Polymer 93 (2016) 198–203

Contents lists available at ScienceDirect

Polymer

journal homepage: www.elsevier.com/locate/polymer

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

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ARTICLE INFO

Article history:
Received 2 February 2016
Received in revised form
30 March 2016
Accepted 11 April 2016
Available online 13 April 2016

Keywords:
Liposomes
Self-assembly
Nanocontainers
Star-shaped polycation

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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http://dx.doi.org/10.1016/j.polymer.2016.04.025
0032-3861/

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%).

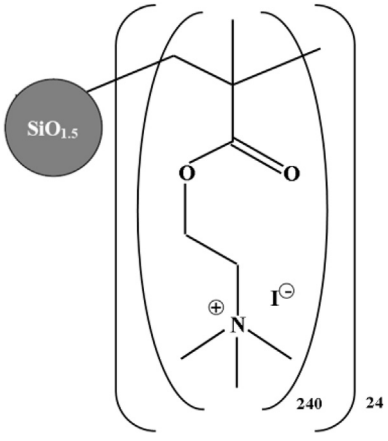

Fig. 1. Cationic SPE.

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