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pH-Sensitive liposomes with embedded ampholytic derivatives of cholan-24-oic acid

Anton S. Popov, Anna A. Efimova,* Alexey V. Kazantsev, Dmitry A. Erzunov, Nikolay V. Lukashev, Irina D. Grozdova, Nikolay S. Melik-Nubarov and Alexander A. Yaroslavov

Department of Chemistry, M. V. Lomonosov Moscow State University, 119991 Moscow, Russian Federation. Fax: +7 495 939 0174; e-mail: ephimova@genebee.msu.su

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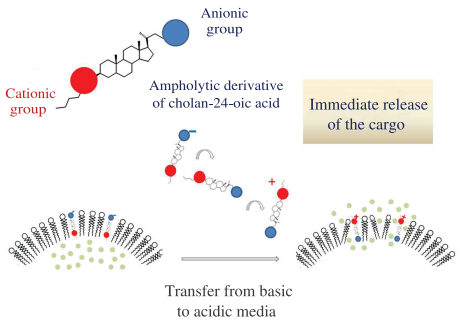
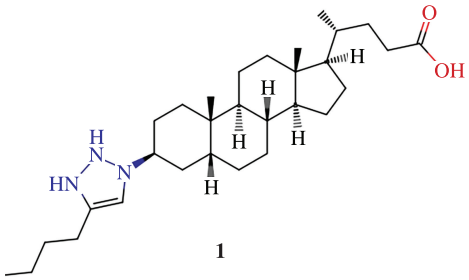
Stimulus-sensitive liposomes have been prepared from zwitterionic dioleoylphosphocholine and ampholytic molecular switches with carboxylic anionic groups and the triazole or isobutylamino cationic ones attached to the opposite ends of the steroid core. When the pH of outer solution was altered from slightly alkaline to slightly acidic, the switches changed their orientation in the liposomal membrane, which induced temporal defects formation and the release of a drug model load. The low-toxic pH-sensitive isobutylamino derivative–dioleoylphosphocholine liposomes demonstrated fast cargo release.



Keywords: pH-sensitive liposome, cytotoxicity, controlled drug delivery, molecular switch, stimulus-sensitive.

The use of vesicles like liposomes or cerasomes for encapsulation and delivery of drugs was suggested decades ago.[1–5] Nowadays, much attention is paid to liposomes as bilayer lipid vesicles capable of releasing water-soluble drugs under the action of an external stimulus.[6,7] Among other stimuli, pH value is of particular interest since a lower pH is typical of tumors and inflammation areas.[8–10] After acidification, it typically takes dozen minutes for the pH-sensitive liposomes to liberate their cargo.[11–19] An increase in the release rate is accompanied by the elevation of the liposome cytotoxicity.[11,20] Therefore, a need remains for low toxic pH-sensitive liposomes with the fast response to a decrease in pH.

In this work, we investigated two types of liposomes containing derivatives of cholan-24-oic acid as ampholytic molecular switches (AMSs) with anionic and cationic groups attached to the opposite ends of the steroid core. 3β-(4-Butyl-1*H*-1,2,3-triazol-1-yl)-5β-cholan-24-oic acid **1** with carboxylic and triazole moieties has been described by our group.[21] This substance was embedded in the membrane of the egg yolk lecithin liposomes and was found to change its orientation adapting to acidity or basicity of the surrounding aqueous solution. This rearrangement led to formation of temporal defects in the liposomal membrane and leakage of an encapsulated water-soluble drug. The content of 3 mol% AMS **1** in the membrane ensured low cytotoxicity of the liposomes accompanied by extended drug leakage. An increase in the content of compound **1** could accelerate the release, however the cytotoxicity of the modified liposomes remained to be controlled. Another AMS with carboxylic and secondary amino groups, namely 3β-(isobutylamino)-5β-cholan-24-oic acid **2**, was synthesized in this work. The two molecular switches have similar structure though differ in the nature of cationic groups. This distinction could affect their toxicity and kinetics of the pH-induced drug release.



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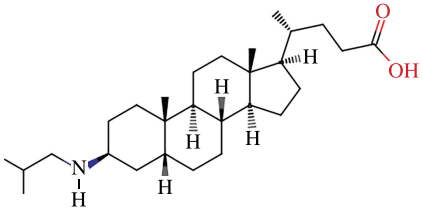

2

First, liposomes from zwitterionic electroneutral dioleoylphosphocholine (DOPC) were modified by AMS **1** (for details, see Online Supplementary Materials), with its molar content in the liposome membrane being varied from 0.03 to 0.15. The size of the liposomes fluctuated from sample to sample but always remained within a range of 40 ± 10 nm. The AMS **1**–DOPC liposomes of all the compositions demonstrated aggregative stability at physiological salt concentration. Thus, in a buffer with 0.15 M NaCl the size of the liposomes remained unchanged for at least 14 h after preparation. It was also estimated that the AMS **1**–DOPC liposomes were stable at different temperatures and pH values, since upon the temperature and pH variations the particle size did not change (for details, see Online Supplementary Materials).

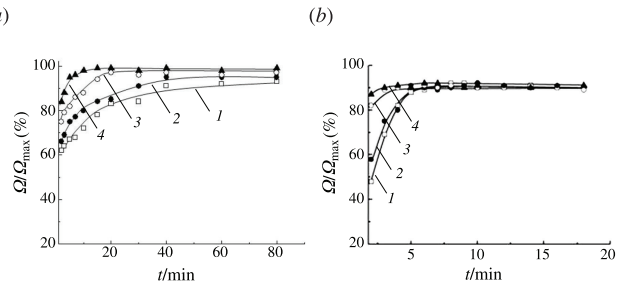

Figure 1 Time-dependent changes in conductivity of the NaCl-loaded (*a*) AMS **1**–DOPC and (*b*) AMS **2**–DOPC liposomes after an outer solution was acidified to 6.0. Molar content of compound **1** was (*1*) 0.03, (*2*) 0.04, (*3*) 0.075 and (*4*) 0.15; the content of compound **2** was (*1*) 0.03, (*2*) 0.06, (*3*) 0.1 and (*4*) 0.12. The total DOPC+AMS concentration was 1 mg ml^{-1}.

The liposomes were loaded with an aqueous solution of NaCl as a model for a water-soluble drug, following the procedure described.[22,23] A leakage of the salt from liposomes to a surrounding solution was monitored *via* measurement of the suspension conductivity. The maximum increase in the conductivity due to irreversible liposome destruction was achieved after addition of a 10-fold excess of Triton X-100 as a surfactant.[24] Since the pH values measured for pathological areas of living organs lie in the range 5.9–6.9,[8–10] the NaCl-loaded liposomes were prepared at pH 8.5 and then the pH value was reduced to 6.0. pH-Induced increase in the relative conductivity of the liposome suspensions with different molar content of compound **1** is demonstrated in Figure 1(*a*).

An increase in the molar content value for AMS **1** resulted in acceleration of the NaCl leakage, amount of NaCl released within the first minute as a minimal measurement period increased from 63 to 84% with rising of the content from 0.04 to 0.15, while a maximum release was achieved after 40, 20 and 10 min for the molar content values of 0.04, 0.075 and 0.15, respectively. Therefore, incorporation of additional compound **1** into the liposomal membrane favored the releasing ability of the AMS **1**–DOPC liposomes.

With these encouraging results, the cytotoxicity of the AMS **1**–DOPC liposomes to human breast adenocarcinoma MCF-7/R cells was evaluated using a conventional methyl-tetrazolium blue assay[24] (for details, see Online Supplementary Materials). The plot of cell viability *vs*. liposome concentration [Figure 2(*a*)] reflects low toxicity of the control DOPC liposomes [Figure 2(*a*), curve *1*] as well as the liposomes with 0.03 molar part of compound **1** [Figure 2(*a*), curve *2*], both curves lie above the 50% viability (LC_{50}), which is a quantitative measure of cytotoxicity. However, an increase in the AMS **1** molar ratio above 0.03 enhanced the liposome cytotoxicity [Figure 2(*a*), curves *3*–*5*], which represented a serious restriction for the use of liposomes with compound **1** in the drug encapsulation or delivery

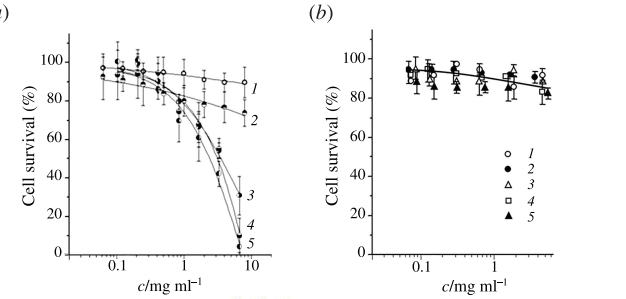

Figure 2 Viability of MCF-7/R cells *vs*. (*a*) the AMS **1**–DOPC and (*b*) AMS **2**–DOPC liposomes concentration. Molar content of compound **1** was (*1*) 0 (control), (*2*) 0.03, (*3*) 0.04, (*4*) 0.075 and (*5*) 0.15; the content of compound **2** was (*1*) 0 (control), (*2*) 0.03, (*3*) 0.06, (*4*) 0.1 and (*5*) 0.12.

Table 1 EPM and ζ-potential of the AMS **2**–DOPC liposomes with 0.03 molar part of compound **2** *vs*. pH value of surrounding solution.

pH	EPM/cm μm s^{-1} V^{-1}	ζ-Potential/mV
8.0	–0.93 ± 0.12	–12 ± 2
7.5	–0.47 ± 0.11	–6 ± 1
6.4	0.30 ± 0.11	4 ± 1
6.0	0.84 ± 0.13	10 ± 1
5.0	2.63 ± 0.02	33 ± 2

and initiated the consideration of another AMS **2** with carboxylic as well as secondary amino groups.

First, it was necessary to make sure that compound **2** embedded in the DOPC membrane responded to a change in pH; therefore, the AMS **2**–DOPC liposomes with a molar content of compound **2** equal to 0.03 were prepared in a buffer solution at pH 8.0 and then transferred to buffer solutions with pH values from 7.5 to 5.0. Table 1 demonstrates how the electrophoretic mobility (EPM) and ζ-potential, as parameters associated with the liposome surface charge calculated using the Smoluchowski electrokinetic model, for the mixed AMS **2**–DOPC liposomes changed upon acidification of the outer solution. When the solution was progressively acidified, the mixed liposomes lost their negative charge and then became positively charged. These changes resulted from rotation of the AMS molecules in the liposomal membrane, which developed in response to alteration of the outer solution pH.

Next, the kinetics of pH-induced NaCl release from the AMS **2**–DOPC liposomes was considered. As follows from the data of Figure 1(*b*), a switch of pH from 8.0 to a lower value was accompanied by elevation of the NaCl amount released from the liposomes within the first minute. This 'primary emission' increased from 45 to 87% of the maximum value when the molar content of compound **2** in the membrane was altered from 0.03 to 0.12, while the maximum salt release was achieved 5 min after the pH switch for all the formulations with the AMS **2** molar content of 0.03–0.12.

Finally, the AMS **2**–DOPC liposomes were tested for their cytotoxicity to MCF-7/R cells (for details, see Online Supplementary Materials). All the formulations showed an effect comparable to that for the DOPC liposomes devoid of AMS **2** [Figure 2(*b*)].

In summary, the liposomes composed of conventional zwitterionic DOPC and AMS **2** with carboxylic and secondary amino groups demonstrate a negligible cytotoxicity at the compound **2** molar content up to 0.12. Decrease in pH of the outer solution to 6.0 induces a leakage of the NaCl solution from liposomes, while 87% of the salt flow out of the liposomes with a 0.12 molar ratio of AMS **2** within the first minute after the pH switch. The mixed AMS **2**–DOPC liposomes seem to be promising for the encapsulation and controlled delivery of drugs.

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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.2021.11.019.

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