Vesicle-Templated Polymer Hollow Spheres

Jutta Hotz and Wolfgang Meier*

Institut für Physikalische Chemie, Departement Chemie, Universität Basel, Klingelbergstrasse 80, CH-4056 Basel, Switzerland

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A cross-linking polymerization of hydrophobic monomers within the surfactant bilayer of vesicles leads to the formation of a quasi-two-dimensional polymer network. Depending on the dimensions of the templating vesicles, polymer hollow spheres can be produced with diameters ranging from several tens of nanometers up to hundreds of micrometers. The hollow sphere morphology of the polymer particles is proved by confocal laser scanning microscopy, scanning electron microscopy, and static and dynamic light scattering. After extraction of the surfactant matrix, the polymer particles contract considerably without loss of their spherical shape. The extent of the contraction depends sensitively on the cross-linking density of the polymer network and shows a similar scaling behavior as branched polymers.

Introduction

Liposomes or vesicles are closed bilayer aggregates, formed from phospholipids and surfactants. They have found increasing interest due to their various applications in pharmaceutical or cosmetic processes and as simple models for biological cells or cell membranes.1 To enhance their limited stability and to control the permeability of their surfactant bilayer, different methods are used to support the bilayer surfaces by synthetic polymer scaffolds. For example, this can be realized by using polymerizable surfactants,2 incorporating polymers during the formation of vesicles,3 and surface grafting of water soluble polymers.1,4

Another way is offered by the compartmental structure of these colloidal structures: vesicles or liposomes are able to solubilize hydrophobic substances in the interior of their surfactant bilayer. Using hydrophobic monomers, subsequent polymerization leads to the formation of polymer chains entrapped in the hydrophobic part of the membrane. Previous studies employed styrene or alkylacrylates as hydrophobic monomers to characterize these new stabilized structures.5–7 First kinetic investigations and light scattering measurements indicate that the polymerization occurs with a high reaction rate8 and the structure and dimensions of the templating vesicles are not influenced by the included polymers.5,6

In contrast to polymerized vesicles formed by polymerizable surfactants, the polymer scaffold in the interior of the membrane still allows lateral mobility of the lipid molecules and does not alter the transbilayer movement of low molecular substances.5 This makes these polymer-containing vesicles an ideal model system to study the interactions of pharmaceutically active substances or proteins with biological membranes.

There is a relative paucity of information regarding the structure of the polymers formed in these systems, e.g., about the arrangement of the polymer within the bilayer. It is obvious, that only a homogeneous distribution of the polymer chains within the bilayer leads really to a polymer hollow sphere, while incoherent polymer structures would not build up a closed polymer shell. Polymerization in the presence of monomers bearing more than one polymerizable group leads to the formation of a polymer network which should be able to retain the structure of a polymer hollow sphere even after extraction of the surfactant matrix.

Due to spatial limitations within the hydrophobic part of the membrane (thickness ~3–5 nm) the wall of the hollow sphere can be regarded as being constituted from a two-dimensional polymer network. Such materials have recently attracted a great deal of attention.9 Their conformation may eventually show a flat low-temperature and a crumpled high-temperature phase.

In this work we describe the cross-linking polymerization of hydrophobic methacrylate monomers in the interior of the surfactant bilayer of dimethyldioctadecylammonium chloride (DODAC) vesicles. Extraction of the surfactant matrix allows the isolation of the resulting polymer particles. The influence of the dimensions of the templating vesicles and the cross-linking density of the polymer network structure on conformation and dimension of the resulting particles is investigated using static and dynamic light scattering and different methods of microscopy.

Experimental Section

Materials. Dimethyldioctadecylammonium chloride (DODAC) was twice recrystallized from acetone and freeze-dried from benzene to remove water traces. The monomers 1-methacyrloyloxybutane (MAOB, Fluka) and 1,2-bis(methacyrloyloxy)ethane (MAOE, Fluka) were distilled over calcium hydride and stored at 4 °C. The initiator azobisisobutyronitrile (AIBN) was purified by precipitation from methanol.

Preparation of Vesicles. Multilamellar vesicles (MLV) were prepared by hydrating (vortex mixing) dry surfactant (DODAC) in doubly distilled water at 60 °C. A surfactant concentration of 3.7 × 10⁻² mol L⁻¹ was routinely employed. Small unilamellar vesicles (SUV) were prepared by ultrasonification in water (T = 60 °C). The ultrasonic preparation method led to small vesicles with a broad size distribution. Furthermore, the mean vesicle diameters are highly dependent on temperature and sonication time during preparation. For controlled size variations, therefore, the extrusion method was used.

The unilamellarity of the vesicles can be significantly enhanced by repetitive freezing and thawing the MLV suspension prior to extrusion,¹⁰ This was obtained by freezing the MLVs in liquid nitrogen and thawing the samples in a water bath at the same temperature as used for hydration. The freeze–thaw cycle was repeated five times. Afterward the vesicles were extruded using a stainless steel extrusion device (LiPex Biomembranes, Vancouver, BC). The extrusions were performed under nitrogen overpressure (~10 bar) through two polycarbonate filters (25 mm diameter) of pore sizes ranging from 400 to 100 nm.

For cross-linking density investigations only sonicated vesicles are used. Examinations with controlled vesicle size variations are performed with freeze–thawed structures.

Polymerization. A 60 mL (3.7 × 10⁻² mol L⁻¹) portion of vesicle solution was swollen with 0.128 g (9 × 10⁻⁷ mol) of the linear monomer (MAOB) and 0.178 g (9 × 10⁻⁸ mol) of the cross-linking agent (MAOE) and a small crystal of the initiator (AIBN). Purified argon was bubbled through the solution to eliminate oxygen.

The mixture was tempered 2 h at 55 °C prior to UV-initiated polymerization (Ultratech 400 W, λ = 254 nm, Osram AG; time of irradiation, 2 h).

The surfactant was removed by precipitating the polymer in methanol. The polymer was redissolved in tetrahydrofuran (THF) and the precipitation was repeated five times until 1H NMR indicated the absence of surfactant. Finally, the polymer was dissolved in benzene and freeze-dried, thus leading to a fine, white polymer powder.

In the presence of nonreacted monomers, or traces of oil like hexane and octane, at higher temperature a fusion of the vesicles to large, untomicrometer-sized, unilamellar vesicles was induced. Therefore, temperature-induced polymerization (T = 55 °C, 2–4 h) led to a broad distribution of the size of the formed polymerized vesicles with diameters ranging from nanometers to several hundred micrometers.

To isolate the polymer scaffold of these giant vesicles, they were separated from the aqueous solution and repeatedly washed (5 x) with large excess of THF and subsequently with methanol (5 x). Finally the product was dried for 24 h in a vacuum.

Light Scattering. The static and dynamic light scattering experiments were performed using a commercial goniometer ALV-Langen equipped with a frequency doubled Nd:YAG laser (ADLAS, wavelength λ = 532 nm) at scattering angles between 30° and 150° (T = 25 °C). An ALV-5000/E correlator calculates the photon intensity autocorrelation function g(τ). The samples were prepared by filtering through Millipore filters (0.22 μm, Millex GV for aqueous solutions, 0.5 μm, Millex LCR for toluene).

The data of the dynamic light scattering were analyzed using a Williams–Watts function. Static light scattering data were obtained by extrapolation in a Zimm plot. The refractive index increment in the samples for toluene was determined to be dn/dc = 0.0104.

Dynamic light scattering investigations were routinely employed to determine the hydrodynamic radius of the samples.

Microscopy. Confocal Laser Scanning Microscope (CLSM). An optical section of the hollow spheres can be obtained by confocal laser scanning microscopy (Noran Oydes). Oil immersion lenses with high numerical aperture (NA) are used to provide good resolution in x-, y-, and z-direction (63×/1.4 NA Planapochromat). The resolution of the images was, however, restricted because of the fast diffusion of the single polymer sphere in the oil phase caused by local temperature fluctuations in the immersion oil, which were induced by the laser beam.

Scanning electron microscopy (SEM) was performed using a Philips XL30 SEM. For more information about the internal structure of the hollow spheres, dry polymer samples were sheared between two glass slides to obtain fragments which, for example, allow the determination of the thickness of the polymer shell.

Atomic Force Microscope (AFM). The surface investigations were performed using a Nanoscope III (Digital Instruments, Inc.) microscope. Pure polymer was fixed on a commercial carrier and studied at room temperature.

Light microscopy (LM) was performed using a Leica DMRP microscope. The size of the giant polymer hollow spheres was determined using a calibrated length gauge. To quantify the contraction of large vesicles upon extraction of the surfactant, a small aliquot of these large polymerized vesicles in water was filled into a glass chamber. This chamber was placed on the stage of the microscope, and the size of the polymer containing vesicles was determined. To extract the surfactant, the water was removed and the particles were washed with THF (5 x) and methanol (5 x). After that procedure, the remaining polymer particles were assumed to be surfactant-free and their dimensions could be determined.

Results and Discussion

Temperature-induced free radical polymerization of alkyl methacrylates in a DODAC–vesicle suspension leads to a fusion of the small unilamellar vesicles to giant unilamellar vesicles. These large structures, with diameters up to several hundred micrometers, are well suited to get information about the polymer structure formed within the aggregates. In particular, polymerization of a mixture of monofunctional and bifunctional hydrophobic monomer results in the formation of a cross-linked polymer network structure, which should be able to retain this structure even after extraction of the lipid matrix (see Figure 1).

Confocal laser scanning microscopy (CLSM) is an appropriate method for optically sectioning three-dimensional specimens in order to examine their morphological features. The confocal micrograph in Figure 2 shows an optical section at the equator of a polymer particle after

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extraction of the surfactant matrix. Figure 2 proves that only a thin shell of polymer is present, i.e., really a polymer hollow sphere has been formed during polymerization in the vesicle. The shell thickness can be estimated from the micrograph to be about 2–4 μm for a polymer particle with a diameter of approximately 150 μm. The resolution of the image is, however, limited due to movement of the sample in the immersion oil, caused by local thermal fluctuations induced by the scanning laser beam. Investigations of dry polymer particles have not been possible due to the low contrast in the reflected light mode.

The possibility to isolate also dry polymer particles allows a direct visualization by using scanning electron microscopy. Figure 3a shows, for example, a SEM micrograph of a polymer particle of similar size as the one before. In contrast to previous studies on polystyrene-containing vesicles,6 in our system the spherical shape of the templating vesicle is clearly preserved, even after extraction of the surfactant and subsequently drying of the particles. We never observed a loss of their specific structure, i.e., a collapse into an erythrocyte-like structure as reported in ref 6 although the radius of the particles decreases considerably during extraction and/or drying (see Table 2). This is probably due to the cross-linked polymer network structure of our samples which introduces an additional elastic contribution to the free energy of the polymer acting against the deformation necessary for collapsing. A similar shrinkage without loss of spherical shape has already been reported previously upon polymerization of giant vesicles formed by butadienic lipids (ref 14).

Smaller spherical fragments attached to the surface of the otherwise rather flat surface of the sphere (e.g., in the upper part of Figure 3a) are probably due to smaller vesicles, frozen in during fusion by the polymerization reaction. Indeed, throughout a larger ensemble of particles nearly all different stages of fusion occurring in the growing templating vesicles can be observed.

Shearing the sample between two glass slides, the particles can be broken into pieces. SEM investigations of the fragments confirm not only the hollow sphere morphology of the polymer, as displayed by the hemispherical shell of Figure 3b, but also a more accurate determination of the shell thickness is possible. Figure 3c shows, for example, a magnified section of a particle fragment which allows the determination of this thickness. Typical values for our samples are found in the range of 200–300 nm, i.e., significantly thicker than the templating surfactant bilayer (~3–5 nm). The rather large uncertainty arises probably from the broad size distribution of the particles. Up to now, the influence of sphere dimensions on shell thickness is unknown. This increase of the thickness of the polymer shell is in qualitative agreement with the cross-linked polymer network structure of our samples which introduces an additional elastic contribution to the free energy of the polymer acting against the deformation necessary for collapsing.

Figure 2. Confocal laser scanning micrograph of a polymer hollow sphere (cross-linking density $p = 0.8$). The length of the bar corresponds to 50 μm.

Figure 3. (a) Scanning electron micrograph of a typical dry polymer hollow sphere. The length of the bar corresponds to 50 μm. (b) Scanning electron micrograph of a semispherical fragment of a polymer hollow sphere obtained by shearing polymer particles. The length of the bar corresponds to 50 μm. (c) Magnified section of a particle fragment, showing the polymer shell. The length of the bar corresponds to 2 μm.
**Table 1. Experimental Data from Static and Dynamic Light Scattering Examinations for Two Different Cross-Linking Densities ρ**

<table>
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<tr>
<th>ρ = C_{cross-link}/C_{linear} mol/mol</th>
<th>M_w, g/mol</th>
<th>R_g, nm</th>
<th>R_h, nm</th>
<th>ρ</th>
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<tr>
<td>8.3 × 10^{-1}</td>
<td>1.179 × 10^7</td>
<td>12</td>
<td>12</td>
<td>1.00</td>
</tr>
<tr>
<td>1.1 × 10^{-2}</td>
<td>1.018 × 10^7</td>
<td>20</td>
<td>19</td>
<td>1.05</td>
</tr>
</tbody>
</table>

* M_w, weight average molecular weight of the polymer particles; R_g, radius of gyration of the polymer particles; R_h, hydrodynamic radius of the polymer particles; ρ, ρ-parameter with ρ = R_g/R_h.

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with the observed contraction of the particles upon extraction of the surfactant and/or drying of the samples: within the vesicle structure, the polymer network formed within the hydrophobic part of the surfactant bilayer, is forced into a quasi-two-dimensional conformation. Without this matrix the network chains tend to relax, at least partially, into an entropically favored three-dimensional arrangement thus leading to the observed contraction. The same has been observed in ref 14.

Information about the morphology of smaller polymer particles formed from small unilamellar vesicles (diameters up to several hundred nanometers) can be obtained from static and dynamic light scattering. Table 1 shows the results for two systems differing in the cross-linking density (i.e., the ratio of mono- and bifunctional monomers used). Similar to the observations above, the pure, surfactant-free polymer particles in toluene have significantly smaller radii than the templating vesicles and the extent of the contraction depends sensitively on the cross-linking density (see below).

Furthermore, also the light scattering results (Table 1) support the hollow sphere morphology of the particles: the radius of gyration R_g from static light scattering and the hydrodynamic radius R_h from dynamic light scattering are found to be identical for both samples investigated, within the experimental error. The ratio ρ = R_g/R_h = 1.0 is characteristic for spherical shells. This so-called ρ-parameter is a highly structure sensitive property, reflecting the radial density distribution of the particle. Typical values for ρ have been shown to be random coils ρ = 1.5–1.8, solid spheres ρ = 0.78,15 and hollow spheres with a infinitely thin shell ρ = 1.0.16 More detailed information about the polymer structure could be obtained from the analysis of the angular dependence of the particle scattering factor P(q).17 In this context, however, the polymer particles must be large enough, i.e., q⁴R_g > 1, which was not the case for our samples. It is obvious, that further investigations with larger particles (R_g ≈ 100 nm) are necessary.

The SEM investigations of the large polymer particles already indicate that their surface is, despite the contraction of the samples upon extraction of the surfactant, rather flat (see Figure 3a). More detailed information about this surface topography can be obtained using AFM. The results are shown in Figure 4. As was to be expected for a polymer particle, no long range order can be detected even though the surface pleating displays a certain preferential direction in certain areas. Compared to the hagediometer of the particles investigated (100–200 μm), the surface is found to be rather flat with fluctuations in the range of ±5 nm.

As a result of the preparation of the polymer within the interior of the surfactant bilayer of the vesicle (thickness about 3–5 nm), these systems can be regarded as an example for two-dimensional polymers (at least in the case of the giant vesicles). Such, so-called tethered surfaces have been extensively investigated in recent years.8 These two-dimensional analogues of linear polymer chains have been predicted to exhibit a low-temperature flat phase with long range order in the normals and out of plane fluctuations leading to a certain roughness.18,19 A high-temperature crumpled phase similar to that of coiled linear polymer chains has been predicted but has been found in simulations only in systems without self-avoidance.18–25 It has been shown that internal disorder destabilizes the flat phase.18 This is eventually relevant for our randomly cross-linked polymer networks.

While the mean square radius of gyration of a self-avoiding crumpled membrane consisting of N monomers...
should scale like $R_g^2 \sim N^{0.19-22}$ the flat phase is expected to exhibit a $R_g^2 \sim N^{18-25}$ behavior.

Since the polymer network formed upon polymerization in our vesicles is restricted to the hydrophobic part of the surfactant bilayer (thickness constant), the number $N$ of monomers building up the network structure should be proportional to the squared radius of the templating vesicle. On removal of the surfactant bilayer which forces the quasi-two-dimensional polymer network into its fully extended state, the polymer relaxes into either a crumpled or flat phase.

Figure 5 shows the variation of the radius of the extracted polymer particles as a function of the radius of polymer-containing vesicles. The data of Figure 5 have been determined by light microscopy (large vesicles) and dynamic light scattering (small particles). As was to be expected from the SEM and AFM investigations, the exponent of $0.97 \pm 0.05$ indicates, in agreement with the results of the simulations of refs 19 and 23, that the studied polymer particles are flat with a certain roughness on a local scale. If indeed a crumpling transition occurs with rising temperature and/or decreasing in plane shear modulus, e.g., obtained by variation of the cross-linking density (or meshsize) of the polymer network, has to be clarified in the future.

As already indicated above (see Table 1) the mesh size of the polymer network influences considerably the extend of the contraction of the particles upon extraction of the surfactant. Figure 6 shows the influence of the ratio $p = C_{\text{cross-link}}/C_{\text{linear}}$, being a measure for the cross-linking density of the polymer network formed upon polymerization, on the extent of the shrinkage $g = R_{\text{h, polymer}}^2 / R_{\text{h, vesicle-polymer}}^2$, with $R_{\text{h, polymer}}$ the hydrodynamic radius of the extracted polymer particles and $R_{\text{h, vesicle-polymer}}$ the hydrodynamic radius of the polymer containing vesicles (Table 2).

As can be seen from Figure 6, $g$ shows a power law dependence with an exponent of $-0.57 \pm 0.04$; i.e., the contraction increases with increasing cross-linking density. For higher cross-linking densities ($C_{\text{cross-link}}/C_{\text{linear}} \approx 1$) the extracted particles shrink to about 10% of the original size of the templating vesicles.

Cross-linking polymerization of hydrophobic monomers in the interior of the surfactant bilayer of vesicles allows the preparation of polymer hollow spheres with dimensions ranging from several nanometers up to several hundred micrometers. After extraction of the surfactant matrix the polymer relaxes from a quasi-two-dimensional network within the bilayer to an at least three-dimensional conformation, thus leading to a considerable contraction of the hollow spheres.

Conclusions

Cross-linking polymerization of hydrophobic monomers in the interior of the surfactant bilayer of vesicles allows the preparation of polymer hollow spheres with dimensions ranging from several nanometers up to several hundred micrometers. After extraction of the surfactant matrix the polymer relaxes from a quasi-two-dimensional network within the bilayer to an at least three-dimensional conformation, thus leading to a considerable contraction of the hollow spheres.
This contraction obviously follows the same scaling laws as observed for branched polymers. Interestingly, despite the shrinkage, the polymer shell of the particles remains rather flat with a certain roughness on a local scale. This is in good agreement with simulation results on two-dimensional networks. If, however, a transition into a crumpled state can occur with increasing temperature and/or varying cross-linking density will be subject of future studies.

We believe, however, that these polymer structures probably open new possibilities for applications in, for example, encapsulating nanoparticles or drugs. Nevertheless, in this context, clearly more information is needed about the influence of chemical constitution and structure and size on the resulting properties of the particles.

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