Nanoreactors from Polymer-Stabilized Liposomes

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We introduce new nanometer-sized bioreactors based on liposomes functionalized by incorporated natural channel proteins. Additionally the lipid bilayers of the underlying liposomes can be stabilized by a cross-linking polymerization of hydrophobic methacrylate monomers in the interior of the membranes. As a representative example we functionalize (polymer-stabilized) 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine liposomes by reconstituting a channel-forming protein from the outer cell wall of Gram-negative bacteria. The protein used (OmpF) acts as a size-selective filter, which allows only passage of molecules with a molecular weight below 400 g mol^{-1}. Substrates below this size may still have access to enzymes encapsulated in the nanocontainers. We demonstrate this using the enzyme β-lactamase, which is able to hydrolyze the antibiotic ampicillin. Interestingly the enzyme and the membrane channel preserve their activity even in the presence of the hydrophobic methacrylate monomers and after their cross-linking polymerization.

Introduction

A current research field in material science is to formulate new types of responsive materials on a nanometer-sized scale. Although it is quite a challenge in the laboratory, nature provides already optimized solutions for this. For example, the outer cell wall of bacteria, such as *Escherichia coli*, contains channel proteins that allow specific substrates to diffuse into the periplasmic space from where they are uptaken actively to the inside.[1] Bacteria can open and close these channels upon specific triggers,[2] and they have mechanisms to eject material outside. It is tempting to use such an optimized system to create a synthetic nanoreactor with controlled permeability and the ability to react on external stimuli.

A typical and widespread model system for nanocapsules are liposomes.[3,4] They can easily be formulated using the extrusion technique. However, such liposomes are generally rather unstable.[3,4] A stabilization of the liposomes by polymerization of reactive lipid molecules is feasible but destroys the lateral mobility of the lipids required for the functionality of certain membrane proteins. Recently it was shown that polyelectrolyte multilayer nanocapsules can be coated with lipid membranes.[5] Such supported vesicles are more stable, but due to interactions with the underlying polyelectrolyte skeleton, their shells may have defects, i.e., not all of them are completely tight.[5] Another solution is to formulate block copolymer vesicles which later can be cross-linked via polymerizable groups at the water-soluble blocks.[6,7]

Here in this work we suggest a different type of stabilization. We add hydrophobic methacrylate monomers to aqueous liposome dispersions which penetrate into the hydrophobic interior of the lipid membranes (see for example refs 8−12). These monomers can easily be polymerized inside the membranes by an UV-induced free radical polymerization. As shown previously this may lead to the formation of a 2-D polymer network in the middle of the membrane on which the lipid molecules can freely glide.[13] Similar to the network of erythrocytes the polymer acts as a scaffold which stabilizes the membrane. This has recently been quantified using giant, free-standing, planar lipid membranes which became extremely stable after polymerization.[13]

Here we demonstrate that inside such liposomes water-soluble enzymes can be encapsulated thereby leading to a small bioreactor. We chose β-lactamase as a model enzyme. β-Lactamase is part of the natural defense system of bacteria and hydrolyses β-lactam antibiotics such as ampicillin.[14−16] However, the low permeability for substrates across lipid membranes limits drastically the turn over of encapsulated enzymes[17−20] which have thus rather limited applications. To overcome this limit, we propose

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(1) Schirmer, T. *J. Struct. Biol.* **1998**, *121*, 101.

(2) Delcour, A. H. *FEMS Microbiol. Lett.* **1997**, *151*, 115. Van Gelder, P.; Dumas, F.; Winterhalter, M. *Biophys. Chem.* **2000**, *85*, 153.

(3) Lasic, D. D. *Liposomes*: *From Physics to Applications*; Elsevier Science Publications B.V.: Amsterdam, 1993.

(4) Ringsdorf, H.; Schlarb, B.; Venzmer, J. *Angew. Chem.* **1988**, *100*, 117.

(5) Moya, S.; Donath, E.; Sukhorukow, G. B.; Auch, M.; Bäumler, H.; Lichtenfeld, H.; Möhwald, H. *Macromolecules* **2000**, *33*, 4538.

(6) Nardin, C.; Hirt, T.; Leukel, J.; Meier, W. *Langmuir* **2000**, *16*, 1035.

(7) Nardin, C.; Thoeni, S.; Widmer, J.; Winterhalter, M.; Meier, W. *Chem. Commun.* **2000**, 1433.

(8) Murtagh, J.; Thomas, J. K. *Faraday Discuss. Chem. Soc.* **1986**, *81*, 127.

(9) Kurja, J.; Noelte, R. J. M.; Maxwell, I. A.; German, A. L. *Polymer* **1993**, *34*, 2045.

(10) Poulain, N.; Natache, E.; Pina, A.; Levesque, G. *J. Polym. Sci., Part A*: *Polym. Chem.* **1996**, *34*, 729.

(11) Morgan, J. D.; Johnson, C. A.; Kaler, E. W. *Langmuir* **1997**, *13*, 6447.

(12) Hotz, J.; Meier, W. *Langmuir* **1998**, *14*, 1031.

(13) Meier, W.; Graff, A.; Diederich, A.; Winterhalter, M. *Phys. Chem. Chem. Phys.* **2000**, *2*, 4559.

(14) Zimmermann, W.; Rosselet, A. *Antimicrob. Agents Chemother.* **1977**, *12*, 368.

(15) Nikaido, H.; Rosenberg, E. *J. Bacteriol.* **1983**, *153*, 241.

(16) Nikaido, H.; Rosenberg, E.; Foulds, J. *J. Bacteriol.* **1983**, *153*, 232.

(17) Kato, Y.; Hosokawa, T.; Hayakawa, E.; Ito, K. *Biol. Pharm. Bull.* **1993**, *16*, 740.

(18) Shimizu, M.; Miwa, Y.; Hashimoto, K.; Goto, A. *Biosci. Biotechnol. Biochem.* **1993**, *57*, 1445.

(19) Nema, S.; Kiick, D. M.; Avis, K. E. *PDA J. Pharm. Sci. Technol.* **1994**, *48*, 231.

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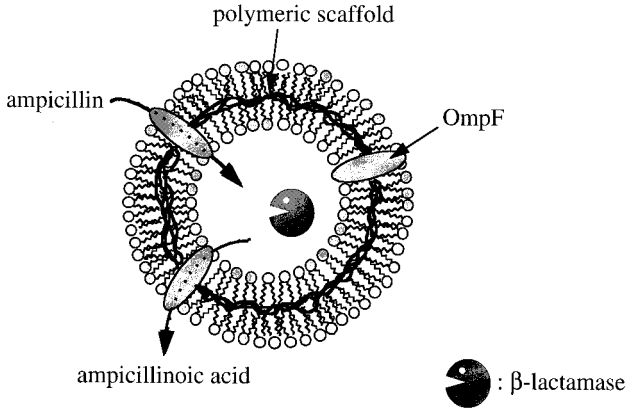

Figure 1. Schematic representation of a polymer-stabilized nanoreactor with encapsulated enzyme.

a novel technique to control the permeability of the liposomes using naturally occurring membrane channels with selected features. As an example we use OmpF, a channel protein from the outer cell wall of *E. coli*. The resulting nanoreactors are stabilized by polymerization of hydrophobic monomers inside their lipid bilayers. A schematic representation of the resulting nanoreactors is shown in Figure 1. The influence of the presence of hydrophobic monomers and subsequent free-radical polymerization on the functionality of the channel proteins and the enzyme activity is quantified.

Experimental Section

Materials. The lipid, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC), was purchased from Avanti Polar Lipids Inc. (Birmingham, AL). The monomers, *n*-butyl methacrylate (BMA, Fluka) and ethylene glycol dimethacrylate (EGDMA, Fluka) were distilled over calcium hydride and stored at − 4 °C. The β-lactamase was obtained from Fluka and stored at −20 °C under argon. Ampicillin was purchased from Sigma and used without further purification. The protein, OmpF from the outer membrane of *E. coli* was purified according to previous protocols[21] and stored at 1 mg mL^{-1} in 1% octyl-POE detergent (Alexis, Lauchringen, Switzerland).

Preparation of OmpF-Containing Liposomes and Encapsulation of the β-Lactamase. Two milliliters of a POPC stock solution in chloroform (10 mg mL^{-1}) was evaporated under high vacuum over 6 h. Afterward 20 μL of a 1 mg mL^{-1} stock solution of OmpF in 1% detergent octyl-POE (OPOE) was added, vortexed to provide a homogeneous mixture, and dried again for a short period (molar ratio POPC:OmpF $\approx 5 \times 10^4$). The dry mixed POPC/protein film was dispersed in 1 mL of buffer containing 10 mM Hepes, 100 mM NaCl, and 0.11 mg mL^{-1} β-lactamase at pH 7.4. This yields a dispersion containing multilamellar, polydisperse liposomes. A freeze−thaw cycle was applied to the dispersion consisting of five cycles of freezing in liquid nitrogen and thawing in a water bath at 30 °C. To obtain unilamellar liposomes of rather uniform size the dispersion was repeatedly extruded through polycarbonate filters (Nucleopore filters (Millipore)) with a pore size of 200 nm. Nonencapsulated β-lactamase was removed chromatographically (Sephadex G-150, Pharmacia) from the porin-containing liposomes.

Dynamic light scattering showed that generally the resulting liposomes (with and without OmpF and/or β-lactamase) had a unimodal size distribution with a polydispersity of about 20%, in good agreement with previously reported values on extruded liposomes.[22−24] Their average hydrodynamic radius was typically around 150 nm.

Polymerization of Hydrophobic Monomers inside the Lipid Membranes. Five milliliters (2 mg mL^{-1} with respect to the lipid) of the liposome solution was mixed with 1.01 mg (9×10^{-4} mol) of BMA and 0.7 mg (4.5×10^{-4} mol) of the cross-linking agent EGDMA. Purified argon was bubbled through the solution to eliminate oxygen. The polymerization was initiated by UV-irradiation (Ultratech 400W, $\lambda = 254$ nm, Osram AG) for 30 min. Previous investigations showed a nearly complete conversion of the monomers under these conditions.[12] A schematic representation of the resulting polymer-stabilized nanoreactors is shown in Figure 1.

***β*-Lactamase Assay.** The enzyme β-lactamase is able to hydrolyze β-lactam antibiotics such as ampicillin. The activity of the enzyme can be quantified via a secondary reaction. In contrast to ampicillin the product of the hydrolysis, the ampicillinoic acid, can reduce iodine to iodide. This can readily be monitored by iodometry, i.e., via the decolorization of a starch−iodine complex.[25,26] To check the functionality of the system, we added 5 μL of a 20 mM ampicillin solution (in 10 mM Hepes, 100 mM NaCl, pH 7.4) to 0.5 mL of the nanoreactor dispersion and incubated the resulting mixture for the desired time interval. 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, and 50 mL of 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. The hydrolysis reaction was stopped by adding 0.5 mL of this starch−iodine reagent to the reaction mixture,[25] and the absorbance of the starch−iodine complex at 623 nm (where the complex shows an absorption maximum) was measured as a function of time using a Hewlett-Packard 8452A spectrophotometer. The standard curve for the assay was linear over the relevant ampicillinoic acid concentration range from 0.1 to 50 μM (see also refs 25 and 26). To convert the rates of decolorization of the starch−iodine complex into enzyme reaction velocities, the iodine consumption of the system after the respective incubation time was determined using the Beer−Lambert law.[26]

Dynamic Light Scattering. Dynamic light scattering experiments were performed using a commercial goniometer ALV-Langen equipped with a frequency-doubled Nd:YAG laser (ADLAS, $\lambda = 532$ nm at 20 °C). An ALV-5000/E correlator calculates the photon intensity autocorrelation function $g_2(t)$. The samples were prepared by filtering through Millipore filters (0.22 μm, Millex GV for aqueous solutions). The data were analyzed using CONTIN. Dynamic light scattering investigations were routinely employed to determine the hydrodynamic radius of the samples.

In agreement with previous investigations, the polymerization did not lead to any measurable changes in size and size distribution of the liposomes.[12] To demonstrate the polymer-scaffold-induced stabilization of the liposomes, we performed lysis experiments with the detergent OPOE. Prior to the light scattering investigations, the liposomes were incubated for 12 h at room temperature with OPOE. Additional control measurements after 48 and 72 h generally reproduced the results. The data for the liposomes before and after polymerization are shown in Figure 2. A titration of the POPC liposomes leads to a rapid breakdown, and above a molar ratio OPOE/POPC of about 5:1, only mixed surfactant−lipid micelles are detected with a hydrodynamic radius of $R_h = 15$ nm. In contrast to that, the polymer-containing liposomes are resistant to lysis and their hydrodynamic radius remained constant at 168 nm at least up to a molar ratio of OPOE: POPC of 40:1 where we stopped the experiment. This is expected since the polymer shells formed inside the lipid bilayers have approximately the same dimensions as the liposomes.

It has to be emphasized that the polymer-containing liposomes can be isolated from the aqueous phase by lyophilization. The resulting white powder can subsequently be redispersed in

(20) Schumacher, I.; Arad, A.; Margalit, R. *Biotechnol. Appl. Biochem.* **1999**, *30*, 225.
(21) Saint, N.; Lou, K. L.; Widmer, C.; Luckey, M.; Schirmer, T.; Rosenbusch, J. P. *J. Biol. Chem.* **1996**, *271*, 20676.
(22) van Zanten, J. In *Vesicles*; Rosoff, M., Ed.; Marcel Dekker: New York, 1996; Vol. 62, p 240.
(23) van Zanten, J. H.; Monbouquett, H. G. *J. Colloid Interface Sci.* **1991**, *146*, 330.
(24) Ruf, H.; Georgalis, Y.; Grell, E. *Methods Enzymol.* **1989**, *172*, 364.
(25) Zimmermann, W.; Rosselet, A. *Antimicrob. Agents Chemother.* **1977**, *12* (3), 368.
(26) Novick, R. P. *Biochem. J.* **1962**, *83*, 236.

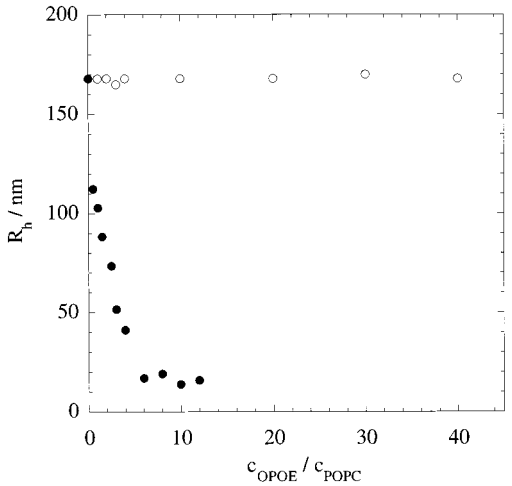

Figure 2. Hydrodynamic radius R_h as a function of the molar ratio of surfactant (OPOE) to lipid (POPC), c_{OPOE}/c_{POPC}.

aqueous media by sonification thus yielding particles of the same size as the original liposomes. Obviously the polymer-stabilized liposomes preserve their hollow sphere morphology even in the dry state.

Permeability of the Lipid Bilayers. We tested the permeability of the liposomes before and after polymerization to ensure tightness of their shells. Here we encapsulated the fluorescent dye 6-carboxyfluorescein (10 μM) and added Co^{2+} ions (500 μM) to the external aqueous phase. It is well-known that Co^{2+} acts as a quencher for 6-carboxyfluorescein. Hence permeation of either Co^{2+} ions or the dye across the membranes would cause a reduction in fluorescence intensity. Generally the fluorescence intensity (excitation wavelength 490 nm, measuring wavelength 520 nm) remained constant for at least 2 days after addition of Co^{2+}; i.e., the shells of the particles are completely impermeable on this time scale. If the liposomes are destroyed by addition of OPOE, the fluorescence intensity decreases within a few seconds to zero. It is interesting to note that the fluorescence of the polymer-containing liposomes was also quenched in the presence of OPOE. Obviously the particles become leaky in the presence of the surfactant despite their morphological stabilization (see above).

Results and Discussion

To control the permeability of liposomes, we propose to insert naturally occurring membrane channels with selected features into their shells. As an example we use the porin OmpF, a channel from the outer cell wall of *E. coli*. It has the advantage to be extremely stable and available in large enough quantities to allow technological applications. Generally, porins are transmembrane proteins that form trimeric channels in the outer membrane of Gram-negative bacteria.[27] These water-filled channels allow passive diffusion of small solutes such as ions, nutrients, or antibiotics across the biological membrane. Molecules with a molecular weight above 400 g mol^{-1} are sterically excluded from the OmpF channels.[27]

For our experiments we encapsulated the enzyme β-lactamase (M_w 50 000 g mol^{-1}) in the aqueous core domain of POPC liposomes. β-Lactamase hydrolyzes β-lactam antibiotics such as ampicillin (M_w 349 g mol^{-1}). The activity of the enzyme reaction can be monitored via a secondary reaction. In contrast to ampicillin, the product of the hydrolysis, the ampicillinoic acid, can reduce iodine to iodide. This can readily be monitored after quenching the hydrolysis reaction by iodometry, i.e., via the decolorization of a starch−iodine complex.[25,26] The results are shown in Figure 3 together with the control experiments

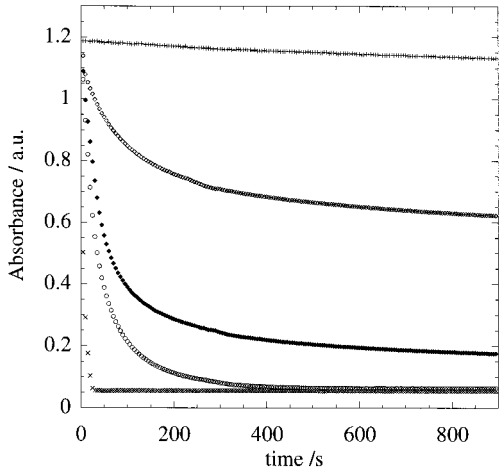

Figure 3. Time profile of the absorbance of the starch−iodine complex at 550 nm after incubation in the presence of 10 μL of a 1 mM ampicillin solution (10 mM Hepes, 100 mM NaCl, pH 7.4): ×, control with free, nonencapsulated enzyme; +, control with POPC liposomes without OmpF channels. OmpF-containing POPC liposomes incubated with ampicillin for (◊) 10 min, (♦) 20 min, and (○) 30 min.

for the free, nonencapsulated enzyme and liposomes in the absence of OmpF channels. For liposomes made in the absence of porin, the absorbance remained constant on the time scale of the experiment. As expected the ampicillin is not able to diffuse across the lipid membranes of the liposomes. Therefore it is not hydrolyzed by the enzyme and the iodine is not reduced. In contrast to that for the OmpF-containing liposomes the absorbance of the complex decreases with time due to the reduction of the iodine by the ampicillinoic acid present in the system. The saturation values reflect the overall amount of ampicillinoic acid produced during the respective incubation time. Furthermore, inspection of Figure 3 shows that for a given incubation time the amount of hydrolyzed ampicillin and, hence, the reaction rate of encapsulated enzyme is lower than that in the control experiment using the free enzyme under the given conditions. This is due to the slow diffusion of ampicillin and ampicillinoic acid through the rather limited number of narrow OmpF channels in the membranes of the liposomes. (An estimation of the amount of lipid added and the number of liposomes formed versus the amount of OmpF protein added yields approximately 2 OmpF trimers per liposome!)

From these data it is possible to calculate the amount of hydrolyzed ampicillin. For comparison we take the amount of ampicillin hydrolyzed by the free enzyme under these conditions as a reference (i.e., 1 μmol mg^{-1} min^{-1}). Figure 4 shows the results for the nanoreactors at various external substrate concentrations together with that for the free enzyme and the liposomes without reconstituted channel proteins. Obviously below an external ampicillin concentration of about 0.5 μM the relative activity (expressed as the ratio of hydrolysis rates) of the OmpF-containing liposomes is considerably decreased compared to the free enzyme (i.e., 58 ± 10% and 86 ± 10% for 0.2 and 0.4 μM, respectively). This suggests that the turnover of the enzyme is limited at these concentrations by the translocation through the channels. At higher substrate concentrations, however, this difference vanishes, demonstrating clearly the absence of the membrane as a permeation barrier. Translocation of ampicillin through the channels is proportional to the ampicillin concentration. Hence, at higher concentrations β-lactam hydrolysis

(27) Nikaido, H. *Mol. Microbiol.* **1992**, *6* (4), 435.

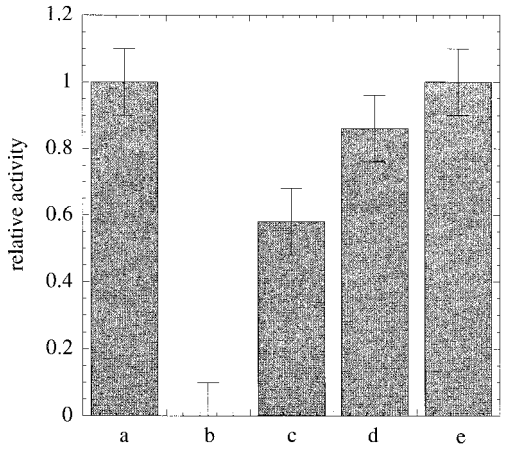

Figure 4. Relative activity (hydrolyzed ampicillin/hydrolyzed ampicillin by the free enzyme) of the encapsulated enzyme in liposomes and OmpF-containing liposomes ($c_{ampicillin}$, external ampicillin concentration): a, free enzyme; b, liposomes without OmpF; c, liposomes with OmpF (at $c_{ampicillin} = 0.2\ \mu M$); d, liposomes with OmpF (at $c_{ampicillin} = 0.4\ \mu M$); e, liposomes with OmpF (at $c_{ampicillin} = 0.8\ \mu M$).

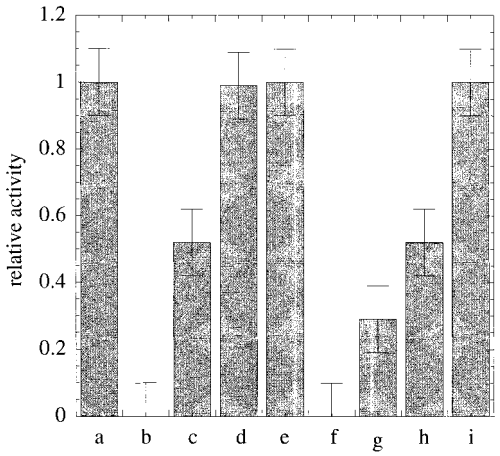

Figure 5. Relative activity (hydrolyzed ampicillin/hydrolyzed ampicillin by the free enzyme) of the encapsulated enzyme in monomer-containing and polymerized nanoreactors ($c_{ampicillin}$, external ampicillin concentration): a, free enzyme; b, monomer-swollen liposomes without OmpF; c, monomer-swollen liposomes with OmpF (at $c_{ampicillin} = 0.2\ \mu M$); d, monomer-swollen liposomes with OmpF (at $c_{ampicillin} = 0.4\ \mu M$); e, monomer-swollen liposomes with OmpF (at $c_{ampicillin} = 0.8\ \mu M$); f, polymer-stabilized liposomes without OmpF; g, polymer-stabilized liposomes with OmpF (at $c_{ampicillin} = 0.2\ \mu M$); h, polymer-stabilized liposomes with OmpF (at $c_{ampicillin} = 0.4\ \mu M$); i, polymer-stabilized liposomes with OmpF (at $c_{ampicillin} = 0.8\ \mu M$).

is no longer limited by the rate of antibiotics diffusion through the OmpF channels.

These results indicate clearly that the permeability of the shells of liposomes can be controlled by channel proteins. This suggests use of such functionalized liposomes for protection of sensitive enzymes against a hostile outside environment (e.g., protease). However, due to the noncovalent interactions responsible for their formation the underlying liposomes have only a limited stability and may undergo structural changes.[3] Many applications require more stable particles. Therefore, in a second set of experiments we investigate whether such functionalized liposomes can be stabilized by a polymeric scaffold without loss of their activity. Hence we swell their lipid bilayers with a mixture of *n*-butyl methacrylate (BMA) and the cross-linker ethylene glycole dimethacrylate (EGDMA) (molar ratio BMA:EGDMA = 2:1), which can subsequently be polymerized by a UV-induced free radical polymerization.[12] Previous investigations have shown that the polymerization leads to the formation of a 2-D polymer network structure inside lipid bilayers without disrupting their structure (see also Figure 1 for a schematic representation).[12,13] Moreover polymer-containing liposomes are considerably stabilized against surfactant lysis and are able to preserve their hollow sphere morphology even after lyophilization and redispersion in aqueous buffers (for details see Experimental Section).

Figure 5 shows the relative activity (i.e., ratio of reaction rates) of the functionalized liposomes in the presence of the hydrophobic monomers and after the cross-linking polymerization together with the control experiments for the corresponding systems without reconstituted OmpF. For the monomer-swollen and polymer-stabilized liposomes made in the absence of porin, no activity can be detected since the ampicillin cannot diffuse across the shells of the reactors. In the presence of reconstituted OmpF, the activity of the monomer swollen nanoreactors is always the same as that of the monomer-free system within the experimental error. Again above approximately 0.5 μM ampicillin the nanoreactors adopt the same activity as the free enzyme. This clearly indicates that the monomers do not denature the proteins or inhibit their function.

After polymerization, however, the activity of the functionalized liposomes decreases considerably. Since the polymerization conditions (i.e., incubation of 5 mL of the β-lactamase stock solution in the presence of 1.01 mg of BMA and 0.7 mg of EGDMA for 1 h at room temperature and 30 min of UV-irradiation) do not lead to any detectable reduction of the activity of the free enzyme, this effect seems to originate from the OmpF channels. This is in agreement with a recent study on amphiphilic block copolymer membranes with reconstituted OmpF.[28] There it was shown that a polymerization may induce a closure or an expulsion of some of the reconstituted channels during the cross-linking reaction, probably due to internal stress occurring in the membrane in the course of the polymer chain reaction.[28]

To quantify the time dependence of ampicillin hydrolysis by the nanoreactors, the decolorization was measured after different incubation times for a given ampicillin concentration. The results for the nanoreactors, the nanoreactors in the presence of the hydrophobic monomers and after polymerization, are shown in Figure 6. As can directly be seen after a lag time of about 10 min,[26] the hydrolysis rate was linear corresponding to a reaction kinetics of zero order. Since the same lag time is also observed in the case of the nonencapsulated enzyme, it seems to be related to the priming of the enzymatic reaction. Obviously for a given ampicillin concentration outside the nanoreactors, a steady state is rapidly established at which the rate of antibiotic diffusion through the OmpF channels and the β-lactam hydrolysis are equal thus resulting in a constant ampicillinoic acid release. While this release is the same for the monomer-free and the monomer-swollen nanoreactors, that for the polymerized system is decreased by a factor of 1.7 due to partial closure of OmpF channels. Nevertheless the results indicate that the nanoreactors are interesting as a new type of delivery device for applications in pharmacy and diagnostics. Especially as such systems often require a constant release of substances over an extended period of time.

(28) Nardin, C.; Winterhalter, M.; Meier, W. *Angew. Chem.* **2000**, *112*, 4747. *Angew. Chem., Int. Ed.* **2000**, *39*, 4599.

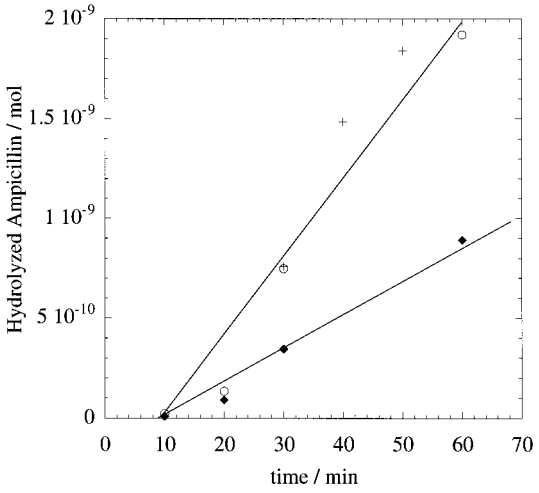

Figure 6. Amount of hydrolyzed ampicillin as a function of time: +, liposomes with OmpF; ○, monomer-swollen liposomes with OmpF; ♦, polymer-stabilized liposomes with OmpF.

Conclusions

We have presented a route to prepare a new kind of effective bioreactor. As a representative example we incorporated the bacterial channel protein OmpF into the shells of POPC liposomes. The lipid membranes of the system can subsequently be stabilized by polymerization of hydrophobic monomers inside the lipid bilayers. This leads to the formation of a 2-D polymer network structure in the interior of the bilayers without disrupting the integrity of the membranes. Interestingly the functionality of the membrane protein is preserved despite the artificial surrounding within monomer-containing and/or polymer-stabilized membranes. For solutes with a molecular weight below 400 g mol^{-1}, the incorporated channels allow a free molecular exchange between the interior of the resulting nanocapsules and the bulk medium.

In our present study we encapsulated the enzyme β-lactamase in the interior of the liposomes. It is important to note that this enzyme preserves its full activity in the presence of the hydrophobic methacrylate monomers and even after their cross-linking polymerization inside the lipid bilayers. Due to their size selectivity the functionalized shells of the (polymer-stabilized) liposomes provide a selective permeability, which allows protecting of the guest enzyme against a hostile outside environment (e.g., protease) and simultaneously controls the diffusion of substrates and products.

Moreover, it has to be emphasized that the system introduced in the current study is a representative example of this new type of nanometer sized bioreactors. In fact, nature provides many more specific, unspecific, or ligand gated channels which can be reconstituted in the same way providing a unique tool to control the permeation across nanoreactor shells. We believe that the principle of using the protective ability of such nanocontainers in combination with controlled permeability either by natural or genetically modified channels or pumps will have many future applications. For example, the encapsulation could be extended to antibodies and then decrease the immunogenicity of the enzyme when injected in plasma. This allows using enzymes designated to have a therapeutic role from various sources.

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