Liposomes Remain Intact When Complexed with Polycationic Brushes

Alexander A. Yaroslavov,*,[†] Andrei V. Sybachin,[†] Marc Schrinner,[‡] Matthias Ballauff,[‡,§] Larisa Tsarkova,[‖]
Ellina Kesselman,[⊥] Judith Schmidt,[⊥] Yeshayahu Talmon,[⊥] and Fredric M. Menger*,[#]

*Department of Chemistry, M. V. Lomonosov Moscow State University, Leninskie Gory 1-3, 119991 Moscow,
Russian Federation, Physikalische Chemie I, Universität Bayreuth, 95440 Bayreuth, Germany, Helmholtz-Zentrum
Berlin für Materialien und Energie, Hahn-Meitner-Platz 1, D-14109 Berlin, Germany, Physikalische Chemie II,
Universität Bayreuth, 95440 Bayreuth, Germany, Department of Chemical Engineering, Technion-Israel Institute of
Technology, 32000 Haifa, Israel, and Department of Chemistry, Emory University, Atlanta, Georgia 30322*

Received February 10, 2010; E-mail: yaroslav@libro.genebee.msu.ru

Spherical bilayer lipid vesicles (liposomes) have been used extensively for drug delivery.[1] Drug encapsulation by liposomes allows in vivo protection from degradative enzymes and thus an enhanced circulation time and bioavailability. Liposomes immobilized on suitable implant surfaces could conceivably act as capacious depots for pharmaceutically active compounds, ensuring their controllable leakage into the surrounding tissue. Furthermore, immobilized liposomal nanocontainers could provide viable fluidic solutions, especially in niche areas where ultraminiaturization and biocompatibility are of critical importance.[2] Surfaces onto which liposomes have been adsorbed[2,3] include those provided by mica (native and silanized),[4,5] glass,[6] silica,[7] gold,[8] clay,[9] liquid crystals,[10] platinum,[11] sulfonated polystyrene,[12] self-assembled monolayers of 1-octadecanethiol,[13] supported human serum albumin,[14] and supported lipid bilayers.[15] Because of liposome–surface and liposome–liposome interactions, fusion and rupture events are common during adsorption, particularly at higher coverages. Thus, when liposomes bind to a solid substrate, they tend to form a planar bilayer when the surface is hydrophilic and a planar monolayer when the surface is hydrophobic.[16] Successful attempts to preclude this problem (e.g., via tethering)[17] have been published, but still a need remains for simple systems that strongly adsorb liposomes but at the same time do not disrupt the liposome integrity. In this communication, we describe an integrated system consisting of soft nanocontainers (anionic liposomes) immobilized on "spherical polycationic brushes" (SPBs), defined as colloidal particles with grafted cationic macromolecules. SPBs have recently been used as effective building blocks for new functional materials.[18,19] We demonstrate herein that immobilized liposomes retain their original size, shape, and encapsulating power.

SPBs (Figure 1) were prepared in three steps along the lines given previously.[20] First, cationic polystyrene particles (∼100 nm in diameter) were synthesized by emulsion polymerization of styrene in the presence of a cationic surfactant, cetyltrimethylammonium bromide (CTAB, Fluka) and a cationic initiator, 2,2′-azobis(2-amidinopropane) dihydrochloride (V50, Wako Chemicals). Next, the latex particles were covered with a very thin layer of the photoinitiator 2-[p-(2-hydroxy-2-methylpropiophenone)]ethylene glycol methacrylate (HMEM; see the Supporting Information). Lastly, polycationic chains were grown on the surface of the latex particles by grafting of a cationic monomer, (2-(acryloyloxy)ethyl)trimethylammonium chloride, under UV–vis radiation. Accord-

ing to dynamic light scattering (DLS), grafting increased the diameter of the particles to 230 nm, indicating a 65 nm thickness of the cationic corona (L_c in Figure 1). In a typical experiment, an SPB suspension was mixed with a suspension of unilamellar anionic liposomes (∼50 nm) prepared conventionally by sonication and composed of egg lecithin (EL) and a doubly anionic phospholipid, cardiolipin (CL^{2-}), in a molar ratio of 19:1 (Figure 1).

Injection of the EL/CL^{2-} liposomes into an SPB suspension caused the charge on the cationic SBPs to decrease, as registered by laser electrophoresis (Figure 2a). When sufficient anionic liposomes were added, the electrophoretic mobility (EPM) of the liposome/SPB complex declined to zero, indicative of total charge neutralization. Further addition of liposomes created a mild negative charge on the complex. The diameter of the complex, as measured by DLS, increased sharply to 1300 nm at the charge-neutralization point, where the EPM = 0 (Figure 2a). Further liposome addition caused a decrease in size to ∼300 nm, which is 70 nm larger than that of the initial SPB species. In summary, both the electrophoresis and DLS data conclusively demonstrated the presence of liposome/SPB complexation.
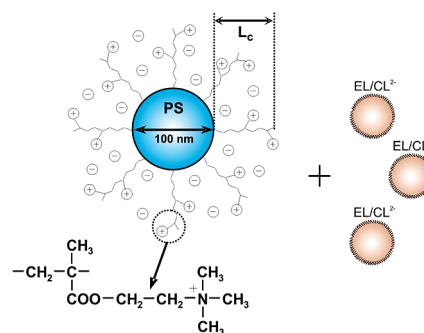
Figure 1. Schematic representation of a spherical polycationic brush (SPB) with a polystyrene (PS) core added to anionic liposomes composed of egg lecithin (EL) and cardiolipin (CL^{2-}).

In order to determine the number of liposomes that ultimately bind to a single SPB particle, we performed fluorescence measurements of EL/CL^{2-} liposomes with 0.5 wt % N-fluorescein-isothiocyanyldipalmitoylphosphatidylethanolamine, a fluorescent lipid, incorporated into the bilayer during the liposome preparation. A series of mixtures with various concentrations of labeled EL/Cl^{2-} liposomes (0.3–1.3 mg/mL) and a constant SPB concentration of 1.5 mg/mL was prepared, incubated for 30 min, and centrifuged; the fluorescence intensities of the clear supernatants were then measured. Figure 2b shows negligible fluorescence in the supernatants up to 0.65 mg/mL lipid, after which the fluorescence rises

† M. V. Lomonosov Moscow State University.
‡ Physikalische Chemie I, Universität Bayreuth.
§ Helmholtz-Zentrum Berlin für Materialien und Energie.
‖ Physikalische Chemie II, Universität Bayreuth.
⊥ Technion-Israel Institute of Technology.
Emory University.

steeply as a result of increasing amounts of unbound lipid. These fluorescence "titrations" indicate that a maximum of ~36 liposomes are able to bind to each SPB particle under the conditions close to the neutralization point.
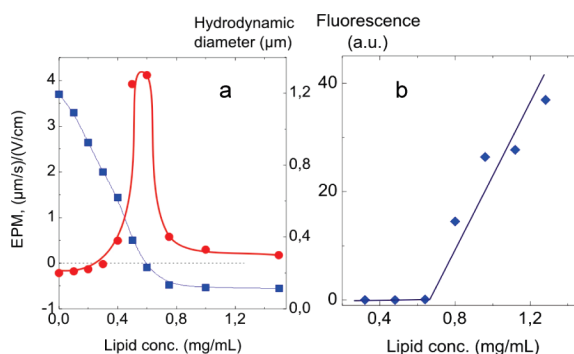
Figure 2. (a) Effect of EL/CL^{2-} liposomes on the EPM (left-hand axis) and hydrodynamic diameter (right-hand axis) of the SPB particles. (b) Liposome concentration dependence of the relative fluorescence of supernatants after separation of SPB/liposome complexes. SPB concentration = 0.38 mg/mL; $[SPB^+] = 10^{-4}$ M; 10^{-2} M borate buffer (pH 9).

The resulting $SPB/EL/CL^{2-}$ liposome supra-aggregates were visualized with the aid of cryo-transmission electron microscopy (cryo-TEM), which preserves the morphology of soft objects.[21] Figure 3a shows a cryo-TEM micrograph of SPB particles by themselves in an aqueous buffer. Polycationic chains projecting from the polystyrene core are visible. A cryo-TEM image of the EL/CL^{2-} liposomes by themselves (Figure 3b) gave sizes in agreement with the DLS measurements. Figure 3c is a cryo-TEM image of two SPB/liposome complexes taken immediately after their formation, in which multiple liposomes cover each SPB particle. Clearly, the liposomes retain their normal shape upon complexation with the cationic brushes. A cryo-TEM image taken 2 h after complex formation (not shown) was identical to Figure 3c, indicating complex stability over this time period.
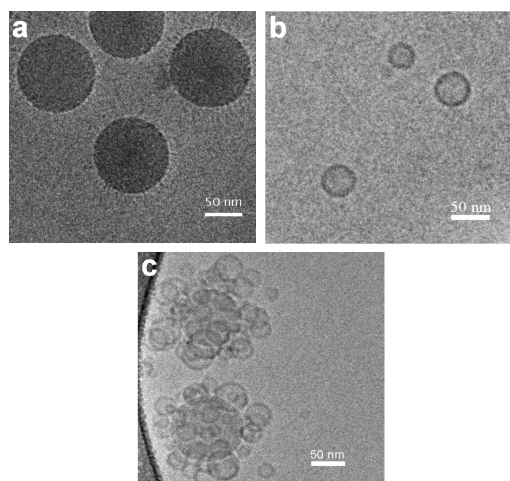
Figure 3. Cryo-TEM images of (a) a 1.5 mg/mL SPB suspension, (b) a 0.1 mg/mL EL/CL^{2-} liposome suspension, (c) and SPB/liposome complexes prepared by mixing a 1.5 mg/mL SPB suspension and a 0.1 mg/mL liposome suspension.

As mentioned above, a key question relates to the integrity of the liposomes when complexed with the brushes, i.e., whether the liposomal membrane remains intact or whether pores and other defects appear in the membrane. To address this question, we prepared a suspension of EL/CL^{2-} liposomes filled with a 1 M NaCl solution. Formation of defects, if such occurred, would result in leakage of salt from the liposomes into the surrounding solution, where the salt could be detected by conductometry. Initially, a control experiment in which a NaCl-loaded liposome suspension was treated with a Triton X-100 solution was carried out; as expected, we observed NaCl leakage and a sharp rise in conductivity. However, when NaCl-loaded liposomes were mixed with an SPB suspension, only a negligible increase in conductivity was detected. Thus, the anionic EL/CL^{2-} liposomes maintained their ability to encapsulate when bound to the grafted polystyrene.

For comparison, we added NaCl-loaded EL/Cl^{2-} liposomes to a suspension of latex particles lacking the grafted polycationic chains. Cationic polystyrene core lattices obtained in the first step of the above-described synthetic procedure were used for the experiment. With the aid of electrophoresis, the anionic liposomes were found to adsorb onto these cationic particles; however, the interaction was accompanied by NaCl leakage and a consequent rise in conductivity. The result demonstrates a principal difference in the fate of anionic liposomes bound to the two types of cationic colloidal particles: disruption of liposomes upon direct adsorption onto a rigid cationic surface but conservation of liposome integrity upon adsorption onto a surface bearing mobile cationic chains.

In summary, evidence of electrostatic adsorption of anionic liposomes onto the surface of spherical particles bearing grafted linear cationic macromolecules has been provided. The size, shape, and encapsulation ability of the liposomes remain unchanged upon adsorption onto SPBs. The supramolecular aggregates, representing an assembly of self-assemblies, have potential applications in the drug delivery field.

Supporting Information Available: Synthesis of liposomes and SPBs and the structure of HMEM. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Gregoriadis, G. *Trends Biotechnol.* **1995**, *13*, 527.
(2) Christensen, S. M.; Stamou, D. *Soft Matter* **2007**, *3*, 828.
(3) Richter, R. P.; Berat, R.; Brisson, A. R. *Langmuir* **2006**, *22*, 3497.
(4) Klacar, S.; Dimitrievski, K.; Kasemo, B. *J. Phys. Chem. B* **2009**, *113*, 5681.
(5) Teschke, O.; de Souza, E. F. *Langmuir* **2002**, *18*, 6513.
(6) Benes, M.; Billy, D.; Benda, A.; Speijer, H.; Hof, M.; Hermens, W. Th. *Langmuir* **2004**, *20*, 10129–10137.
(7) Nordlund, G.; Loenneborg, R.; Brezinski, R. *Langmuir* **2009**, *25*, 4601.
(8) Cho, N.-J.; Wang, G.; Edvardson, M.; Glenn, J. S.; Hook, F.; Frank, C. W. *Anal. Chem.* **2009**, *81*, 4752.
(9) Undabeytia, T.; Nir, S.; Gomara, M. J. *Langmuir* **2004**, *20*, 6605.
(10) Brake, J. M.; Daschner, M. K.; Abbott, N. L. *Langmuir* **2005**, *21*, 2218.
(11) Reimhult, E.; Holoeck, F.; Kasemo, B. *Langmuir* **2003**, *19*, 1681.
(12) Pereira, E. M. A.; Petri, D. F. S.; Carmona-Ribeiro, A. M. *J. Phys. Chem. B* **2002**, *106*, 8762.
(13) Ha, T. H.; Kim, K. *Langmuir* **2001**, *17*, 1999.
(14) Randino, A.; Cambria, A.; Sarpietro, M. G.; Satriano, C. *J. Colloid Interface Sci.* **2000**, *231*, 66.
(15) Seantier, B.; Kaseno, B. *Langmuir* **2009**, *25*, 5767.
(16) Zhang, L.; Hong, L.; Yu, Y.; Bae, S. C.; Granick, S. *J. Am. Chem. Soc.* **2006**, *128*, 9026.
(17) Yoshina-Ishii, C.; Boxer, S. G. *J. Am. Chem. Soc.* **2003**, *125*, 3696.
(18) Ballauff, M. *Prog. Polym. Sci.* **2007**, *32*, 1135.
(19) Schrinner, M.; Ballauff, M.; Talmon, Y.; Kauffmann, Y.; Thun, J.; Möller, M.; Breu, J. *Science* **2009**, *323*, 617.
(20) Mei, Y.; Wittemann, A.; Sharma, G.; Ballauff, M.; Koch, Th.; Gliemann, H.; Horbach, J.; Schimmel, Th. *Macromolecules* **2003**, *36*, 3452.
(21) Weisman, S.; Hirsch-Lerner, D.; Barenholz, Y.; Talmon, Y. *Biophys. J.* **2004**, *87*, 609.

JA1012323