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Interaction of polyelectrolyte coated beads with phospholipid vesicles
Interactions entre billes recouvertes de polyélectrolytes et vésicules phospholipidiques

Andreas Fery [a,*], Sergio Moya [b], Pierre-Henri Puech [c], Françoise Brochard-Wyart [c], Helmuth Mohwald [a]

[a] *Max Planck Institute für Kolloide und Grenzflächen Golm, Am Mühlenberg, 14476 Golm, Germany*
[b] *Laboratoire de chimie des interactions moléculaires, Collège de France, 11, place Marcellin Bertellot, 75231 Paris cedex 05, France*
[c] *Laboratoire physico-chimie Curie, UMR 168, institut Curie, 11, rue P. et M. Curie, 75005 Paris, France*

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Abstract

Colloidal particles coated by polyelectrolyte multilayers of alternatingly positive and negative charge are shown to interact strongly with lipid vesicles. We have studied two cases: (i) the interaction between beads and small unilamellar vesicles (vesicles diameter smaller than the particles one), where we found evidence for coating of the beads with lipid bi- or multilayers in the form of an increase in bead diameter and changes in the beads surface potential; (ii) the interaction of beads with giant vesicles (vesicles larger than the particles), where we observed by fluorescence microscopy the spreading of the vesicle on the bead manipulated with an optical tweezer. Giant fluctuations of the vesicles are suppressed due to the adhesion of the vesicle to the bead and direct observation of the coating process shows that lipid coverage is not limited to the direct vesicle-bead contact area, but is rather extended to the entire bead. ***To cite this article: A. Fery et al., C. R. Physique 4 (2003).***
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Résumé

Des particules colloïdales recouvertes de multicouches de polyélectrolytes alternativement positives et negatives interacgissent fortement avec des vésicules lipidiques. Nous avons étudié deux cas : (i) interactions entre des billes et des vésicules unilamellaires de taille plus petite que celle des billes, où nous avons mis en évidence un recouvrement des billes par une ou plusieurs bicouches lipidiques, par le biais d'une augmentation du diamètre des billes et de changements de leur potentiel de surface ; (ii) interactions entre des billes et des vésicules géantes (plus grandes que les billes) où nous avons observé, par microscopie de fluorescence, l'étalement d'une vésicule sur une bille (cette dernière étant manipulée à l'aide d'une pince optique). Dans ce dernier cas, les fluctuations géantes de la vésicule sont supprimées à cause de l'adhésion sur la bille, et l'observation directe du recouvrement lors du processus d'étalement montre que la couverture lipidique de la bille n'est pas limitée seulement à la zone de contact bille/vésicule, s'étend à la bille entière. ***Pour citer cet article : A. Fery et al., C. R. Physique 4 (2003).***
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Mots-clés : Adhésion ; Membranes ; Polyélectrolytes ; Particules ; Mouillage

* Corresponding author.
E-mail address: andreas.fery@mpikg-golm.mpg.de (A. Fery).

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1. Introduction

The approach of building up polyelectrolyte multilayers by adsorbing polyelectrolytes of alternating charge was first demonstrated by Decher et al. on flat substrates [1]. The adsorption process is generally believed to be driven by the electrostatic interaction of the polyelectrolytes with the oppositely charged substrate and the entropy gain due to the release of counterions. It could be shown that the adsorption process results in charge reversal of the surface which is the basis for continuing the process beyond a single layer, by alternatingly using polyelectrolytes of opposite charge, which is known as the layer-by-layer (LbL) technique. At the same time, the self-repulsion of the polyelectrolyte is limiting the thickness of a single polyelectrolyte layer to 1–3 nm depending on the adsorption conditions, which enables excellent control of the total layer thickness. The LbL approach has subsequently been extended to colloidal particles. Latex beads, emulsions, organic crystals, silica particles, in the range from 100 nm to several micrometers can be coated [2–4]. Here the use of dissolvable template particles has opened the way for creating free standing polyelectrolyte capsules by dissolution of the template particles after the multilayer buildup [2].

One interesting property of polyelectrolyte films is that they are well suited as substrates for supported lipid bilayers. Several authors have investigated the properties of lipids adsorbed on flat substrates coated with polymers [5–13]. The main finding is that a polymer layer serves as a soft cushion that is perturbing the structure of the supported bilayer less than a hard surface which results in a higher lipid mobility. The spacing between the hard substrate and the bilayer is increased as well, which is of particular importance when the incorporation of transmembrane proteins in supported membranes is attempted.

We have been investigating in how far this approach can be extended to colloidal particles covered with polyelectrolyte and subsequently to hollow polyelectrolyte shells.

The lipid coating on beads would provide a way to specifically functionalise them. In parallel, techniques like DSC, NMR, or fluorescence correlation spectroscopy could be used for the characterization of the supported membranes.

In a previous work, two of the authors have shown that the covering of polyelectrolyte coated beads with lipids is possible [14]. The aim of this work is to study the interaction between vesicles and latex beads as a function of lipid composition and vesicle curvature, which is necessary to understand the coating process and at the same time gives insight into interactions between hard objects (latex beads, capsules) and vesicle membranes.

2. Experimental conditions

Polystyrene sulfonate (PS) latex particles of 640 nm diameter used in the experiments with small unilamellar vesicles were prepared as described in [15]. Melamin-formaldehyde (MF) particles of 4.7 micrometers average diameter used for experiments with giant unilamellar vesicles were obtained from Microparticles GmbH (Berlin, Germany). PSS (Poly(styrene sulfonate, sodium salt)) of molecular weight 70 000, PAH (Poly(allylamin hydrochloride)) of molecular weight 8000–11 000 and PDADMAC (Poly(diallyldimethyl ammonium chloride)) of medium molecular weight were obtained from Aldrich. PAH and PDADMAC were used as received, PSS was purified from low molecular weight impurities by dialysis against Millipore water (membrane cut-off molecular weight 14 000) and subsequently lyophilized. DPPA (Dipalmitoyl diphosphatidic acid) and DPPC (Dipalmitoyl phosphatidyl choline) were obtained from Avanti Polar Lipids. DOPC (Dioleyl phosphatidyl choline) was obtained from Aldrich.

N-(3-sulfopropyl)-4-(4-(didecylamino) styryl) pyridinium (Di6ASP-BS) (kindly provided by Mireille Blanchard-Desce, UMR 6510, Université de Rennes, France) was used as a fluorescent membrane marker.

Polyelectrolyte solutions were prepared by adding 1 mg/mL of polyelectrolyte to 0.5 M aqueous NaCl solutions. For all aqueous solutions, water purified by a Millipore purification system was used and the resistivity was checked to be higher than 18.2 MΩ/cm. Both PS-latex- and MF-particles were coated using the layer by layer method. In the case of PS latices, the first adsorbed layer was PAH; in the case of MF-particles, PSS was used as the first layer. The number of layers was for each experiment at least 8 to rule out substrate influences. Each incubation step of the particles in the polyelectrolyte solution lasted for 20 minutes and was followed by 2–3 washing cycles with Millipore water. To exchange solution, PS particles were centrifuged at 10 000 g for 10 minutes, MF particles were centrifuged at 5000 g for 5 minutes, afterwards, the supernatant was taken off, and the particles were redispersed by adding water for washing cycles or the polyelectrolyte solution for adsorption cycles.

For this study, both the interactions of PE-beads with small unilamellar vesicles (SUV) and with giant unilamellar vesicles (GUV) were investigated. SUVs were prepared from DPPC, DPPA and mixtures of DPPA and DPPC in the following way: in a first step, the lipid powder was dissolved in chloroform (concentration 0.5 mg/mL). The lipid-solution was subsequently vacuum dried and afterwards water was added up to a lipid concentration of 1 mg/mL. The solutions were sonicated for at least 5 minutes. Vesicle size was checked by single particle light scattering (SPLS) to be in the range between 200 and 300 nm diameter. GUVs were obtained using the electroformation method [16–18]. Lipid powder was in a first step dissolved in chloroform (p.A. quality, Aldrich) up to a concentration of 10 mg/mL. From this mother solution which was stored at $-20\,^{\circ}\mathrm{C}$ and used not longer than 2 weeks after preparation, a spreading solution of two thirds chloroform and one third methanol

(p.A. quality, Aldrich) with a lipid concentration of 1 mg/mL was prepared. This solution was cast onto ITO plates (15–25 µL of solution per cm^2) that served as electrodes for the electroformation. The electrodes were separated by 1 mm teflon spacers, the gap was filled with solution, sealed by an inert paste (Critoseal, Fisher Bioblock) and a sinusoidal AC-potential was applied with a frequency of 10 Hz and an amplitude of 1.1 V for 6 h. GUVs were prepared both in water and in 1 : 2 water-glycerol mixtures to increase the solvent viscosity. Previous work has shown that addition of glycerol does not affect the vesicle stability [19,20]. The viscosity of the water-glycerol mixture was 32 centipoise. 100 mM sucrose was added to the solution and the osmolarity was measured using a commercial osmometer (SD2300, Plasmos, Germany).

Zeta potential measurements were carried out using a commercial Zeta-sizer (Malvern Zetasizer 4, Malvern, USA). Single particle light scattering measurements were performed on a home built apparatus, detailed information on the setup can be found in [21]. For the experiments using giant unilamellar vesicles, a home built laser tweezer was used that is described in [22].

3. Results and discussion

3.1. Experiments with SUVs

In a first series of experiments, PE covered Polystyrene beads with both PAH and PDADMAC as an outermost layer were incubated with SUVs for 20 minutes to allow for contact between vesicles and beads. Incubation took place in an excess of lipids, such that incomplete coverage due to depletion of lipids could be ruled out (100 µL of 1 volume percent PS-latex solution was added to 1 mL of lipids at a lipid concentration of 1 mg/ml). After incubation remaining vesicles were removed by 3 washing cycles and zeta potential measurements and single particle light scattering measurements were performed. Table 1 summarizes the zeta potentials measured for bare particles and after incubation.

Before lipid adsorption, particles with PAH and particles with PDADMAC as an outermost layer showed zeta potentials of +40 mV. Incubation with pure DPPA resulted in a reversal of the zeta potential to −40 mV, incubation with pure DPPC resulted in weakly positive zeta potential and mixtures of DPPC and DPPA resulted in negative zeta potential. These results can be explained by a covering of the particles with lipids.

For the cases of pure DPPA and DPPA-DPPC mixtures, where a reversal of the zeta potential was observed, the buildup of multilayer structures was proven to be possible by SPLS [14]. Thus the layer thickness per PAH/lipid layer could be determined and it could also be ruled out that the observed zeta potential reversal after lipid incubation was caused by desorption of PAH rather than adsorption of lipids. While the sensitivity of SPLS is not sufficient to unambigously measure the thickness increase due to the single lipid adsorption step, a linear thickness increase for subsequently adsorbing PE/lipid layers was found both for PDADMAC and PAH.

Remarkably, for pure DPPA, the thickness increase per lipid-PDADMAC layer was 5 nm, which is well compatible with the thickness of a PDADMAC layer of 1 nm and a lipid bilayer of 4 nm. PAH showed smaller thickness increases under the same conditions. This suggests that for DPPA single bilayers could be adsorbed [14].

In contrast, for mixtures of 10% DPPA and 90% DPPC, thickness increases of 15 nm per lipid-PAH or lipid-PDADMAC pair were found, which suggests that adsorption continued beyond a single bilayer. However the structure of the adsorbed lipid layers in this case could not be determined due to the limited resolution of SPLS.

3.2. Experiments with GUVs

In a second series of experiments, the case of vesicles made from zwitterionic lipids interacting with PE covered beads was examined more closely by directly investigating the coating process by means of fluorescence microscopy. GUVs consisting of 99 mol% DOPC and 1 mol% Di6ASP-BS, a membrane marker that has been shown to incorporate well into DOPC membranes [20] were prepared as explained above. The dye was incorporated by mixing with the mother lipid solution. In this way, lipid covered areas could be distinguished from uncovered areas by fluorescence microscopy. All experiments were carried out at room temperature where DOPC is in the fluid phase.

First experiments showed that exposure of particles to GUVs resulted in covering of the particles as seen by a homogenous fluorescence intensity of the particles after the incubation. Particles already covered showed no adhesive interaction with

Table 1
Zeta potentials measured before and after incubation of particles with SUVs of different composition. Incubation with DPPA and DPPA/DPPC mixtures results in reversal of the Zeta-potential, incubation with DPPC in a pronounced decrease

Particle type	Before incubation (outer layer PAH)	After incubation with DPPA	After incubation with 90%DPPC/10%DPPA	After incubation with DPPC
Zeta potential (mV)	+40	−36	−22	+5

vesicles. However, the coating process could not be followed directly in this case, since particles were quickly covered by smaller vesicles which were present in the solution and, in rare cases where GUVs would hit uncovered particles, the process was too fast to be followed with standard video equipment.

Therefore, GUVs were not prepared from aequous solutions, but rather from water-glycerol mixtures of a viscosity of 32 centipoise. In this way, the velocity of the covering process could be slowed down as well as the degree of mixing of the solution containing the vesicles with the solution containing the beads could be reduced, which eliminated the problem of competition between small vesicles and GUVs.

For the experiment, GUVs were prepared in sucrose solution and sedimented for at least one hour in isoosmolar glucose solution. This reduce the amount of small vesicles as well as bringing the vesicles into a medium in which they sediment to the chamber bottom which was essential for the use of a laser tweezer for bead manipulation as explained below. After the sedimentation step, 40 µl of PE covered beads and 10 µl of vesicles in equiosmolar glucose solution were carefully injected into a home built chamber consisting of 2 coverslides separated by a teflon spacer. After injection, the chamber was sealed to avoid evaporation and thus changes in osmolarity during the experiment. The chamber was mounted onto an inverted microscope equipped with both a fluorescence and a laser tweezer setup. Particles that showed no fluorescence could be picked up with the laser tweezer and brought into contact with GUVs. Fig. 1 shows a typical covering event.

In all cases observed, GUVs showed strong thermal shape fluctuations before contact with the beads, indicating that the membrane tension was close to 0. After the covering process, the vesicle membrane was tensed and shape fluctuations were no more visible. As well, in all cases the particles showed an *homogeneous* fluorescence intensity after the coating process. In no case a rupture of the vesicle membrane as a result of the covering process was observed. Furthermore, beads already covered with lipid showed no adhesion to GUVs upon contact. Vesicles already tensed due to a covering process with one particle showed no adhesion on uncovered beads.

Dietrich et al. [23] have recently treated the covering process of latex beads by phospholipid vesicles both theoretically and experimentally. The main idea of their model is a simple energy consideration:

If a spherical particle is adhering to a vesicle as shown in Fig. 2(b), there are two main contributions to the total energy change from the non-adhering situation shown in Fig. 2(a). The energy is lowered, as the system gains an adhesion energy proportional to the contact area between vesicle and bead $A_{\mathrm{Adh}} = 2\pi a^2 z$ (where a is the vesicle radius and z the dimensionless ratio of the

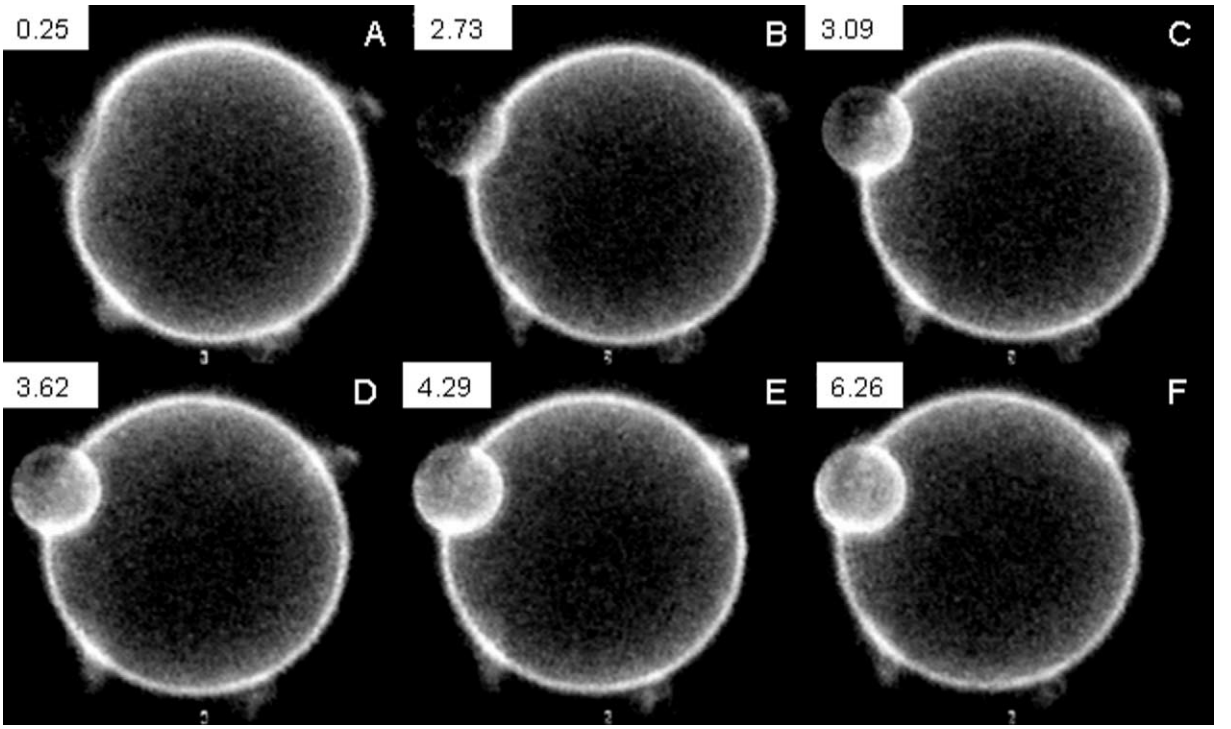

Fig. 1. Covering process directly observed by fluorescence microscopy: initially the bead that is brought into contact is non-fluorescent (A). Subsequently, the lipids start to spread on the bead resulting in coverage as seen by increasing fluorescence of the particle (B–E). In the final state (F) the bead is fully covered and has partly penetrated the vesicle, resulting in tensening of the membrane. The inset in each picture gives time in seconds.

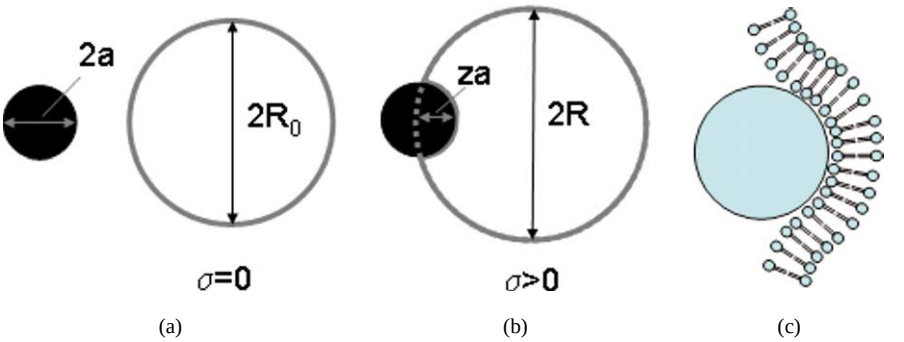

Fig. 2. Vesicle-bead adhesion model according to Dietrich et al.: (a) Before adhesion takes place, the vesicle of radius R_0 is tensionless, a is the bead radius; (b) Adhesion results in establishing a contact area between bead and particle which is accompanied by a penetration z^*a of the particle into the vesicle. Thus the radius of the vesicle has to increase in order to ensure volume conservation, which results in an increase in vesicle membrane tension; (c) sketch of the presumed molecular situation (lipid size not to scale).

bead indentation to the vesicle radius as sketched in Fig. 2(b)). At the same time during the process, the volume of the vesicle is fixed due to the constraint of constant osmotic pressure. Therefore, the area of the vesicle must be increased, which creates a membrane tension σ that increases the total energy by a contribution $A_{\mathrm{Ves}}\sigma$. Deserno and Gelbart refined this treatment by including the curvature energy of the membrane, which is however not relevant in the case of micron sized particles [24].

These models match our observations, except for the fact that both authors assume that the covering of the beads is limited to the direct contact area between the particle and the vesicle. We find indeed that the whole particle surface is covered, as can be seen from Fig. 2, where the fluorescent region of the bead is not limited to the region inside the vesicle radius.

In principle, the appearance of fluorescence on bead regions outside the contact area could also be caused by adsorption of dye from solution rather than spreading of the vesicle membrane. However, the timescale at which a bead becomes fluorescent due to dye adsorption from solution is on the order of at least 10 minutes under the experimental conditions, as checked by bringing a bead close to contact and resting at this position (data not shown). In the case of direct contact, the bead is fully covered within several seconds. The adsorption of dye from solution could only be speeded up by a local increase of dye concentration close to the vesicle due to the tensioning of the vesicle. We would not expect this to happen, since the expulsion of dye from a tensed membrane would lead to a further increase of the membrane tension and increase both the energy of the membrane and the amphiphilic dye molecules that would be forced into water/glycerol environment.

Thus we conclude that the fluorescence is more likely caused by a spreading process of the lipids on the bead surface and that the simple contact picture used for calculating the energy changes in [23] is not appropriate for situations in which the interaction energies are big, as it is expected for the case investigated here. Still the fundamental phenomenon of limitation of the covering process by membrane tension that is proposed in [23] is found in this system as well. Like in these experiments, contact energies could not be determined due to the fact that the initial and finite membrane tensions are unknown. Here, experiments using the micropipette technique [25] are on the way for quantifying the adhesion energies. This should give the basis for a more profound description.

These findings can also explain the pronounced difference in the thickness values found for DPPC-PAH and DPPA-PAH in the experiments on SUVs. While in the first case, vesicles can remain intact when adsorbed on the beads, which leads to thickness values for the lipid layer beyond a single bilayer, in the second case, due to the stronger interaction between the charged groups of PAH and the oppositely charged headgroups of DPPA, the critical tension necessary to stop the spreading process might be beyond the rupture tension of the membrane. This would result in the observed bilayer-coverage. Unfortunately comparative experiments with charged GUVs could not be performed in the present study to directly confirm this hypothesis. However, adhesion energies between lipid monolayers of positively charged DODAB and zwitterionic DMPE on negatively charged SiO_2 surfaces have been determined recently by Graf [26] to be in the range of 10–15 mN/m and 2–3 mN/m respectively. These orders of magnitude support our hypothesis, since typical rupture tensions are on the order of 10 mN/m [25].

4. Conclusion and perspectives

As we showed here, interaction of lipid vesicles with polyelectrolyte covered beads leads to coverage of the particle surface with lipids. Depending on the type of lipid chosen, a single bilayer or more complicated structures can be formed. Both cases are of potential interest for applications: for relatively weak interactions like zwitterionic lipids with PAH or PDADMAC, the simple exposure of the vesicles to beads can be used to create a defined membrane tension. Since the process to establish the

tension is self-organized, this is experimentally considerably less demanding than methods like the micropipette aspiration and can be an alternative in cases where high precision is not required.

In the case where interaction energies are high enough to provoke membrane rupture, exposure of vesicles to suitably coated beads could be used to trigger the release of the vesicle interior. This approach is especially interesting in mixtures of vesicles of various membrane composition, since the release is driven by the interactions of the membrane with the bead surface, which can be chosen as needed by the experimentalist. In this way, in a mixture of charged and zwitterionic vesicles, the rupture of only one species could be triggered by adding beads, while the other species would remain intact. An exciting perspective in this context is also the use of specific key-lock interactions rather than electrostatics.

Finally PE-beads or even more PE-shells covered by lipid layers represent an interesting system for the incorporation of membrane proteins, which goes beyond the subject of the present study.

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