Advances in Colloid and Interface Science 226 (2015) 54–64

Contents lists available at ScienceDirect

Advances in Colloid and Interface Science

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

Historical perspective

Multi-liposomal containers

A.A. Yaroslavov [a,*], A.V. Sybachin [a], O.V. Zaborova [a], A.B. Zezin [a], Y. Talmon [b], M. Ballauff [c], F.M. Menger [d,*]

[a] M.V.Lomonosov Moscow State University, Department of Chemistry, Leninskie Gory 1-3, 119991 Moscow, Russian Federation
[b] Department of Chemical Engineering, Technion—Israel Institute of Technology, 32000 Haifa, Israel
[c] Helmholtz-Zentrum Berlin für Materialien und Energie, Hahn-Meitner-Platz 1, D-14109 Berlin, Germany
[d] Department of Chemistry, Emory University, Atlanta, GA 30322, USA

ARTICLE INFO

Available online 2 September 2015

Keywords:
Anionic liposome
Spherical polycationic brush
Multi-liposomal complex
Flip-flop
Lateral lipid segregation
pH-sensitivity

ABSTRACT

Small unilamellar liposomes, 40–60 nm in diameter, composed of anionic diphosphatidylglycerol (cardiolipin, CL^{2-}) or phosphatidylcerine (PS^{1-}) and zwitter-ionic egg yolk lecithin (EL) or dipalmitoylphosphatidylcholine (DPPC), electrostatically complex with polystyrene microspheres, ca. 100 nm in diameter, grafted by polycationic chains ("spherical polycationic brushes", SPBs). Polymer/liposome binding studies were carried out using electrophoretic mobility (EPM), dynamic light scattering (DLS), fluorescence, conductometry, differential scanning calorimetry (DSC), and cryogenic transmission electron microscopy (cryo-TEM) as the main analytical tools. By these means a remarkably detailed picture emerges of molecular events inside a membrane. The following are among the most important conclusions that arose from the experiments: (a) binding of liposomes to SPBs is accompanied by flip-flop of anionic lipids from the inner to the outer leaflet of the liposomal membrane along with lateral lipid segregation into "islands". (b) The SPB-induced structural reorganization of the liposomal membrane, together with the geometry of anionic lipid molecules, determines the maximum molar fraction of anionic lipid (a key parameter designated as ν) that ensures the structural integrity of liposomes upon complexation: $\nu = 0.3$ for liposomes with conically-shaped CL^{2-} and $\nu = 0.5$ for liposomes with anionic cylindrically-shaped PS^{1-}. (c) The number of intact liposomes per SPB particle varies from 40 for ($\nu = 0.1$) to 13 ($\nu = 0.5$). (d) By using a mixture of liposomes with variety of encapsulated substances, multi-liposomal complexes can be prepared with a high loading capacity and a controlled ratio of the contents. (e) In order to make the mixed anionic liposomes pH-sensitive, they are additionally modified by 30 mol% of a morpholinocyclohexanol-based lipid that undergoes a conformational flip when changing pH. Being complexed with SPBs, such liposomes rapidly release their contents when the pH is reduced from 7.0 to 5.0. The results allow loaded liposomes to be concentrated within a rather small volume and, thereby, the preparation of multi-liposomal containers of promise in the drug delivery field.

Contents

* Corresponding authors.
E-mail addresses: yaroslav@genebee.msu.ru (A.A. Yaroslavov), menger@emory.edu (F.M. Menger).

http://dx.doi.org/10.1016/j.cis.2015.08.011
0001-8686/

1. Introduction

Spherical bilayer lipid vesicles (liposomes) have been widely used as nano-scale containers for encapsulation, delivery and release of biologically active (medicinal) substances [1,2]. Hydrophobic guests can be incorporated within the liposomal membrane, whereas hydrophilic guests situate in the aqueous liposomal cavity [3,4]. A traditional approach, where individual drug-loaded liposomes operate independently, proved to be very fruitful in allowing enhanced bioavailability of poorly soluble drugs [2,5]; in protecting drugs against premature biodegradation [6,7]; in modifying the liposome surface by "vector" molecules and ensuring liposome binding to the target cells [8,9]; in increasing the circulation time of liposomes in the blood stream [2,10]; and in improving the drug uptake by the target cells [11]. The scientific and patent literature describes antitumor, antifungal, and antiviral liposomal drugs [2,12,13], among the most cited of which are liposomal forms of the antitumor antibiotic doxorubicin, Doxil/, Caelyx and DaunoXome [14–16]. However, other principal issues are still waiting for their solutions. Concerns remain over the limited capacity of conventional liposomal containers; over problems with multi-component drugs for combination therapy; and over the slow release of drugs in the area of therapeutic action [17–19].

Immobilization of liposomes on a suitable surface could result in a more capacious depot for biologically active compounds and an increase in their therapeutic effects. Liposomes were adsorbed onto mica [20], glass [21], silica [22], gold [23], polymers [24] and supported lipid bilayers [25]. Unfortunately, due to liposome–surface and liposome–liposome interactions, fusion and rupture vents are common during adsorption particularly at higher coverage. Successful attempts at intact liposome immobilization, described in the literature, include pre-modification of liposomes and/or surface. For example, liposomes and the surface were modified by complementary single-stranded oligonucleotide [26]. Immobilization of liposomes was accompanied by formation of a double-stranded oligonucleotide bridge which, by protecting liposomes from direct contact with the surface, allowed the liposomes to retain their integrity. Liposomes and the surface modified by poly(ethyleneglycol) chains with terminated biotin groups could be conjugated via addition of protein avidin and formation of liposome/biotin–avidin–biotin/surface bridges [27]. In another example, modification of liposomes by charged polymeric nanoparticles provided the electrostatic binding of the modified liposomes to the surface of polystyrene latex particles while, at the same time, prevented fusion of bound liposomes [28]. Plasma treatment of metal surfaces created carboxylic groups, which were used for covalent binding of amino-containing liposomes [29]. A simpler technology includes a preliminary aggregation of liposomes with subsequent immobilization of the aggregates [30]. However, the aggregation is often accompanied by liposome disruption and a premature loss of the encapsulated drug [31]. Additionally, the aggregation is difficult to control in that it leads to irreproducible multilayer liposome adsorption [30]. A need thus arises for carriers devoid of such stability problems.

In the present review, we describe electrostatic adsorption of anionic liposomes on the surface of polymeric microspheres with grafted polycationic chains, known as "spherical polycationic brushes" (SPBs). We show that the liposomes retain their integrity after adsorption and the resulting liposome/brush complex does not dissociate into its components in physiological solution with [NaCl] = 0.15 M. By varying the anionic lipid fraction in the liposomal membrane, we can manipulate the amount of bound liposomes. By using liposomes with different entrapped contents, the multi-liposomal complexes with desirable content ratios can be prepared. Complexation decreases the cytotoxicity of the polycationic brushes to approximate that of the initial anionic liposomes. This makes the brush-based multi-liposomal containers promising in the drug delivery field.

2. Complexation of the polycationic brushes with conventional liposomes composed of anionic and electroneutral lipids

In order to prepare SPBs, cationic poly(trimethylaminoethylmethacrylate) ammonium chloride macromolecules were grafted from the surface of 100 nm monodispersed polystyrene latex particles as described elsewhere [32]. Dynamic light scattering showed a mean hydrodynamic diameter of the brush equal to 230 nm with a thickness of a cationic corona (L_c) of $(230-100)/2 = 65$ nm (see structure in Fig. 1). Concentrations of SPBs are expressed as moles of cationic units per liter ([SPB^+]) throughout the text. The brushes were coupled with liposomes composed of electrically neutral egg yolk lecithin (EL) and diphosphatidyl glycerol (cardiolipin, CL^{2-}), a lipid bearing two anionic headgroups and four alkyl tails. Their chemical structures are shown in Fig. 2 (I and II). A molar content of negative CL^{2-} headgroups $\nu = 2[CL^{2-}]/(2[CL^{2-}] + [EL])$ was specified from 0.05 up to 0.4. The bilayer membrane of these "liquid" liposomes is, at room temperature, in the fluid (or liquid crystalline) state. This fluid state is characterized by considerable lipid chain disorder and an ability of lipid molecules to migrate within each membrane leaflet (lateral mobility) and between them (transmembrane migration or flip-flop).

Binding of anionic EL/CL^{2-} liposomes to SPBs was monitored by a fluorescence method. SPB suspensions were mixed with increasing concentrations of EL/CL^{2-} liposomes whose membranes contained a fluorescence tag, N-fluorescein-iso-thiocyanyldipalmitoylphosphatidylethanolamine (FITC-DPPE) shown in Fig. 2 (III). SPB/liposome complex particles were separated by centrifugation, and the fluorescence intensities of supernatants were measured. The amount of unbound liposomes vs. total amount of added liposomes was then plotted as for several values of ν (0.05 to 0.4) (Fig. 3). It is seen that there are no unbound liposomes until the SPB particles are saturated, at a concentration designated as C_{lip}, beyond which the number of unbound

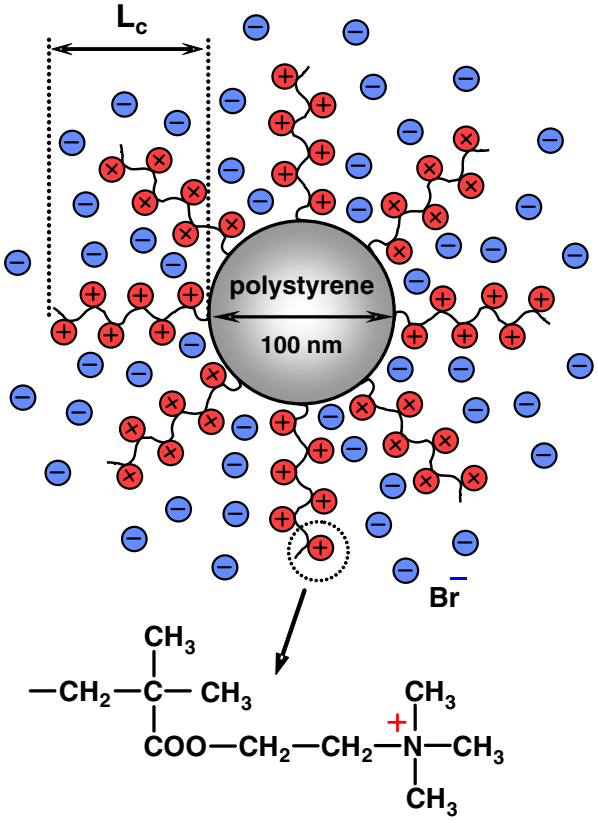

Fig. 1. The polycationic brush (schematical presentation).