Lipid Coating on Polyelectrolyte Surface Modified Colloidal Particles and Polyelectrolyte Capsules

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Received August 31, 1999

ABSTRACT: Dipalmitoyldiphosphatidic acid (DPPA), dipalmitoyldiphosphatidylcholine (DPPC), and sphingosine were adsorbed onto polyelectrolyte coated colloids and capsules forming composite lipid−polyelectrolyte layers. The stepwise coating was performed either by adsorption of preformed vesicles onto the capsule surface or by a solvent exchange protocol. The lipid assembly was monitored by ζ-potential measurements. Single particle light scattering, flow cytometry, and fluorescence studies of Förster energy transfer have been used to quantify the lipid coating. Confocal microscopy images of capsules coated with fluorescent lipids demonstrated a homogeneous coverage of the capsule surface. Differential scanning calorimetry shows a phase transition temperature characteristic for lipid layer structures. It was concluded that the adsorbed DPPA form bilayers while DPPC may form multilayers. It was further shown that on top of the lipid layers further polyelectrolyte layers could be assembled. The permeability of 6-carboxyfluorescein (6-CF) through the composite layer structure was studied by means of confocal laser scanning microscopy. The permeation time through the composite layer was of the order of 10^1-10^2 min, while in the absence of lipids 6-CF equilibrated faster than the time resolution of the technique.

Introduction

Biological membranes are largely composed of lipids and proteins. They represent one of the major structural components of biological cells. Lipid vesicles and planar bilayers have been widely used as models for biological membranes, to study lipid phase transitions, lipid lateral diffusion, permeation, lipid protein interactions, etc.[1] One shortcoming of liposomes and planar membranes as models for biomembranes is that those are free systems while biomembranes are supported by a polymer network, i.e. the cytoskeleton, or in the case of bacteria and plants the cell wall. For this reason, lipid membranes in contact with polymers and polyelectrolytes are interesting model systems for biomembranes.[2−11]

A method for the fabrication of polyelectrolyte coated colloids and subsequently of micro- and nanosized hollow polyelectrolyte capsules has been recently introduced.[12−15] The method employs the electrostatic interaction between oppositely charged stepwise adsorbed polyelectrolyte species.[16] A polyelectrolyte film is formed on a colloidal template. The template is dissolved after deposition of the film, forming a hollow polyelectrolyte capsule. So far, either weakly crosslinked melamine formaldehyde (MF) colloidal particles or biological cells have been used as templating cores.[17,18] In the case of melamine particles a reduction of the pH value or in the case of the biocolloids a treatment with an oxidizing solution is applied to dissolve the core. The resulting capsules have a high chemical and mechanical stability. The capsules may have potential applications as drug delivery systems. In comparison with the widely used liposomes, the capsule wall is readily permeable for small polar compounds while macromolecules cannot penetrate through the polyelectrolyte layers.[19,20]

The combination of lipid and polyelectrolyte layers may be useful for the understanding of the principles of the interaction of membranes with biopolymers, such as proteins, DNA, or carbohydrates.[21] As far as capsules are concerned, the lipid assembly may result in a composite capsule wall structure with unique properties taking profit of the stability of the support and the permeation features of lipid membranes. Polyelectrolytes have already been used to modify liposomes for drug formulation in particular to enhance the efficiency and the circulation lifetime.[22,23] The combination of lipids and polyelectrolyte layers may also result in structures with novel interesting mechanical, optical, and dielectric properties.[24,25]

Therefore, the adsorption of lipid bilayers onto polyelectrolyte coated colloids and capsules consisting of polyelectrolyte multilayers was studied by means of electrophoretic mobility measurements (EPM), single particle light scattering (SPLS), flow cytometry, electrorotation, differential scanning calorimetry (DSC), and confocal laser scanning microscopy (CLSM).

Experimental Details

Materials. The sources of chemicals were as follows: poly(styrenesulfonate, sodium salt) (PSS), M_w 70 000, Aldrich; poly(allylamine hydrochloride) (PAH), M_w 8000−11 000, Aldrich; poly(diallyldimethylammonium chloride) (PDADMAC), medium molecular weight, Aldrich; DPPA (dipalmitoyldiphosphatidic acid), Avanti polar lipids; sodium hypochlorite solution, ca. 12% chlorine, Hedinger; 6-carboxyfluorescein (6CF), DPPC (dipalmitoyldiphosphatidylcholine), sphingosine, and fluorescently labeled DPPC (L-α-phosphatidylcholine (NBD-β-aminohexanoyl)-γ-palmitoyl) were purchased from Sigma.

PAH was used as received, whereas PSS was dialyzed against Milli-Q water (cutoff 14 000) and lyophilized before

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10.1021/ma9914974 CCC: $19.00
Published on Web 05/17/2000

use. PAH was covalently labeled with either fluorescein isothiocyanate (FITC) or rhodamine isothiocyanate (Molecular Probes) as described in ref 26.

The water used in all experiments was prepared in a three-stage Millipore Milli-Q Plus 185 purification system and had a resistivity higher than 18.2 MΩ cm.

Negatively charged polystyrene sulfate latex particles (diameter = 640 nm) were prepared as described elsewhere.[27]

Methods. *Capsule Preparation*. Human erythrocytes were obtained from the blood bank, Charité, Berlin. The cells were fixed in a 2.5% glutardialdehyde solution for 1 h. Then PSS and PAH were absorbed on the cells applying the stepwise assembling protocol.[13,16] After each adsorption step the samples were centrifuged in an Eppendorf rotor (Hettich Universal 30F) and washed three times in water. After adsorbing 10 layers of polyelectrolytes, the colloids were exposed for 10 min to the 1:10 diluted NaOCl deproteinizing solution from Hedinger. The deproteinizer destroys the cellular template, leaving behind polyelectrolyte hollow capsules.[15] The sample was three times washed with a 10^{-2} M NaCl solution.

Vesicle Formation. Lipid vesicles were prepared as follows. First, the lipids were dissolved in chloroform (1 mg/2 mL). The chloroform was evaporated in a rotavap at 40 °C, and afterward water was added up to a final lipid concentration of 1 mg/mL. The lipid water solution was sonicated for 5 min with a Sonorex Super Digital. Lipid vesicles have been prepared from DPPA, sphingosine, and a mixture of DPPC 90% and DPPA 10% (w/w).

Confocal Microscopy. Confocal micrographs were taken with a confocal laser scanning microscope "Aristoplan" from Leica, equipped with a 100× oil immersion objective. For confocal microscopy L-α-phosphatidylcholine (NBD-β-aminohexanoyl)-γ-palmitoyl at a concentration of 5% w/w was used as a fluorescent label for the lipid layer visualization.

Differential Scanning Calorimetry. Differential scanning calorimetry was performed with the Micro Calorimetry System from Microcal, Inc. Control measurements were performed using pure DPPA and DPPC vesicle dispersions with a concentration of 1 mg/mL for each lipid.

Single Particle Light Scattering. SPLS measurements were conducted on a home-built photometer equipped with an argon laser, Innova 305 from Coherent.[28] Polystyrene sulfate latex particles covered with lipid and polyelectrolyte layers were employed for SPLS measurements.

Electrophoretic Mobility. The electrophoretic mobility was measured by means of a Malvern Zetasizer 4. The ζ-potential was calculated by the conversion of the electrophoretic mobility, μ, with the Smoluchowski relation: $\zeta = \mu\eta/\epsilon$, where η and ϵ are the viscosity and permittivity of the solvent, respectively.

Fluorescence Spectroscopy. The Förster energy transfer between FITC labeled PAH and rhodamine labeled PAH was recorded by means of a Fluorolog Spex device. Excitation was set at 488 nm, i.e., the FITC absorption maximum. For measurements of the transfer efficiency polystyrene latex particles coated with polyelectrolyte films were used.

Flow Cytometry. The particle fluorescence intensity distributions were recorded with a flow cytometer, FACScan from Becton Dickinson, employing fluorescently labeled DPPC as a marker. Fixed erythrocyte cells covered with 10 polyelectroyte layers (PSS/PAH) were used for these measurements.

Results and Discussion

Lipid Assembly. Two different protocols have been used to adsorb lipids onto polyelectrolyte multilayer coated colloids. In the first protocol the electrostatic attraction between charged phospholipid vesicles and oppositely charged colloidal surfaces is used to adsorb the lipids onto the polyelectrolyte multilayer. The hypothesis was that the charged vesicles would adhere to the particle surface and subsequently would spread (Figure 1A). Lipid vesicles were mixed with a capsule dispersion and incubated for 20 min in order to let adsorption occur. Then the samples were centrifuged for 20 min at 30 000*g*, and the supernatant was removed. The centrifugation and resuspension procedure was repeated three times to remove any remaining vesicles from the bulk.

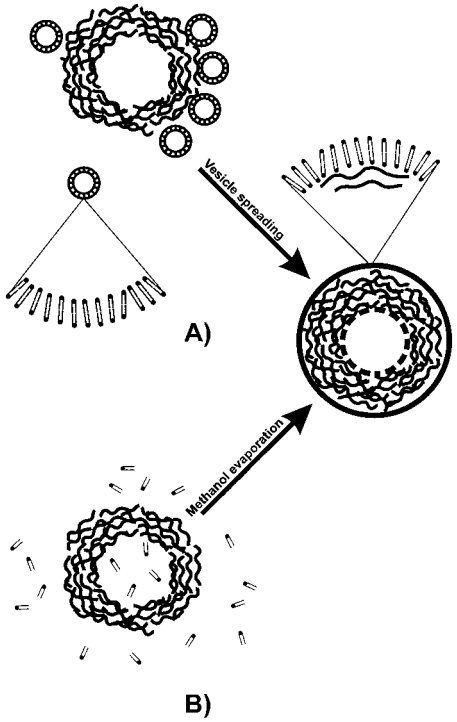

Figure 1. Scheme of lipid layer formation onto polyelectrolyte capsules: (A) via adsorption and spreading of preformed vesicles; (B) via deposition of lipids onto polyelectrolyte capsules from a lipid solution in methanol. In this case a bilayer may be formed at the inside as well.

The idea of the second protocol was to directly form lipid layers on the polyelectrolyte support by means of adsorbing lipid molecules from a saturated solution in methanol. Capsules were suspended into a 1 mg/mL solution of lipids in methanol. The methanol was slowly evaporated at 60 °C until the volume was reduced to approximately 1/20 of its original value. Then 100 μL of water was added in aliquots of 20 μL. After adding the water the sample was kept for 20 min in the water bath. The lipid adsorption onto the polyelectrolyte surface is supposed to occur in parallel with vesicle formation (Figure 1B). The solvent exchange protocol was used for capsules only to avoid any interaction of the latex core with the methanol.

As will be outlined below, significant differences between lipid layers assembled with the two different protocols have not been found so far.

The lipid adsorption on the polyelectrolyte coated colloid particles and capsules was studied by means of EPM measurements, SPLS, CLSM, and flow cytometry. To record surface charge changes upon the adsorption of lipids, EPM measurements were conducted. DPPA, DPPC, and a mixture of 90% DPPC and 10% DPPA were adsorbed on cell templated capsules having PAH as the top layer, resulting in a ζ-potential of +40 mV. However, positively charged sphingosine was adsorbed on negatively charged capsules, having PSS as top layer with a ζ-potential of around −35 mV. As shown in Figure 2, after lipid adsorption the ζ-potential changes from +40 to −40 mV for the negatively charged DPPA to approximately zero for the zwitterionic DPPC and to an intermediate negative value for the DPPA−DPPC

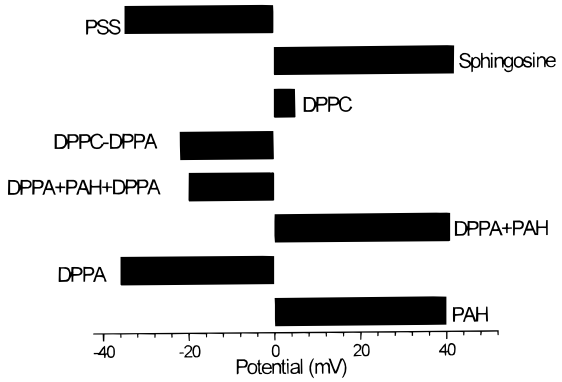

Figure 2. ζ potential of capsules and polyelectrolyte coated latices as a function of lipid and polyelectrolyte adsorption.

mixture. Upon adsorption of sphingosine, the ζ-potential changed from −35 to about +40 mV. The change in the ζ-potential of the capsules leads to the conclusion that lipid adsorption occurred. Hence, composite structures consisting of stepwise adsorbed lipids and polyelectrolytes can be produced. EPM measurements were also used to check multiple adsorption of lipids and polyelectrolytes. Also in Figure 2 it can be seen that, after adsorbing PAH on polyelectrolyte coated colloids already covered with DPPA, the ζ-potential becomes again positive. A second DPPA adsorption step yields then a negative ζ-potential.

Although the ζ-potential may indicate stepwise adsorption, it would be desirable to have an independent prove of the regular growth of the composite layers. Such a proof will be provided below by means of CLSM, SPLS, and flow cytometry data.

Up to now it has been demonstrated that lipid adsorption is likely to occur. As a second step it is interesting to know whether the adsorbed lipids cover the capsule (or particle) surface homogeneously. CLSM provides a suitable means to study the three-dimensional distribution of fluorescent species. The confocal image in Figure 3 of a DPPA(5% w/w labeled DPPC) coated capsule shows a continuous lipid fluorescence with an almost constant intensity over the whole particle surface. This homogeneity in the lipid coverage was also found for the DPPC−DPPA mixture and sphingosine. Another PAH layer has then been adsorbed onto the DPPA coated capsules. The fluorescence distribution again has a constant intensity and is continuous. This proves that the lipids remain attached to the capsule wall even after PAH adsorption. Hence, it can be concluded that the presence of lipids on the capsule walls is confirmed, and within the resolution of the confocal microscopy technique a homogeneous lipid coverage of the capsule surface is seen.

Once adsorption and homogeneous coverage have been established, it was interesting to learn whether the lipids form ordered layer structures when adsorbing onto the capsules. An important characteristic of lipid layers is the phase transition from the gel to the liquid crystalline state. DSC measurements can provide information on the phase of the adsorbed lipid by measuring the phase transition enthalpy. Changes in the enthalpy and phase transition temperature could give as well additional information on the extent of coupling of the lipids to the polyelectrolyte support. For these measurements DPPA and DPPC were absorbed on polyelectrolyte capsules with PAH as the top layer by vesicle adsorption and methanol exchange, respectively.

Figure 3. Fluorescent confocal microscopy image of a polyelectrolyte capsule in the equatorial plane covered with DPPA deposited from a methanol solution. The width of the image is 12 μm. Similar confocal images were obtained after DPPA, DPPA−DPPC, and sphingosine adsorption. One or more additionally deposited polyelectrolyte layers on top of the lipid layer did not remove the fluorescence either.

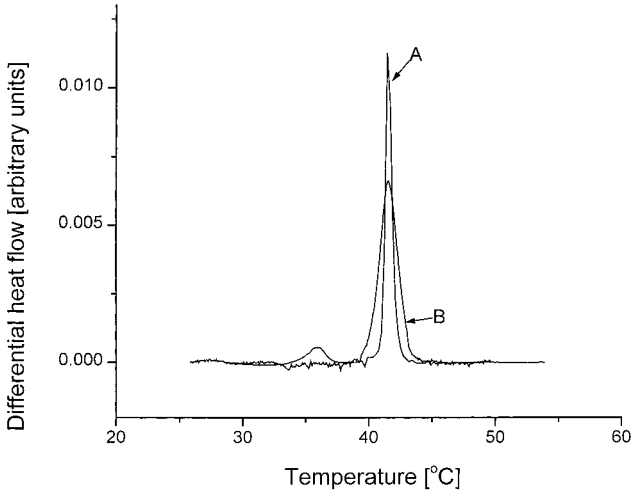

Figure 4. Calorimetry measurements. Differential heat flow of (A) DPPC vesicles and (B) polyelectrolyte capsules covered with DPPC.

After lipid absorption another layer of PAH was assembled to provide a symmetric environment to the lipid layers. In Figure 4A it can be seen that DPPA assembled on capsule surfaces observes a gel to liquid crystalline phase transition which is 8 °C below the phase transition temperature of DPPA vesicles suspended in water. This lower value of the phase transition temperature of assembled DPPA compared with a pure vesicle

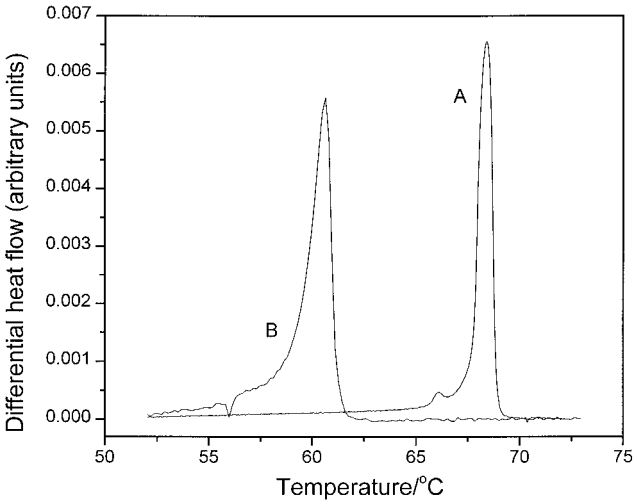

Figure 5. Calorimetry measurements. Differential heat flow of (A) DPPA vesicles and (B) polyelectrolyte capsules covered with DPPA.

preparation could be attributed to a disordering effect of the polyelectrolyte molecules on the lipids as a result of the dominating polyelectrolyte lipid headgroup interaction. An alternative interpretation could be to assume an increase in the lipid headgroup ionization due to the interaction with the amino groups of PAH.

As shown in Figure 4B, the phase transition temperature of the adsorbed DPPC remains at 42 °C, which exactly compares to the phase transition temperature of pure DPPC vesicles. The absence of a shift in the phase transition temperature can be understood considering the rather weak electrostatic interactions of the zwitterionic DPPC with the charged polyelectrolyte molecules. Nevertheless, the pretransition disappears, and the phase transition peak broadens. This can be interpreted as a decrease of cooperativity in the assembled lipid layers. To confirm this hypothesis, more experiments are necessary to achieve a better understanding of these phenomena. In any case, the presence of a phase transition indicates that the lipids form a distinct separate phase as bilayers or multilayers and do not occur as single molecules adsorbed on polyelectrolyte moieties.

The regularity of the layer growth upon stepwise lipid and polyelectrolyte adsorption was studied by means of SPLS and flow cytometry. SPLS measurements were conducted on PS latex particles covered with five PSS/PAH polyelectrolyte layers as a precursor film. The SPLS data demonstrated an increase of the intensity of the scattered light from single particles with increasing layer number. This increase in intensity was converted to a layer thickness increase by applying the Raleigh−Debye−Gans theory[28] assuming a refractive index for the polyelectrolyte/lipid multilayer of 1.44.[29] Figure 6A shows indeed a continuous layer thickness increase at subsequent adsorption of PDADMAC/DPPA pairs. The average increase of film thickness is about 5 nm per PDADMAC/DPPA pair. This value indicates a bilayer structure of DPPA between polycation layers. Figure 6B shows the stepwise adsorption of a DPPC−DPPA mixture together with PAH. A film thickness increase after lipid absorption of 15 nm was found. This possibly indicates a multilayer lipid structure. It cannot be excluded either that the resulting lipid layers have patches due to an incomplete spreading of the weakly

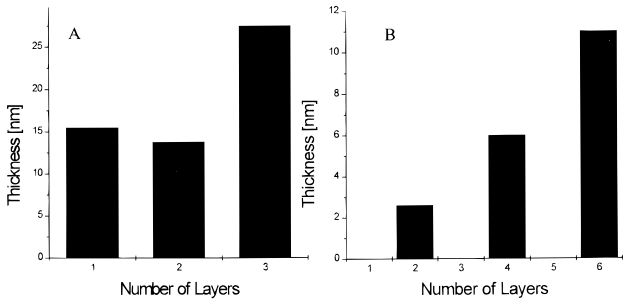

Figure 6. Layer thickness increase as a function of lipid adsorption. (A) DPPA was assembled via the vesicle adsorption protocol in alternation with PDADMAC. The thickness increase refers to 2, 4, and 6 layers of DPPA and PDADMAC. (B) DPPC together with 10% DPPA was assembled via the vesicle adsorption protocol in alternation with PAH. The thickness increase refers to one lipid layer, a lipid−PAH pair, and a second lipid layer on top of the lipid PAH pair.

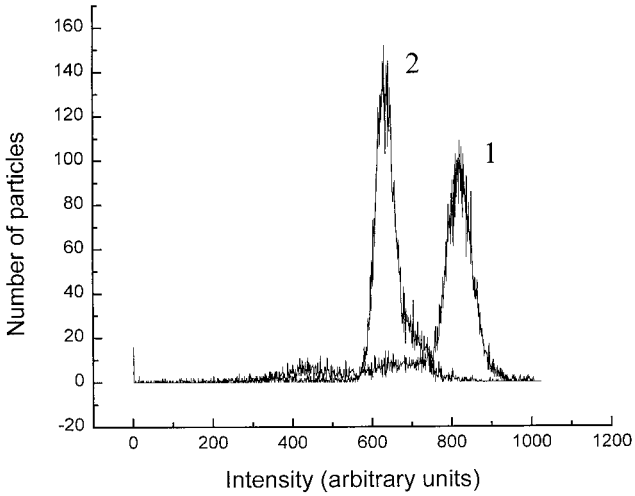

Figure 7. Fluorescence intensity distribution of glutaraldehyde fixed human red blood cells covered with a DPPC−DPPA polyelectrolyte composite layer: 1, $(PSS/PAH)_5$/DPPC−DPPA; 2, $(PSS/PAH)_5$/DPPC−DPPA/PAH. The DPPC−DPPA mixture contained 10% DPPA. The fluorescent label concentration (NBD-labeled DPPC) was in all cases 5% w/w.

charged vesicles. After the adsorption of PAH some of the lipids are removed, and the apparent film thickness decreases by almost 2 nm.

By means of flow cytometry, the fluorescence and the scattering intensity of single particles can be measured simultaneously. In Figure 7 the fluorescence intensity distribution of glutaraldehyde fixed polyelectrolyte coated human red blood cells covered additionally with lipids is plotted employing fluorescently labeled lipid species. The general finding was that always one main peak in the fluorescence distribution was observed. (In some cases two progressively smaller peaks at the double and triple of the main peak intensity were found.) Almost no particles with fluorescence less than that of the main peak were detected. The simultaneously recorded scattering intensity demonstrated that the occasional peaks with 2 and 3 times increased fluorescence intensity could not be attributed to the aggregation of two or three particles because the scattering intensity was the same as for single particles. Particles with an incomplete lipid coverage have not been found. The adsorption of PAH to the already coated with a DPPC−DPPA mixture polyelectrolyte capsules resulted in a decrease of the fluorescence intensity. These findings are in agreement with the SPLS data provided above which demonstrated

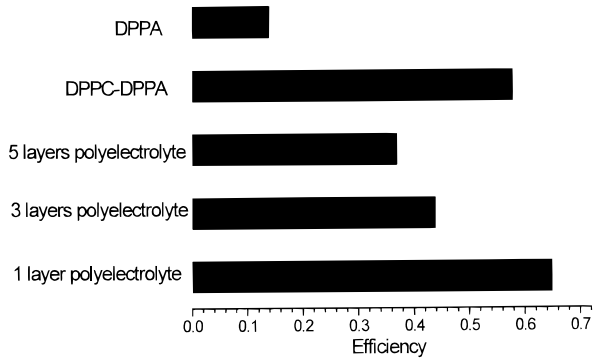

Figure 8. Föster energy transfer through polyelectrolyte and lipid layers intercalated between the donor (FITC−PAH) and acceptor (rhodamine labeled PAH) layer.

the removal of some extra lipids upon adsorption of another polyelectrolyte layer possibly resulting in a more regular lipid layer structure.

Although SPLS has established the regular layer growth, the accuracy of the thickness increase determination is limited by the assumption of a refractive index. The presence of adsorbed water layers between or on lipid layers could significantly modify the value of the refractive index. Therefore, it is useful to apply an independent method to estimate the thickness of the intermediate lipid layers. For this reason Förster energy transfer between fluorescein labeled PAH as the donor and rhodamine labeled PAH as the acceptor has been used to estimate the distance between two PAH layers separated by one or more layers of adsorbed lipids and polymers. The fluorescence data were plotted as the apparent transfer efficiency $(I_a - 0.4I_d)/(I_a + I_d - 0.4I_d)$ where I_a and I_d are the maximum fluorescence intensities at the emission maximum of the acceptor and the donor, respectively. The factor $0.4I_d$ takes into account the fluorescence of the donor at the acceptor maximum. The apparent transfer efficiency would correspond to the real one if the fluorescence quantum efficiencies of donor and acceptor were equal and if the acceptor were excited directly Figure 8 illustrates the transfer efficiency across one polyelectrolyte layer, three polyelectrolyte layers, five polyelectrolyte layers, DPPA, and DPPC−DPPA embedded between the donor and the acceptor layer. As can be seen, there is a continuous decrease of the transfer efficiency when the number of polyelectrolyte layers between the labeled polymers is increased. In the case of DPPA the transfer efficiency falls between the values obtained for one layer and three layers of polyelectrolyte. Considering that the thickness of one polyelectrolyte layer[13] is 1.5−2 nm, it can be estimated that the thickness of a DPPA layer is about 4−5 nm. This justifies the SPLS calculations and indicates a lipid bilayer intercalated between two adjacent positively charged PAH layers. For DPPC the transfer was found to be smaller than for five but larger than for three intermediate polyelectrolyte layers. This would yield for DPPC a layer thickness of the order of 8−10 nm and confirms the hypothesis of multilayer adsorption.

Permeability Studies of Lipid Layers Assembled onto Polyelectrolyte Shells. An important aspect of lipid adsorption onto polyelectrolyte layers is the control of the capsule wall permeability. Pure polyelectrolyte capsules are readily permeable for polar compounds of low molecular weight and ions. Hence, it would be desirable to modify the permeability of the latter by means of intercalating lipid layers inbetween the polyelectrolyte layers. The permeability decrease for polar compounds brought about by the lipid layer adsorption might be a good measure for the integrity of the adsorbed lipid layer.

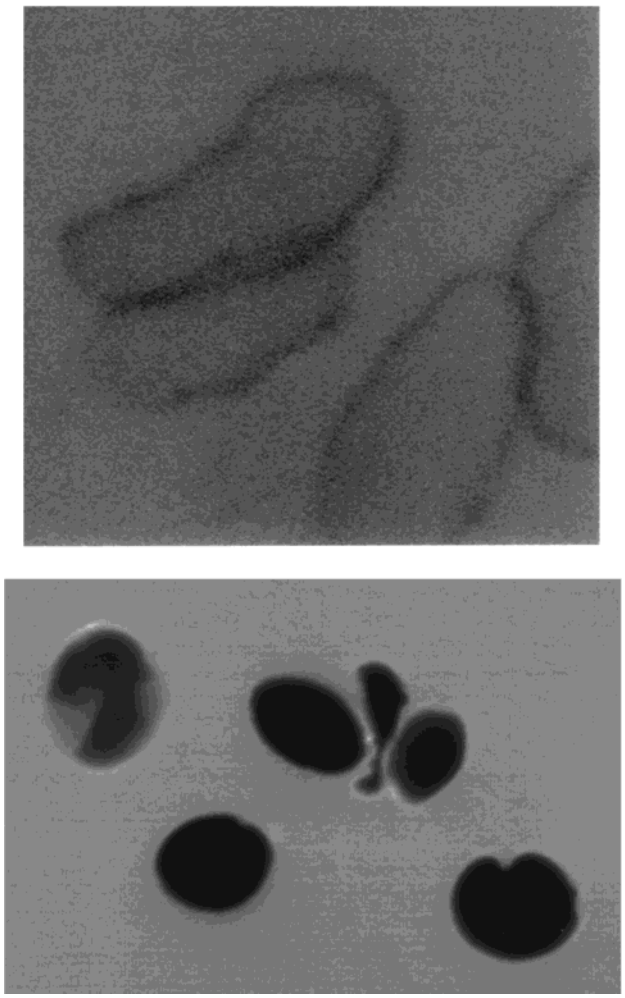

Figure 9. Confocal images of polyelectrolyte capsules suspended in a 0.1 mM 6-CF solution. (A, top) Polyelectrolyte capsules without lipid coverage. The width of the image is 17.5 μm. (B, bottom) Polyelectrolyte capsules covered with one DPPA layer deposited via the vesicle adsorption protocol. The width of the image is 46 μm.

The permeability of the water-soluble dye 6-CF through polyelectrolyte capsules with a wall consisting of 10 PSS/PAH layers was studied by means of CLSM before and after the adsorption of one additional DPPA layer. The confocal image in Figure 9A shows polyelectrolyte capsules without a lipid layer suspended in a 100 μM 6-CF solution. An equal fluorescence intensity inside and outside the capsules can be observed. To estimate the characteristic permeation time for 6-CF, a bleaching protocol was performed. Bleaching was achieved by means of repeatedly scanning the image with high laser power. The image taken immediately after the bleaching showed nevertheless an equal fluorescence inside and outside the capsules. It was concluded that 6-CF equilibrates through the capsule walls faster than the time resolution of the technique being of the order of 10 s. (The time resolution was limited by the time necessary to manually adjust the laser power and the gain of the photomultiplier.) The image in Figure 9B taken after bleaching shows polyelectrolyte capsules additionally coated with DPPA. The dark

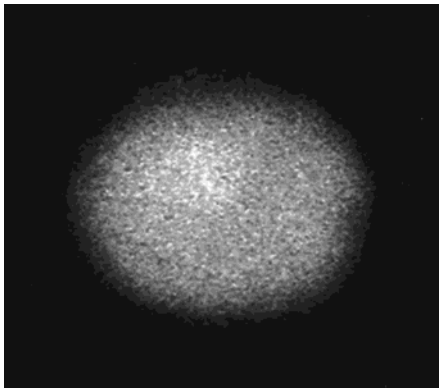

Figure 10. Confocal micrograph of a polyelectrolyte capsule loaded with 100 μm 6-CF and sealed with one DPPA layer adsorbed from a vesicle suspension of DPPA. The width of the image is 32 μm.

appearance of the interior now contrasts with the bright fluorescent outside. It was found that 6-CF equilibrated through the capsule walls at a time scale of the order of 10^1-10^2 min. Hence, it was concluded that the presence of a DPPA layer reduced the permeability of the capsule wall for 6-CF by at least a factor of 10^3. Qualitatively similar observations were made on DPPC and DPPC−DPPA coated polyelectrolyte capsules. Further remarkable is that even after storage for 6 months at 4 °C an apparently tight lipid layer was found. This underlines the stability of the lipid coating. It has to be mentioned that the degree of sealing against 6-CF penetration was not homogeneous. In parallel with almost tight capsules others were observed where quick equilibration of 6-CF took place. This finding indicates the importance of defects in the lipid structure for 6-CF permeability.

Another way of measuring the permeability is to study the release of 6-CF loaded into the lipid coated capsules. To entrap 6-CF, polyelectrolyte capsules were suspended in a 100 μM solution of 6-CF for 1 h. Afterward, the capsules were sealed by adsorption of DPPA vesicles, and the remaining 6-CF outside the capsules was removed by centrifugation. The confocal image in Figure 10 was taken 1 day after the sample preparation. The capsule interior is still strongly fluorescent. The conclusion was that even after 1 day 6-CF is still present within the capsules, indicating the remarkable reduction of the capsule wall permeability brought about by the lipid adsorption.

CLSM can only be used to obtain information on the permeability of fluorescent molecules The ionic permeability of the composite layer has thus been studied by means of electrorotation. This techniques provides the conductance and capacity of thin layers surrounding a micron-sized volume.[30] It was found that the conductivity of the composite polyelectrolyte−DPPC layer is smaller than or at least equal to the lower limit of the method which corresponds to approximately 10^{-7} S/m, while DPPA yielded a conductance of the order of $10^{-4}-10^{-5}$ S/m.[31] The conductance of the polyelectrolyte support was found to be of the order of 10^{-1} S/m. These data confirm that the permeability toward small polar substances is largely reduced by the presence of a lipid layer. Research to characterize in more detail the permeation properties and kinetics of dye release and penetration through the lipid structure is currently under way.

Conclusion

Lipids can be adsorbed onto colloidal particles coated with polyelectrolytes and polyelectrolyte capsules. Charged lipids, such as DPPA, form bilayers while zwitterionic lipids, such as DPPC, may adsorb in form of more than one bilayer. The lipid assembling can be performed either by adsorption of lipid vesicles onto colloidal particles or in the case of capsules also by adsorption of lipids from a methanol solution during a gradual exchange of methanol for water.

In the case of polyelectrolyte capsules the coating with lipids has resulted in a remarkable reduction of the capsule wall permeability. This provides a new means to control the permeability of the capsules, hence encouraging their use as containers for slow release of polar substances. In comparison to liposomes or vesicles, the lipid/polyelectrolyte composite are more stable systems with a size predefined by the size of the polyelectrolyte capsule.

The lipid layers adsorbed on the supporting skeleton of a polyelectrolyte matrix represent an interesting model system which could be used for biophysical studies. They have a high mechanical stability together with determined size and shape. Reconstruction of various biological functions can be envisaged by means of incorporation protein functionalities into the adsorbed lipid layer.

Acknowledgment. The authors thank Dr. K.-H. Lerche from microparticles GmbH for producing soluble polymeric cores. We are grateful to Prof. G. Fuhr, Humboldt-University, Lehrstuhl für Membranphysiology, for using confocal microscopy. P. Klein provided valuable assistance with this technique. The authors thank H. Zastrow for electrophoretic mobility measurements, Dr. R. Georgieva for electrorotation data, and Dr. A. Voigt for capsule preparations. The work was partly supported by a grant from BMBF No. 03CO293A1.

References and Notes

(1) Hoppe, W.; Lohmann, W. *Biophysics*; Springer-Verlag: Berlin, 1983.
(2) Stryer, L. *Biochemistry*; Freeman Wand Co.: New York, 1988.
(3) Möhwald, H.; Dahmen, U.; de Meijere, K.; Brezesinski, G. *Prog. Colloid Polym. Sci.* **1998**, *109*, 3.
(4) de Mejere, K.; Brezesinski, G.; Möhwald, H. *Macromolecules* **1997**, *30*, 2337.
(5) Mobed, M.; Chang, T. M. S. *J. Microencapsulation* **1998**, *15*, 595.
(6) Macdonald, P. M.; Crowell, K. J.; Franzin, C. M.; Mitrakos, P.; Semchyschyn, D. J. *Biochem. Cell Biol.* **1998**, *76*, 452.
(7) Porcar, I.; Garcia, R.; Gomez, C.; Campos, A.; Abad, C. *Polymer* **1997**, *38*, 5107.
(8) Raudino, A.; Castelli, F. *Macromolecules* **1997**, *30*, 2495.
(9) Yaroslavov, A. A.; Sukhishvili, S. A.; Obolsky, O. L.; Yaroslavova, E. G.; Kabanov, A. V.; Kabanov, V. A. *FEBS Lett.* **1996**, *384*, 177.
(10) Thomas, J. L.; Borden, K. A.; Tirell, D. A. *Macromolecules* **1996**, *29*, 2570.
(11) Diederich, A.; Bahr, G.; Winterhalter, M. *Langmuir* **1998**, *14*, 4597.
(12) Sukhorukov, G.; Donath, E.; Davis, S.; Lichtenfeld, H.; Caruso, F.; Popov, V.; Möhwald, H. *Polym. Adv. Technol.* **1998**, *9*, 759.
(13) Sukhorukov, G.; Donath, E.; Lichtenfeld, H.; Knippel, E.; Knippel, M.; Budde, A.; Möhwald, H. *Colloids Surf. A* **1998**, *137*, 253.
(14) Donath, E.; Sukhorukov, G.; Caruso, F.; Davis, S.; Möhwald, H. *Angew. Chem., Int. Ed. Engl.* **1998**, *37*, 2201.
(15) Voigt, A.; Lichtenfeld, H.; Sukhorukov, G.; Zastrow, H.; Donath, E.; Möhwald, H. *Ind. Eng. Chem. Res.*, in press.
(16) Decher, G. *Science* **1997**, *277*, 1232.

(17) Sukhorukov, G.; Donath, E.; Davis, S.; Lichtenfeld, H.; Caruso, F.; Popov, V.; Möhwald, H. *Polym. Adv. Technol.* **1998**, *9*, 759.
(18) Donath, E.; Sukhorukov, G.; Caruso, F.; Davis, S.; Möhwald, H. *Angew. Chem., Int. Ed. Engl.* **1998**, *37*, 2201.
(19) Sukhorukov, G.; Donath, E.; Moya, S.; Susha, A.; Hartmann, H.; Voigt, A.; Möhwald, H. *J. Microencapsulation*, in press.
(20) Sukhorukov, G.; Brumen, M.; Donath, E.; Möhwald, H. *J. Phys. Chem. B* **1999**, *103*, 6434.
(21) Sukhorukov, G. B.; Montrel, M. M.; Petrov, A. I.; Shabarchina, L. I.; Sukhorukov, B. I. *Biosens. Bioelectron.* **1997**, *11*, 913.
(22) Torchilin, V. P. *J. Microencapsulation* **1998**, *15*, 1.
(23) Torchilin, V. P.; Trubetskoy, V. S. *Adv. Drug Delivery Rev.* **1995**, *16*, 141.
(24) Wenzel, A.; Antonietti, M. *Adv. Mater.* **1997**, *9*, 487.
(25) Antonietti, M.; Thuneman A. *Curr. Opin. Colloid Interface Sci.* **1996**, *1*, 667.
(26) Nargessi, R. D.; Schmitt, J. *Methods Enzymol.* **1996**, *122*, 67.
(27) Furusawa, K.; Norde, W.; Lyklema, J. *Kolloid Z. Z. Polym.* **1972**, *250*, 908.
(28) Lichtenfeld, H.; Knapschinsky, L.; Dürr, C.; Zastrow, H. Colloidal Stability investigations by single-particle scattering photometer. *Prog. Colloid Polym. Sci.* **1997**, *104*, 148.
(29) Riegler, H. Submitted to *Langmuir*.
(30) Arnold, W. P.; Schwan, H. P.; Zimmermann, U. *J. Phys. Chem.* **1987**, *91*, 5093.
(31) Georgieva, R. Unpublished results.

MA9914974