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Cationic colloid–anionic liposome–protein ternary complex: formation, properties, and biomedical importance

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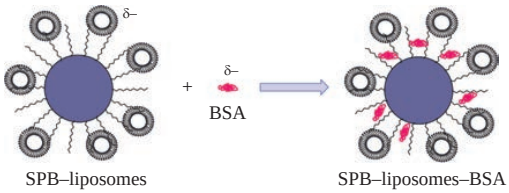
A saturated spherical polycationic brush–anionic liposome binary complex in a 0.15 M NaCl solution can additionally bind a negatively charged protein (albumin) so that the 'doubly saturated' brush–liposome–albumin complex retains in the excess of protein.



Among large number of delivery vehicles for bioactive compounds (polymers, micells, oligosaccharides, *etc*.),[1–3] spherical bilayer lipid vesicles (liposomes) were proved to be effective containers for immobilization and targeted drug delivery.[4,5] Inner cavity of liposomes can be filled with hydrophilic substances while hydrophobic molecules can be incorporated in the area of fatty tails of lipids.[6,7] A typical size of liposomes, 50–100 nm in diameter, significantly restricts their therapeutic application.[8] Additionally, the personalized medicine requires the combined therapy with the use of different bioactive substances at a desirable ratio;[9] this problem cannot be solved within the traditional approach 'one liposome for one type of drug'. To increase the effective volume of the liposomal container and to make it multifunctional, small anionic liposomes loaded by different compounds can be concentrated onto the surface of a 'spherical polycationic brush' (SPB).[10] This approach allows several dozens of intact liposomes to be concentrated on each brush;[11] electrostatic brush–liposome complexes remain stable in physiological solution with C_{NaCl} = = 0.15 mol dm^{-3}.[12] However, in the bloodstream such multiliposomal containers can enter into competitive reactions with proteins, *e.g*. with albumin, which can influence the structure of the multiliposomal container and finally its therapeutic effect.

Here we analyzed the behavior of the electrostatic brush–liposome complex in the presence of albumin to understand the prospects for practical use of the multiliposomal containers. A special attention was paid to the competitive reactions in the brush–liposome–albumin ternary system and the stability of the brush–liposome complex with an excess of albumin.†

The binding of liposomes on the SPB surface in a 10^{-3} M Tris buffer (pH 7) was detected by microelectrophoresis and dynamic light scattering (DLS). Electrophoresis showed the electrophoretic mobility (EPM) of particles (their surface charge) while DLS revealed the size of particles in the mixed SPB–liposome suspension. Addition of the liposome suspension with average EPM of –3.2 μm s^{-1} V^{-1} cm to the SPB suspension led to neutralization of the brush charge and change of the total particle charge from positive to negative in the excess of liposomes (Figure 1, curve *1*). The size of particles in the system grew with increase in the liposome concentration, and then decreased in the overabundant liposome suspension (Figure 1, curve *2*). The largest particles were formed at the liposome concentration which ensured a complete neutralization of the SPB charge. Such profiles of the two curves reflected formation of an electrostatic complex between cationic SPB and anionic liposomes (Figure 2).

Of special interest was to quantify the SPB-to-liposome complexation in a water–salt solution owing to salts in a biological liquid. Since neither microelectrophoresis nor DLS could answer this question, the centrifugation of the complexes prepared in a 10^{-3} M Tris buffer solution with C_{NaCl} = 0.15 mol dm^{-3} (Tris-NaCl

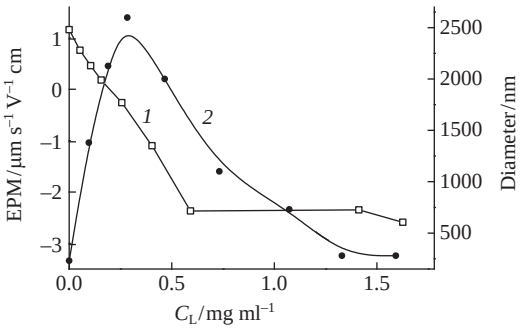


Figure 1 (*1*) EPM and (*2*) size of particles in the mixed SPB and POPS^{1-}/DOPC liposome suspension *vs*. total lipid concentration. C_{SPB+} = = 1×10^{-4} mol dm^{-3}, 10^{-3} M Tris buffer (pH 7).

† SPBs were obtained by graft-polymerization of cationic aminoethyl methacrylate hydrochloride on the surface of 85 nm polystyrene particles[13] with an average contour length of grafted polycationic chains of 75 nm and an average distance between the chains of 5.8 nm. The SPB concentration was found *via* titration with a sodium polystyrenesulfonate solution[14] and expressed in moles of protonated amino groups per liter, C_{SPB+}.

Electroneutral 1,2-dioleyl-*sn*-glycero-3-phosphocholine (DOPC), anionic 2-oleyl-1-palmitoyl-*sn*-glycero-3-phospho-L-serine (POPS^{1-}) and a fluorescent lipid, ammonium salt of 1,2-dioleyl-*sn*-glycero-3-phosphoethanolamine-*N*-(lissamine rhodamine B sulfonyl) (Rh-DOPA) from Avanti, bovine serum albumin (BSA) from Sigma-Aldrich, tris-methoxyaminomethane (Tris) from 'Reakhim' were used as received.

Small unilamellar POPS^{1-}/DOPC liposomes with the molar ratio of anionic POPS^{1-} ν = 0.3 were prepared by the standard sonication technique.[15] The average size of liposomes was 50 nm.

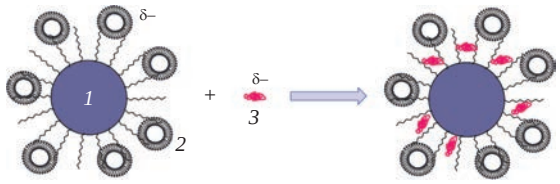


Figure 2 Formation of SPB–liposome–BSA complex: (*1*) 'spherical polycationic brush', (*2*) liposome and (*3*) bovine serum albumin.

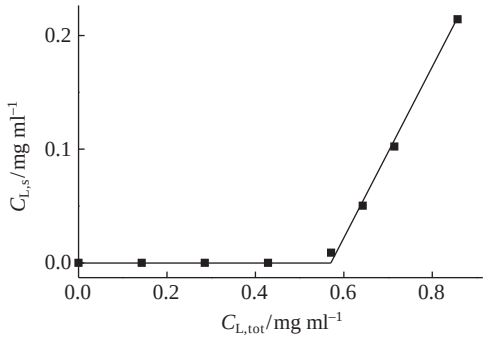


Figure 3 Concentration of POPS^{1-}/DOPC liposomes in the supernatant after separation of SPB–liposome complex *vs.* total liposome concentration. C_{SPB+} = 1×10^{-4} M, 10^{-3} mol dm^{-3} Tris buffer (pH 7), C_{NaCl} = 0.15 mol dm^{-3}.

buffer) with further supernatant analysis were used. The dependence of concentrations of unbound liposomes in supernatant ($C_{L,s}$) on the total concentration of liposomes in the mixed suspension ($C_{L,tot}$) in Figure 3 shows that liposomes are quantitatively adsorbed on the SPB surface up to 0.57 mg ml^{-1} concentration. The termination of the liposome adsorption is governed by strong recharging of the complexes by adsorbed vesicles (see Figure 1) and further electrostatic repulsion of anionic liposomes from anionic complex. The ultimate amount of the ν = 0.3 liposomes capable of binding to one polycationic brush (N) was calculated following the approach described earlier[14] and gave N = 24.

The hydrodynamic diameter of the saturated SPB–liposome complex particles is of 290 nm in a Tris buffer without NaCl and 240 nm in Tris-NaCl buffer. This is obviously due to a partial compression of the polycationic SPB chains after screening their charges by the charges of small salt counter-ions.

Using geometric considerations, the fraction of the SPB surface covered by liposomes was estimated: $\varphi \approx 0.35$. Thus, in a saturated SPB–liposome complex, there is a sizable fraction of the SPB surface free of liposomes. These areas with an uncompensated positive charge could adsorb a negatively charged protein.

The isoelectric point of BSA is p$K_I \approx 5$;[16] the protein globules carry net negative charges in a Tris buffer with pH 7, and the measured EPM value for BSA in Tris was –1.5 μm s^{-1} V^{-1} cm. A quantification of BSA absorbance on bare SPB and saturated SPB–liposome complex was studied by separation of unbound BSA by centrifugation and further supernatant analysis.

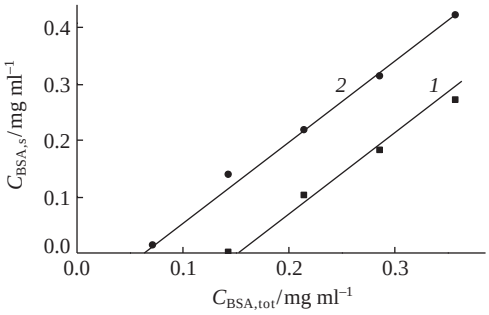


Figure 4 Concentration of BSA in the supernatant after separation of (*1*) SPB–BSA complex and (*2*) SPB–liposome–BSA complex *vs.* total liposome concentration. C_{SPB+} = 1×10^{-4} mol dm^{-3}, ν = 0.3 POPS^{1-}/DOPC liposome concentration 0.8 mg ml^{-1}, 10^{-3} M Tris buffer (pH 7), C_{NaCl} = 0.15 mol dm^{-3}.

A dependence of BSA concentrations in the supernatants ($C_{BSA,s}$) on total BSA concentration ($C_{BSA,tot}$) for the SPB adsorbent shows the quantitative BSA binding up to 0.15 mg ml^{-1} concentration (Figure 4, curve *1*). The protein was quantitatively associated with the saturated SPB–liposome complex up to 0.065 mg ml^{-1} concentration (curve *2*). The protein globules were bound to the positively charged areas on the SPB surface free from liposomes.

To answer the question on the mechanism of BSA binding on the surface of SPB–liposome complex, *viz*. whether BSA covers only the liposome-free areas, or the protein displaces a part of the adsorbed liposomes, the experiments were performed with fluorescent-labeled liposomes.

SPBs are effective quenchers of the fluorescence;[11] so their complexation with the Rh-labeled ν = 0.3 liposomes is accompanied by a decrease in the Rh fluorescence (Figure 5, curve *1*). The minimum level of fluorescence was achieved when the saturated SPB–liposome complex was formed. After that, a suspension of this complex was titrated with a BSA solution; no change in the Rh fluorescence was detected even in an excess of BSA (curve *2*). Lastly, a suspension containing a mixture of the Rh-labeled liposomes and the excess of BSA was titrated with a SPB suspension. The maximum concentration of SPB in this experiment corresponded to that providing formation of the saturated SPB–liposome complex. The fluorescence *vs*. SPB concentration plot (curve *3*) coincided with curve *1* which reflected the titration of the Rh-labeled liposomes by a SPB suspension.

Taking together, the fluorescence results proved the preservation of contacts between SPB and the labeled liposomes in the presence of protein (see Figure 5, curve *2*) and the quantitative adsorption of the labeled liposomes on the SPB surface from the liposome–BSA mixture with an excess of BSA (curve *3*). Thus the electrostatic sorption of BSA on the SPB surface from the liposomes–BSA mixture could not start prior to formation of complex saturated with liposomes. In other words, anionic liposomes were a stronger competitor for binding to SPB in comparison with a negatively charged protein. This could be attributed to lower EPM value of BSA as compared to liposomes.

The size of the SPB–liposome–BSA ternary complex with maximum contents of liposomes and protein ('the doubly saturated complex') was 220 nm (determined by DLS in the Tris-NaCl buffer), which was only slightly less than the size of the saturated SPB–liposome binary complex (240 nm). A decrease in the size of the ternary complex was induced by a shrinkage of the SPB polycationic chains due to neutralization of their charges when

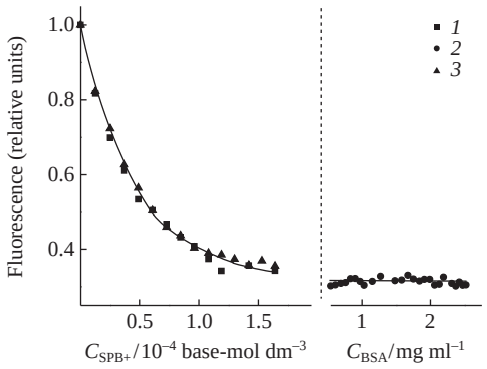


Figure 5 Relative fluorescence of (*1*) labeled liposomes *vs.* SPB concentration, (*2*) the saturated SPB-labeled liposome complex *vs*. BSA concentration and (*3*) a mixture of labeled liposomes and BSA *vs*. SPB concentration. Rh-labeled liposome concentration 1 mg ml^{-1}; 10^{-3} M Tris buffer (pH 7), C_{NaCl} = 0.15 mol dm^{-3}; (*2*) C_{SPB+} = 1.65×10^{-4} mol dm^{-3}; (*3*) C_{BSA} = 3 mg ml^{-1}.

interacting with the negatively charged BSA. The ternary complex demonstrated the very high stability against aggregation: its size did not change for at least 30 h after preparation.

In conclusion, the SPBs adsorb small anionic liposomes in a 0.15 M NaCl solution; the saturated complex contains 24 liposomes per one brush. The saturated binary complex is capable of binding negatively charged albumin over the liposome-free areas. The 'doubly saturated' SPB–liposome–BSA complex is maintained in the excess of protein. These results are important for understanding the mechanism of formation and functioning of multi-liposomal containers in biological media with physiological salt concentration and high protein content.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2018.05.033.

References

1 H. P. James, R. John, A. Alex and K. R. Anoop, *Acta Pharm. Sin. B*, 2014, **4**, 120.
2 H. Jahangirian, E. G. Lemraski, T. J. Webster, R. Rafiee-Moghaddam and Y. Abdollahi, *Int. J. Nanomedicine*, 2017, **12**, 2957.
3 V. V. Spiridonov, A. N. Zakharov, I. G. Panova, M. I. Afanasov, N. S. Perov and I. N. Topchieva, *Colloid Polym. Sci*., 2015, **293**, 1329.
4 T. M. Allen and P. R. Cullis, *Adv. Drug Deliv. Rev*., 2013, **65**, 36.
5 L. Ya. Zakharova, R. R. Kashapov, T. N. Pashirova, A. B. Mirgorodskaya and O. G. Sinyashin, *Mendeleev Commun*., 2016, **26**, 457.
6 M. S. Mufamadi, V. Pillay, Y. E. Choonara, L. C. Du Toit, G. Modi, D. Naidoo and V. M. Ndesendo, *J. Drug Deliv*., 2011, 939851.
7 A. A. Efimova, S. N. Kostenko, V. N. Orlov and A. A. Yaroslavov, *Mendeleev Commun*., 2016, **26**, 99.
8 A. A. Gabizon, H. Shmeeda and S. Zalipsky, *J. Liposome Res*., 2006, **16**, 175.
9 R. X. Zhang, H. L.Wong, H. Y. Xue, J. Y. Eoh and X. Y. Wu, *J. Controlled Release*, 2016, **240**, 489.
10 A. V. Sybachin, O. V. Zaborova, V. N. Orlov, P. I. Semenyuk, M. Ballauff, E. Kesselman, J. Schmidt, Y. Talmon, F. M. Menger and A. A. Yaroslavov, *Langmuir*, 2014, **30**, 2441.
11 A. A.Yaroslavov, A. V. Sybachin, O. V. Zaborova, A. B. Zezin, Y. Talmon, M. Ballauff and F. M. Menger, *Adv. Colloid Interface Sci*., 2015, **226**, 54.
12 A. V. Sybachin, O. V. Zaborova, M. Ballauff, E. Kesselman, J. Schmidt, Y. Talmon, F. M. Menger and A. A. Yaroslavov, *Langmuir*, 2012, **28**, 16108.
13 X. Guo and K. Zhao, *J. Phys.: Condens. Matter*, 2017, **29**, 295101.
14 A. V. Sybachin, M. Ballauff, E. Kesselman, J. Schmidt, Y. Talmon, L. Tsarkova, F. M. Menger and A. A. Yaroslavov, *Langmuir*, 2011, **27**, 5310.
15 A. Yaroslavov, I. Panova, A. Sybachin, V. Spiridonov, A. Zezin, O. Mergel, A. Gelissen, R. Tiwari, F. Plamper, W. Richtering and F. Menger, *Nanomedicine*, 2017, **13**, 1491.
16 O. Yu. Milyaeva, B. A. Noskov, A. V. Akentiev and S.-Y. Lin, *Mendeleev Commun*., 2014, **24**, 264.

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