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Effects of the electrostatic complexation between anionic pH-sensitive liposomes and star-shaped polycations on the release of the liposomal content

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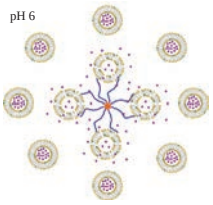
Binding of pH-sensitive anionic liposomes to a star-shaped polycation provides accumulation of plenty of liposomes in a small volume and an increase in the rate and maximal amount of the pH-triggered content release.



A controlled delivery of drugs is a key task of nanomedicine. Spherical bilayer lipid vesicles (liposomes), up to 100 nm in diameter, have proven to be effective containers of biologically active substances.[1–5] A stimuli-responsive release of an entrapped drug may be achieved *via* modification of the liposomal membrane with molecules sensitive to changing of external conditions such as ultrasonication, radiation, temperature, *etc*.[6–10] pH-sensitive containers are of prime importance because they can be 'opened' when pH value decreases, which is typical of pathological physiological pathways, *e.g*. inflammation, solid tumor progression, ischemic injuries of the heart and brain tissues, *etc*.[11,12] Our earlier experiments have demonstrated that a specific lipid-like compound, a 'flipid' capable of flipping its alkyl tails conformation when pH of a medium is lowering, effectively disturbs the lipid bilayer and increases its permeability upon acidification of surrounding solution.[13–15]

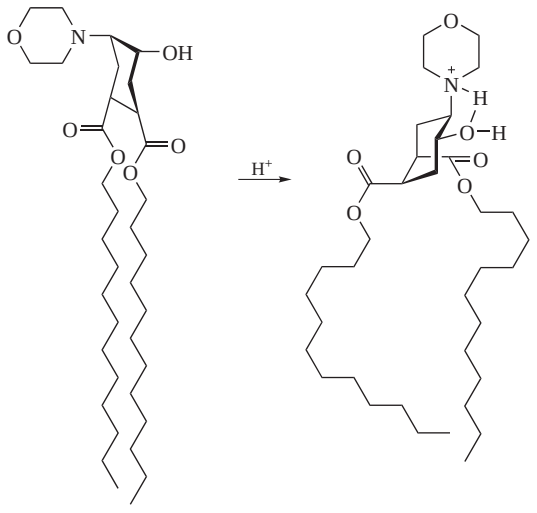


Figure 1 Scheme of protonation-triggered conformational switch in MOCH-flipid.

Here we describe preparation of mixed liposomes composed of anionic and zwitter-ionic lipids as well as a flipping lipid-like compound, their complexation with a star-shaped cationic polyelectrolyte (PE) and examine an effect of pH on the rate of water-soluble content release and on the maximal amount of the released compound.

The flipid (2*t*,4*t*,5*t*)-4,5-bis(dodecyloxycarbonyl)-2-morpholinocyclohexanol (MOCH) was synthesized according to published procedure.[14] The scheme of pH-dependent conformational change in MOCH structure is presented in Figure 1. Flipid-containing liposomes ('fliposomes'[14]) were prepared by sonication technique[16] from a mixture composed of anionic phosphatidylserine (PS^{1-}), zwitter-ionic egg lecithin (EL) and MOCH in a 10^{-3} M Tris buffer solution with pH 7 so that a PS^{1-}/EL/MOCH molar ratio was 1:6:3. Fluorescent-labeled fliposomes were prepared by embedding 0.1 mol% of 1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine-*N*-(lissamine rhodamine B sulfonyl) (Rh-PE) to the fliposomal membrane. In order to control the permeability of the fliposomal membranes, the inner water cavity of fliposomes was filled with 1 M NaCl solution.[17] Synthesis of the star-shaped PE, poly(2-methacryloyloxyethyl) trimethylammonium iodide, with 24 arms and degree of polymerization 240 units per arm was described elsewhere.[18,19]

Binding of anionic PS^{1-}/EL/MOCH fliposomes to the cationic PE was studied using the fluorescence technique as it was described previously.[17] An aqueous suspension of the Rh-PE-labeled fliposomes was added to a solution of PE in a buffer with pH 7. After 25 min the mixed fliposome/PE suspension was centrifuged, and the fluorescence intensity of the supernatant provided the concentration of unbound fliposomes. The experiment was performed for various fliposome concentrations at the

constant PE concentration of 0.0295 mg cm^{-3}, when the molar concentration of cationic PE units [PE] was 10^{-4} M. The complete fliposome binding to PE was observed up to the fliposome concentration 1.2 mg cm^{-3} that gives the maximal average number of 9 fliposomes per one PE star. This result is in good correlation with the data for binding of PS^{1-}/EL binary liposomes to PE described earlier.[17] From there, we can conclude that incorporation of MOCH in the membrane of anionic PS^{1-}/EL binary liposomes does not affect their interaction with PE in a pH 7 buffer solution.

A release of an encapsulated compound from PS^{1-}/EL/MOCH fliposomes was studied by conductometry. For this, the fliposomes loaded with NaCl solution were used. Initially, the fliposomal suspension contained a Tris buffer with pH 7 inside the fliposomes (with additional NaCl) and in the surrounding solution (without additional NaCl). Then, pH 7 of the surrounding solution was reduced to 5 in increments of 0.5 by changing buffer solution. For each specific system, an initial conductivity (Ω_0) and a maximum conductivity (Ω_{max}) after a complete destruction of fliposomes in the presence of an excess of a Triton X-100 surfactant solution[17] were measured. Additionally, a time-dependent conductivity after acidification of suspension was monitored. The error of measurement of the conductance did not exceed 2%.

As an example, the relative time-dependent conductivities [Ω in equation (1)] for suspensions of NaCl-loaded fliposomes free of PE and bound to PE, and for two limiting pH values 7 and 5 of the surrounding solution are presented in Figure 2.

$$\Omega = (\Omega_t - \Omega_0)/(\Omega_{max} - \Omega_0), \quad (1)$$

where Ω_t is a current suspension conductivity. No leakage of NaCl from the free and PE-bound liposomes was detected at pH 7 (curves *1* and *2*), while acidification of the surrounding solution down to pH 5 caused an escape of NaCl solution from both free and PE-bound fliposomes (curves *3* and *4*).

The kinetic curves from Figure 2 supplemented by kinetic curves for other pH values allowed us to estimate the initial rates of NaCl leakage (v). The latter were then recalculated in relative leakage rates:

$$v_{rel} = v/v_{comp,pH\,5}, \quad (2)$$

where $v_{comp,pH\,5}$ is the maximum NaCl escape rate, which in our experiments was observed for the fliposome–PE complex at pH 5. The results are shown in Figure 3 as v_{rel}–pH plot. The decrease of pH from 7 to 5.5 did not induce the NaCl leakage from the free (unbound) fliposomes (in all these cases v_{rel} = 0), while at pH 5 the escape rate was 30% of the maximum. The behavior of PE-bound fliposomes was different. A slight decrease of pH down to 6.5 caused a noticeable NaCl escape. This process is

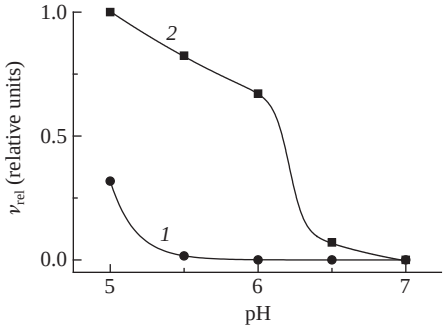


Figure 3 Relative rate of NaCl leakage from the NaCl-loaded fliposomes *vs*. pH of the surrounding solution for (*1*) the PS^{1-}/EL/MOCH (1:6:3) fliposomes and (*2*) the fliposome–PE complex. Fliposome concentration 1 mg cm^{-3}, [PE] = 0.5×10^{-4} M.

permanently accelerated with decreasing pH. In the whole pH range, the escape rate from PE-bound fliposomes exceeded the escape rate from free fliposomes.

From the kinetic curves the maximal conductivities (ω) for the systems at different pH values were found, which were then recalculated into the relative ω values:

$$\omega_{rel} = \omega/\omega_{max,pH\,5}, \quad (3)$$

where $\omega_{max,pH\,5}$ is the maximal conductivity, which was also observed for the fliposome–PE complex at pH 5. In general, a ω_{rel}–pH plot (Figure 4) correlates well with the data from Figure 3. In Figure 4, one can observe no leakage of NaCl from unbound fliposomes when a system is acidified from pH 7 down to pH 5.5 and a release of NaCl at pH 5 (curve *1*). In contrast, a progressive salt release is detected upon gradual acidification of a fliposome–PE suspension (curve *2*).

Thus, we observed a gradual increase in (1) the rate of NaCl escape from fliposomes and (2) the maximum salt release upon acidification of the surrounding solution from pH 7 down to pH 5. Both the salt escape rate and the maximal salt released were much higher for the PE-bound fliposomes as compared with the unbound ones. This can be rationalized as follows. The membrane of the EL/PS^{1-}/MOCH ternary fliposome is in the liquid crystalline state in which the NaCl release occurs *via* permanent defects in the membrane caused by the conformational reorganization of the MOCH molecules when the surrounding solution is acidified (Figure 1). The lower is pH value the higher is the number of defects in the membrane and the rate of NaCl escape from fliposomes to the surrounding solution. After all the MOCH alkyl tails acquire a new conformation, the resulting membrane defects may be 'healed' through the lateral and transmembrane migration of lipid molecules. It is clear that the maximal amount of the

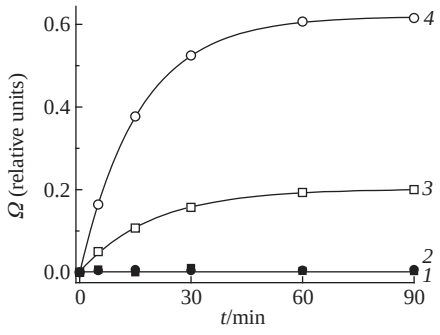


Figure 2 Time-dependent change in conductivity of the NaCl-loaded PS^{1-}/EL/MOCH (1:6:3) fliposome suspensions at (*1*) pH 7 and (*3*) pH 5, and of their complexes with PE at (*2*) pH 7 and (*4*) pH 5. Fliposome concentration 1 mg cm^{-3}, [PE] = 0.5×10^{-4} M.

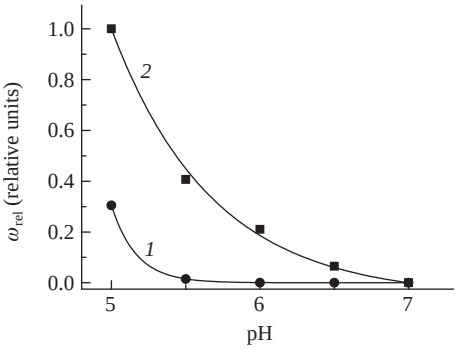


Figure 4 Relative conductivity of the NaCl-loaded fliposome suspension *vs*. pH of the surrounding solution for (*1*) the PS^{1-}/EL/MOCH (1:6:3) fliposomes and (*2*) the fliposome–PE complex. Fliposome concentration 1 mg cm^{-3}, [PE] = 0.5×10^{-4} M.

released NaCl should grow with the increasing number of defects in the membrane, *i.e.* with decreasing pH.

The faster NaCl release from the PE-bound liposomes may be related to the ability of PE cationic star to weaken the liposomal membrane by attracting the electronegative PS^{1-} molecules toward the contact areas,[20] and/or to stabilize the 'conducting' state of the liposomal membrane. Thus, partial PE incorporation into the defects might impede the spontaneous healing of the defects and, consequently, accelerate the NaCl release. Disordering of lipid package in complexes also resulted from pH value sufficient for starting of liposomal cargo release decreasing minimal required number of defects in membrane caused by flipids conformational shift.

Thus, binding pH-sensitive anionic fliposomes to a star-shaped cationic polymer allows one to achieve an accumulation of a plenty of fliposomes in a rather small volume and an increase in the rate of the pH-triggerable release of the water-soluble encapsulated content and the ultimate amount of the released compound. This makes the multi-liposomal containers promising in the drug delivery field.

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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.2016.07.002.

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