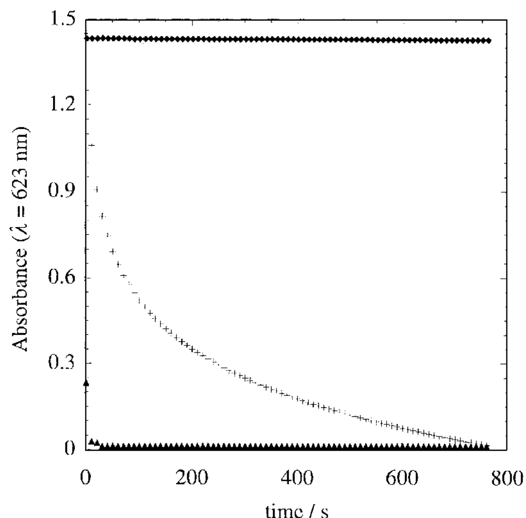

Fig. 3 Time profile of the absorbance of the starch–iodine complex at 623 nm after 30 min incubation in the presence of 10 μL of a 1 mM ampicillin solution (10 mM HEPES, 100 mM NaCl, pH 7.4). +: poly(2-methyloxazoline)–poly(dimethylsiloxane)–poly(2-methyloxazoline) triblock copolymer (PMOXA-PDMS-PMOXA) nanoreactors; ▲: control with free, non encapsulated enzyme; ◆: control with PMOXA-PDMS-PMOXA nanocapsules without OmpF channels.

In conclusion, we have shown that functional OmpF channels can be incorporated into the shells of triblock copolymer vesicles. Despite the artificial surrounding within such a polymerized triblock copolymer shell the functionality of the membrane protein is fully preserved. These channels therefore allow a direct access to enzymes encapsulated in the interior of these vesicles. We are currently investigating the extension of this principle to other enzymes and other transport proteins, with the goal of substrate-specific nanoreactors which could find interesting applications in areas like drug delivery or catalysis.

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Notes and references

† HEPES = 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid.

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