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Complexes of star-shaped cationic polyelectrolytes with anionic liposomes: Towards multi-liposomal assemblies with controllable stability

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ABSTRACT

Complexes were formed via the electrostatic interaction between 30–50 nm anionic liposomes and a star-shaped polyelectrolyte, poly{[2-(methacryloyloxy)ethyl]trimethyl ammonium iodide}, having cationic arms that radiate from a silicon-based central core. The complexation was investigated with attention given to assessing the capacity of the cationic stars for the anionic liposomes (both liquid and solid); the integrity of the complexed liposomes; and the stability of the resulting star/liposome complexes in aqueous salt solutions. We have found that by changing the content of anionic groups in the liposomal membrane as well as the phase-state of membrane, the stability of star/liposome complexes in aqueous salt media can be controlled. The liquid liposomes with 0.1 mol fraction of anionic palmitoyloleoylphosphatidylserine ($POPS^{1-}$), and solid liposomes with 0.1 mol fraction of $POPS^{1-}$, retain their integrity when bound to the stars, with the resulting star/liposome complexes being stable in physiological solution, i.e. [NaCl] = 0.15 M. Multi-liposomal complexes containing up to 12 liposomes per star seem to hold promise as carriers for biologically active compounds.

1. Introduction

Spherical bilayer lipid vesicles (liposomes) are widely used for encapsulation and delivery of drugs [1]. Hydrophilic biologically active compounds are incorporated into the inner water cavity of liposomes, while hydrophobic compounds bind to the lipid bilayer [2–4]. Drug encapsulation by liposomes provides in vivo protection from degradative enzymes and thus an enhanced circulation time and bioavailability [5].

Recently, we have described electrostatic binding of liposomes, composed of anionic and neutral (zwitter-ionic) lipids with a fixed lipid-to-lipid ratio, to a star-shaped polyelectrolyte (SPE) with cationic arms radiating from a small $SiO_{1.5}$ core [6]. The liposomes complexed with the cationic SPE "("stars")" retain their integrity. Each cationic star is able to bind up to a dozen of anionic liposomes. Since the resulting liposome/star complex shows a low cytotoxicity comparable with that of uncomplexed liposomes, such multi-liposomal containers hold promise for possible use as a high-capacity carrier.

In this article, we investigate electrostatic binding of cationic stars to liposomes with a varied content of anionic lipid, from 5 up to 30 mol%, in order to increase the stability of the complexes in water-salt solutions. As shown earlier, an increase in the anionic lipid content stabilizes liposome complexation with linear polycations in water-salt solutions [7]; the same is reasonably to expect for the complexes of anionic liposomes and cationic stars. Additionally, we use two types of liposomes, liquid and solid, with high and restricted mobility of lipid molecules, respectively. Such difference is reflected in the composition of liposome–polycation complexes [8]. By varying the lipid composition and the phase state

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of the liposomal membrane, the liposome-to-star complexation has been optimized in terms of the maximum capacity of the SPE to anionic liposomes, the integrity of the SPE-bound liposomes. and the stability of the resulting complexes in physiological solution with [NaCl] = 0.15 M. The optimized complexes are visualized with the cryogenic transmission electron microscopy (cryo-TEM). Such multi-liposomal complexes, containing up to 12 liposomes per star, are novel carriers for biologically active compounds. This approach suggests that immobilized liposomes can act as a capacious depot for biologically active compounds. Concentrating individual liposomes with encapsulated drugs within a rather small volume allows increasing the efficacy of drug uptake by cells and therefore, the therapeutic effect of drugs. The blood vessels in tumors are more permeable than in normal tissues because of its rapid growth that causes the defect formation [9] so the selective accumulation of particles in sizes up to 500 nm, called the enhanced permeation and retention effect (was first observed by Maeda and coworkers [10]) is one of the key paths of drug delivery. The multi-liposomal containers seem to be promising for "passive targeting" due to selective penetration of 200–400 nm particles in the capillaries of tumors and other inflammation areas [11].

2. Materials and methods

The cationic SPE (Fig. 1) possessing 24 arms with each arm having the number-average degree of polymerization of 240 was used (the detailed characterization and preparation description of the core of the star and the stars themselves can be found in Refs. [12–14]). The concentration of the SPE is given as the molar concentration of its cationic units, [SPE^+].

Zwitterionic dioleoylphosphatidylcholine (DOPC) (I) and dipalmitoylphosphatidylcholine (DPPC) (II), anionic palmitoyloleoylphosphatidylserine ($POPS^{1-}$) (III) and fluorescent N-(lissamine rhodamine B sulfonyl) phosphatidylethanolamine (Rh-PE) (IV) from Avanti Polar Lipids were used as received. Structures of the lipids are presented in Fig. 2.

Small unilamellar anionic liposomes (ca 30–50 nm in diameter) were prepared by sonication from a mixture composed of DOPC or DPPC and $POPS^{1-}$ (see details in the SM) [15]. Fluorescently-labeled liposomes were prepared by the same procedure but with addition of 0.1 wt% of Rh-PE to initial lipid mixtures.

The fluorescence intensity of Rh-labeled liposome suspensions was measured at λ_{em} = 571 nm (λ_{ex} = 557 nm) using a F-4000 Hitachi fluorescence spectrometer (error of measurement is 3%).

The mean hydrodynamic diameters of the SPE stars, liposomes, and SPE/liposome complexes were determined by dynamic light scattering at a fixed scattering angle (90°) in a thermostatic cell with a Brookhaven Zeta Plus (error of measurement is 6%). Software provided by the manufacturer was employed to calculate regularized diameter values. Electrophoretic mobility (EPM) of the SPE, liposomes and SPE/liposome complexes was measured by laser microelectrophoresis in a thermostatic cell, using a Brookhaven Zeta Plus with the corresponding software (error of measurement is 2%).

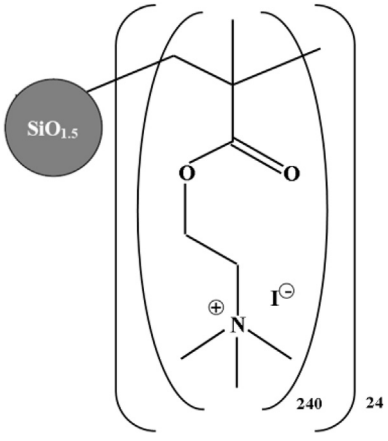


Fig. 1. Cationic SPE.

Permeability of the liposomal membranes toward a simple salt was investigated by measuring the conductivity of NaCl-loaded vesicle suspensions with a CDM83 conductometer (Radiometer) as described in Ref. [16] (error of measurement is 6%).

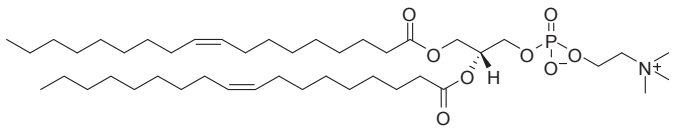
Vitrified specimens for cryogenic transmission electron microscopy (cryo-TEM) were prepared in a controlled environment vitrification system (CEVS), where desirable temperature and humidity were maintained. Briefly, a drop of the SPE solution, or liposome suspension, or mixed SPE/liposome suspension, was placed on a perforated carbon film-coated copper grid, blotted with a filter paper, and plunged into liquid ethane at its freezing point. The vitrified specimens were transferred to a Gatan 626 cryo-cooling holder and observed in either a Philips CM120 or a FEI T12 transmission electron microscope at about −180 °C in the low-dose imaging mode to minimize electron-beam radiation damage. Images were digitally recorded with a Gatan 791 MultiScan cooled-CCD camera (CM120) or with a Gatan US1000 high-resolution cooled-CCD camera (T12). Details may be found elsewhere [17].

Solutions were prepared with double-distilled water that was additionally treated by a Milli-Q Millipore system composed of ion-exchange and adsorption columns as well as a filter to remove large particles. Star-to-liposome binding was examined in 10^{-2} M Tris buffer at 20 °C. Under these conditions, the membranes of DOPC/$POPS^{1-}$ liposomes were in the fluid (liquid-crystalline) state, while the membranes of DPPC/$POPS^{1-}$ liposomes were in the solid (gel) state [18].

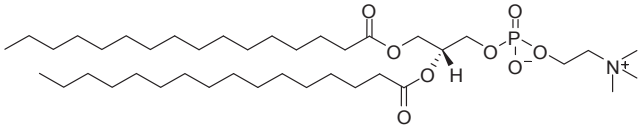
3. Results and discussion

In the previous paper we have described the electrostatic interaction of the SPE with liquid liposomes having a 10-mol% fraction of anionic $POPS^{1-}$ admixed with neutral DOPC [6]. Liquid DOPC liposomes, whose mobile lipid bilayer structurally resembles the cell membrane, are the liposome type used in encapsulation and delivery of drugs [1]. The resulting star/liposome complex remained stable in aqueous solutions with low NaCl concentrations and showed only a slight dissociation in physiological solution with [NaCl] = 0.15 M. One would expect that the stability of electrostatic star/liposome complex in an aqueous salt solution should be enhanced with an increase in the fraction of anionic lipid in the liposomal membrane. Accordingly, we prepared liquid DOPC/$POPS^{1-}$ liposomes with a $POPS^{1-}$ molar fraction $\nu = [POPS^{1-}]/([POPS^{1-}] + [DOPC])$ from 0.1 to 0.3 followed by their complexation with the SPE.

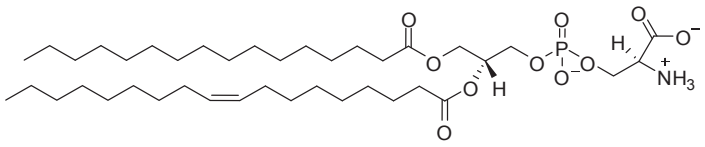
Complexation, accompanied by neutralization of the charge on the stars, was detected by measuring the electrophoretic mobility (EPM) of particles in the system (Fig. 3). For all DOPC/$POPS^{1-}$ liposomes, where the $POPS^{1-}$ content in the liposomal membrane varied from $\nu = 0.1$ up to 0.3, we observed a decrease in the charge of the stars as the liposome concentration increased. Ultimately, an overall change from positive to negative charge on the stars was observed at high liposome concentrations. An increase in the $POPS^{1-}$ content in the liposomal membrane led to a lower concentration of liposomes necessary to achieve charge neutralization on the stars. At the same time, the greater the $POPS^{1-}$ content, the more negative the maximum anionic charge on the star/liposome



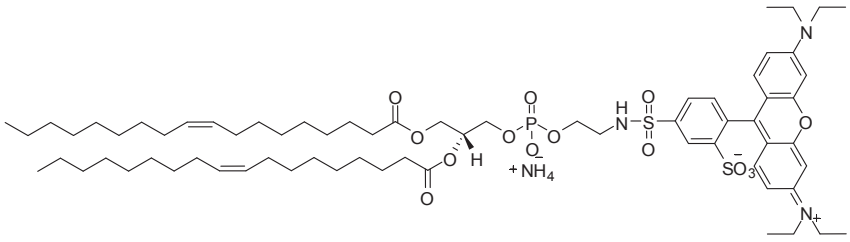
I DOPC



II DPPC



III $POPS^{1-}$



IV Rh-PE

Fig. 2. Lipids (schematic presentation).

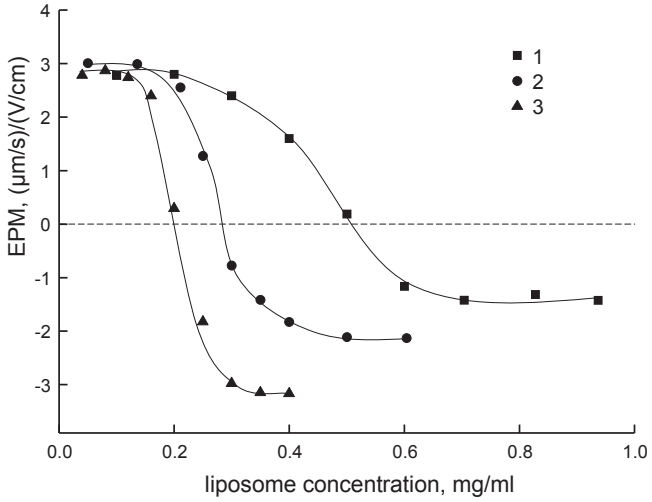


Fig. 3. EPM of the SPE vs. DOPC/$POPS^{1-}$ liposome concentration. ν = 0.1 (1), 0.2 (2) and 0.3 (3). [SPE^+] = 10^{-4} M; 10^{-2} M TRIS buffer solution with pH 7, 20 °C.

complex particles. Charge neutralization (EPM = 0) occurred at 0.51 mg/ml for ν = 0.1 DOPC/$POPS^{1-}$ liposomes with charge saturation at EPM = −1.4 (μm/s)/(V/cm). In contrast, charge neutralization occurred at only 0.2 mg/mL for ν = 0.3 liposomes with charge saturation at EPM = −3.2 (μm/s)/(V/cm).

A total surface charge of colloidal particles is an important factor that determines their resistance to aggregation in aqueous media [19]. Accordingly, the study of star/liposome complex suspension with dynamic light scattering showed aggregates with maximum size at EPM = 0 (cf. Fig. 4 and Fig. 3). Both a positive charge (above

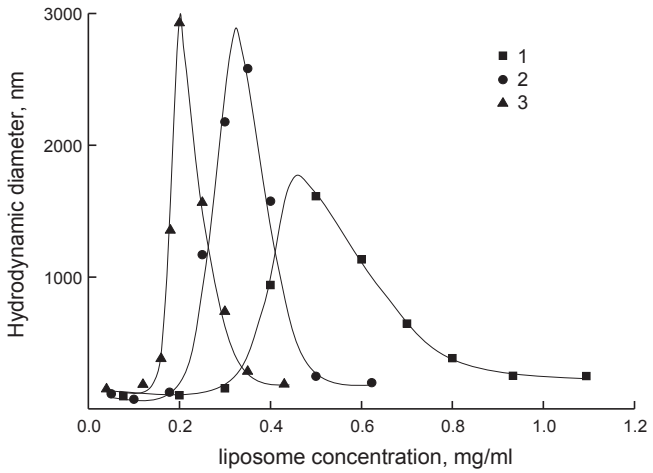


Fig. 4. Hydrodynamic diameter of the SPE vs. DOPC/$POPS^{1-}$ liposome concentration. ν = 0.1 (1), 0.2 (2) and 0.3 (3). [SPE^+] = 10^{-4} M; 10^{-2} M TRIS buffer solution with pH 7, 20 °C.

the EPM = 0 point) and a negative charge (below the EPM = 0 point) cause dissociation of the complexes.

A key question concerning the integrity of star-bound liposomes was examined by conductometry. Suspensions of DOPC/POPS^{1-} liposomes were 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 and increase suspension conductivity. The results for the star-liposome systems were compared with the conductivity of a suspension of NaCl-loaded liposomes completely destroyed in the presence of excess surfactant (Triton X-100) and taken as a unit activity. Only a negligible increase in conductivity was detected when the NaCl-loaded liposomes were mixed with a solution of the cationic star. In other words, complexation with the cationic SPE had no effect on the permeability of DOPC/POPS^{1-} liposomes with $\nu \leq 0.3$. This, of course, is an important result if the complexes are to be used for delivery systems where spontaneous rapid leakage is not acceptable.

The stability of the star/liposome complexes in aqueous salt media was also examined by fluorescence technique. Cationic polymers are known to be effective fluorescence quenchers [20]. Formation and dissociation of star/liposome complexes was controlled by measuring the fluorescence intensity of Rh-PE that had been incorporated into the liposomal membrane. Addition of a star solution to suspensions of fluorescent-labeled DOPC/POPS^{1-} liposomes caused the fluorescence intensity to decrease. Further addition of a NaCl solution to the complex suspensions had different effects depending on the anionic lipid content in the adsorbed liposomes (Fig. 5, curves 1–3). Thus, addition of a NaCl solution to the suspension of complexes formed by liposomes with $\nu = 0.1$ led to a measurable increase in the fluorescence intensity in 0.1 M NaCl solution with a 100% recovery in 0.18 M NaCl solution. These results reflected a dissociation of the SPE-liposome complex into the individual components (stars and liposomes) due to shielding of the liposome and SPE charges by small counter-ions: Na^+ and Cl^-. For complexes of liposomes with $\nu = 0.2$ and 0.3, dissociation became measurable only at [NaCl] = 0.25 M. It was completed at [NaCl] = 0.31 and 0.38 M, respectively. As expected, a higher POPS^{1-} content in liposomes required more concentrated salt solution for the complete complex dissociation. A most important finding is that anionic DOPC/POPS^{1-} liposomes with $\nu \geq 0.2$ remain associated with the stars in physiological solution.

To estimate the number of DOPC/POPS^{1-} liposomes ultimately bound to stars (N), the following experiment was carried out. A suspension of stars was mixed with various volumes of a Rh-labeled DOPC/POPS^{1-} liposome suspension. After 5 min incubation, the star/liposome complexes were separated by centrifugation and the fluorescence intensities of the supernatants were measured. No fluorescence was detected up to a certain liposome concentration (specific for each ν value). At higher concentrations, free (unbound) liposomes were found in the supernatant (Fig. 6, curves 1–3), indicating that the stars had become saturated with liposomes.

Liposome per liter concentration (C_L) for the saturated star/liposome complex can be written as follows:

$$C_L = \left(C_{lip} \times S_1 \times N_A\right) \Big/ \left(2\pi d^2 \times M\right), \quad (1)$$

where C_{lip} is a lipid concentration at saturation (taken from Fig. 6), S_1 is a mean surface area per one lipid molecule ($S_1 = 0.7$ nm^2) [21], d is a diameter of liposomes, M is a mean molecular weight of lipid, and N_A is Avogadro's number. The number of cationic stars per liter (C_S) was calculated as:

$$C_S = \left(\left[SPE^+\right] \times N_A\right) \Big/ (Q \times A), \quad (2)$$

where Q is an average degree of polymerization of SPE arms (Q = 240), A is a an average number of polycationic arms in an SPE (A = 24). Finally, N value, the number of liposomes per star, was quantified as:

$$N = C_L/C_S = \left(C_{lip} \times S_1 \times Q \times A\right) \Big/ \left(\left[SPE^+\right] \times 2\pi d^2 \times M\right), \quad (3)$$

The structure of complexes formed by liquid DOPC/POPS^{1-} liposomes and the cationic stars can be rationalized as follows. The polycationic SPE is covered on the outside by a layer of anionic liposomes, the complexation is electrostatic in nature. No defects in the complexed liposomes are detected. The liposomes can be completely removed from the SPE periphery to surrounding solution in concentrated water-salt solutions.

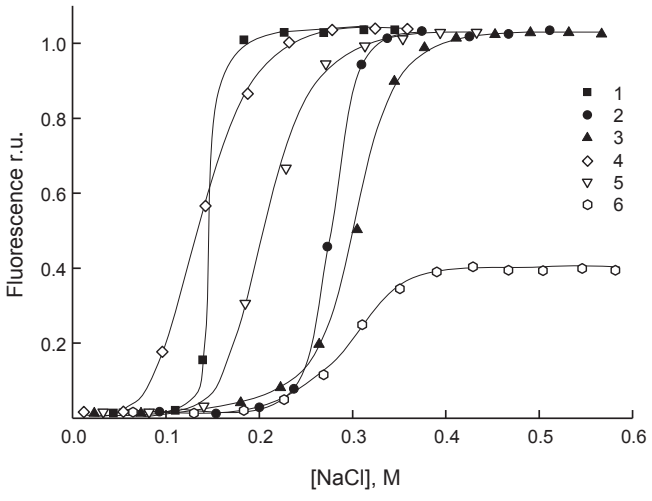


Fig. 5. Changes in the relative fluorescence intensity of Rh-labeled liposomes complexed with the SPE: after NaCl addition. DOPC/POPS^{1-} liposomes: $\nu = 0.1$ (1), 0.2 (2), and 0.3 (3); DPPC/POPS^{1-} liposomes: $\nu = 0.05$ (4), 0.1 (5), and 0.2 (6). [SPE$^+$] = 2×10^{-4} (1), 3×10^{-4} (2), and 5×10^{-4} M (3); total lipid concentration 1 mg/mL; 10^{-2} M TRIS buffer solution with pH 7, 20 °C.

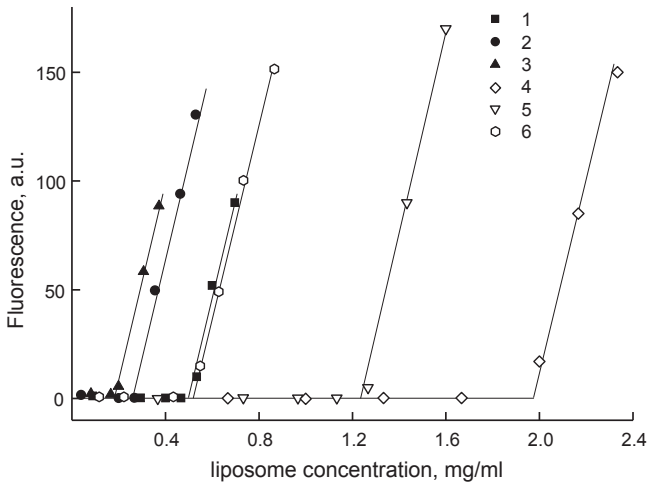


Fig. 6. Relative fluorescence of supernatants after separation of star/liposome complexes vs. liposome concentration. DOPC/POPS^{1-} liposomes: $\nu = 0.1$ (1), 0.2 (2), and 0.3 (3); DPPC/POPS^{1-} liposomes: $\nu = 0.05$ (4), 0.1 (5) and 0.2 (6). [SPE$^+$] = 10^{-4} M; 10^{-2} M TRIS buffer solution with pH 7, 20 °C.

The above calculations reveal that only one or two liposomes bind to each star-shaped macromolecule when the liposome membrane contains 30% anionic lipid ($POPS^{1-}$) admixed with the non-ionic lipid DOPC while N is about 5–6 for at $\nu = 0.1$. Clearly, high $POPS^{1-}$ content subverts the opportunity to exploit the star-bound liposomes for delivery purposes.

In the attempt to obviate this difficulty, we turned from liquid DOPC/$POPS^{1-}$ liposomes (with high mobility of lipids within the bilayer) to solid DPPC/$POPS^{1-}$ liposomes with restricted lateral and transmembrane mobility of lipid molecules. We have shown earlier that the cationic stars adsorb double the number of solid anionic liposomes compared to the liquid liposomes with the same anionic lipid content [6]. One likely reason for such a difference in the adsorption capacity of the SPE toward liquid and solid anionic liposomes is the following: In both liquid DOPC/$POPS^{1-}$ liposomes and solid DPPC/$POPS^{1-}$ liposomes anionic $POPS^{1-}$ molecules are uniformly (fifty–fifty) distributed between the outer and inner leaflets of the membrane [16]. Binding of liquid DOPC/$POPS^{1-}$ liposomes to the SPE induces a migration of $POPS^{1-}$ lipids from the inner to the outer membrane leaflet (flip-flop), so that finally the entirety of anionic $POPS^{1-}$ molecules are concentrated on the outer membrane leaflet where they can form electrostatic bonds with cationic groups of the stars. Since no flip-flop occurs in the star-bound solid DPPC/$POPS^{1-}$ liposomes, twice the number of the solid liposomes is required for neutralization of the SPEs charge, thereby increasing the adsorption capacity of the stars.

Three types of solid DPPC/$POPS^{1-}$ liposomes have been tested: with $\nu = 0.05$, 0.1 and 0.2, each having Rh-PE lipid embedded into the liposomal membrane. Addition of a star solution to suspensions of fluorescently-labeled DOPC/$POPS^{1-}$ liposomes caused the fluorescence intensity to decrease. Further addition of a NaCl solution to the complex suspensions caused different effects depending on the anionic lipid content. In the case of complexes containing liposomes with $\nu = 0.05$ and $\nu = 0.1$, a complete recovery of the fluorescence was observed in 0.25 and 0.35 M NaCl solutions, respectively (Fig. 5, curves 4 and 5). On the other hand, in the case of the complexes containing liposomes with $\nu = 0.2$ the relative fluorescence increased only up to 0.4 in a 0.4 M NaCl solution. Moreover, this value did not change at higher NaCl concentrations (curve 6). The latter indicates only a partial dissociation of complexes when the solid liposomes possessed 20 mol% of anionic $POPS^{1-}$.

An amount of the solid DPPC/$POPS^{1-}$ liposomes ultimately bound to stars (N) was estimated using Rh-labeled liposomes as was described above for the star-liquid liposome system. Fig. 6 shows dependences of the fluorescence intensities in the supernatants after separation of star-liposome complexes on total liposome concentration. From these data, a maximum number of solid liposomes capable of binding to each cationic SPE was calculated: 22 for $\nu = 0.05$, 12 for $\nu = 0.1$ and 6 for $\nu = 0.2$.

By using DPPC/$POPS^{1-}$ liposomes loaded with 1 M NaCl solution, the integrity of solid liposomes upon electrostatic complexation with the cationic stars was assessed. It was found that only liposomes with $\nu = 0.05$ or $\nu = 0.1$ preserved their integrity after being complexed with the stars, whereas liposomes with $\nu = 0.2$ lost approximately 20% of their NaCl within 30 min after complexation.

The fluorescence and conductivity data allows the assumption about the structure of complexes formed by solid DPPC/$POPS^{1-}$ liposomes and the SPE. The solid anionic liposomes adsorb on the top of polycationic chains grafted to a small $SiO_{1.5}$ particles as the liquid DOPC/$POPS^{1-}$ liposomes do. For the solid liposomes with $\nu = 0.05$ and $\nu = 0.1$, the complexation is electrostatic in nature with no defect in the complexed liposomes. Such liposomes can be completely removed from the SPE periphery in concentrated water-salt solutions. In contrast to this, the solid liposomes with $\nu = 0.2$ lose their integrity being bound to the cationic stars. The solid liposomes are known to have more defects in comparison with the liquid ones [22]. Polycationic chains of the SPE can incorporate into the disordering areas in the membranes of solid liposomes, thus making the liposome-to-SPE complexation insensitive to salt concentration. The effect becomes more pronounced with increasing a content of disordering liquid $POPS^{1-}$ in the major solid DPPC.

We see, therefore, that only solid DPPC/$POPS^{1-}$ liposomes with $\nu = 0.1$ meet both key requirements: they form multi-liposomal complexes with the stars, and these complexes remain stable in physiological solution. The solid liposomes with a lower $POPS^{1-}$ content ($\nu = 0.05$) also form multi-liposomal complexes, but they appreciably dissociate in a 0.15 M NaCl solution. At the same time, the liposomes with a higher $POPS^{1-}$ content ($\nu = 0.2$) lose encapsulated compound after their complexation with the SPE.

Summarizing, the liquid DOPC/$POPS^{1-}$ liposomes with $\nu = 0.1$ and the solid DPPC/$POPS^{1-}$ liposomes with $\nu = 0.1$ are optimal constructs for making multi-liposomal electrostatic complexes with the polycationic stars. These liposomes and the resulting complexes were visualized using cryogenic transmission electron microscopy. Typical cryo-TEM micrographs are shown in Fig. 7. The initial liquid liposomes are represented by isolated circles (Fig. 7a) associating after complexation with the SPEs (Fig. 7b). The latter are not visible in Fig. 7,b because of their low electron contrast. These micrographs show that liposomes remain spherical after binding to the stars. In contrast, the initial solid liposomes form non-spherical but polygonal (faceted) structures (Fig. 7c) in agreement with earlier published data [23,24]. Such morphology contributes to formation of lipid packing defects in the liposomal membrane (see above); the effect strengthens when increasing the $POPS^{1-}$ content. These defects become apparent when solid liposomes with high $POPS^{1-}$ content ($\nu = 0.2$ in our case) electrostatically bind to the polycationic stars, and as a consequence leakage of a salt solution from the liposomes takes place into the surrounding solution to increase the conductance.

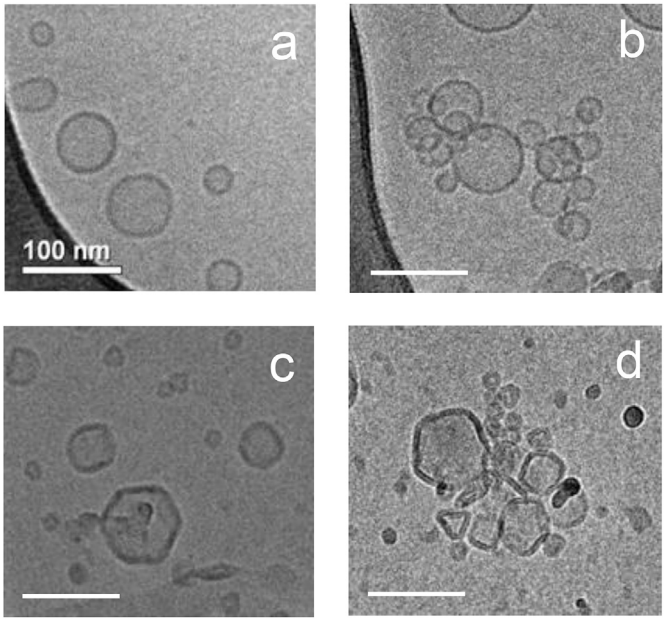


Fig. 7. Cryo-TEM images of liquid DOPC/$POPS^{1-}$ liposomes (a), solid DPPC/$POPS^{1-}$ liposomes (c) and their complexes with the SPE (b and d, respectively). $\nu = 0.1$; $[SPE^{+}] = 10^{-4}$ M; total lipid concentration 2 mg/mL.

4. Conclusions

Earlier described low-toxic complexes formed via the electrostatic interaction between anionic liposomes (both liquid and solid) with a cationic SPE were investigated with the goal of understanding: the capacity of the polycationic star to complex with anionic liposomes; the integrity of the complexed liposomes; and the stability of the resulting star/liposome complexes in aqueous salt solutions. We have found that by changing the content of the anionic lipid of the membrane as well as the phase-state of membrane, the stability of star/liposome complexes in aqueous salt media can be controlled. The liquid liposomes with a molar content of the anionic lipid of $\nu = 0.1$ and solid liposomes with $\nu = 0.1$ retain their integrity when bound to the stars, and the resulting complexes are stable in physiological solution with [NaCl] = 0.15 M. Such multi-liposomal complexes, containing up to 12 liposomes per star, hold promise as carriers for biologically active compounds.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.polymer.2016.04.025.

References

[1] T.M. Allen, P.R. Cullis, Adv. Drug Deliv. Rev. 65 (2013) 36–48.
[2] T. Nii, F. Ishii, Int. J. Pharm. 298 (2005) 198–205.
[3] X. Xu, M.A. Khan, D.J. Burgess, Int. J. Pharm. 423 (2012) 543–553.
[4] A.K. Thompson, A. Couchoud, H. Singh, Dairy Sci. Technol. 89 (2009) 99–113.
[5] A.A. Gabizon, Adv. Drug Deliv. Rev. 16 (1995) 285–294.
[6] A.A. Yaroslavov, A.V. Sybachin, O.V. Zaborova, D.V. Pergushov, A.B. Zezin, N.S. Melik-Nubarov, F.A. Plamper, A.H.E. Müller, F.M. Menger, Macromol. Biosci. 14 (2014) 491–495.
[7] A.A. Yaroslavov, A.A. Efimova, A.V. Sybachin, V.A. Izumrudov, V.V. Samoshin, I.I. Potemkin, Colloid J. 73 (2011) 430–435.
[8] A.A. Yaroslavov, A. Rakhnyanskaya, E.G. Yaroslavova, A.A. Efimova, F.M. Menger, Adv. Colloid Interface Sci. 142 (2008) 43–52.
[9] D.M. McDonald, P. Baluk, Cancer Res. 62 (2002) 5381–5385.
[10] Y. Matsumura, H. Maeda, Cancer Res. 46 (1986) 6387–6392.
[11] V. Torchilin, Adv. Drug Del. Rev. 63 (2011) 131–135.
[12] S. Muthukrishnan, F. Plamper, H. Mori, A.H.E. Müller, Macromolecules 38 (2005) 10631–10642.
[13] H. Mori, A.H.E. Müller, J.E. Klee, J. Am. Chem. Soc. 125 (2003) 3712–3713.
[14] F.A. Plamper, A. Schmalz, E. Penott-Chang, M. Drechsler, A. Jusufi, M. Ballauff, A.H.E. Müller, Macromolecules 40 (2007) 5689–5697.
[15] N. Kasinathan, S.M. Volety, V.R. Josyula, J. Drug Deliv. (2014) 948650.
[16] A.A. Yaroslavov, A.V. Sybachin, M. Schrinner, M. Ballauff, L. Tsarkova, E. Kesselman, J. Schmidt, Y. Talmon, F.M. Menger, J. Am. Chem. Soc. 132 (2010) 5948–5949.
[17] Y. Talmon, in: R.K. Zana, W. Eric (Eds.), Giant Micells, Surfactant Sci. Ser., 140, CRC Press LLC, New York, 2007, pp. 163–178.
[18] A. Therrien, P. Manjunath, M. Lafleur, Biochim. Biophys. Acta 1828 (2013) 543–551.
[19] D. Li, R.B. Kaner, J. Am. Chem. Soc. 128 (2006) 968–969.
[20] J.R. Lakowicz, Principles of Fluorescence Spectroscopy, third ed., Springer, New York, 2006, p. 278.
[21] V. Torchilin, V. Weissig (Eds.), Liposomes: a Practical Approach, second ed., Oxford University Press, New York, 2003.
[22] A.A. Yaroslavov, A.A. Efimova, A.V. Sybachin, Polym. Sci. Ser. A 51 (2009) 638–647.
[23] S. Rangelov, D. Momekova, M. Almgren, Structural characterization of lipid-based colloidal dispersions using cryogenic transmission electron microscopy, in: A. Méndez-Vilas, J. Díaz (Eds.), Microscopy: Science, Technology, Applications and Education, 2010, p. 1724.
[24] A.G. Lee, Biochim. Biophys. Acta. 472 (1977) 237–281.