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Charged additives modify drug release rates from lipidic cubic phase carriers by modulating electrostatic interactions☆

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ABSTRACT

Functionalized lipidic cubic phases (LCPs) have gained considerable interest as drug delivery systems (DDS) in recent years. Among their advantages, one of the most intriguing features that make them interesting as DDS is the possibility of controlling the release of drugs based on electrostatic interactions. Here modulation of the release of a positively charged drug, doxorubicin (DOX) is obtained by incorporation of small amounts of designed charged derivatives of the monoolein host lipid as additives to the otherwise non-charged LCP. By design, the charged hydrophilic head group of the additive is exposed to the aqueous channels, whereas the hydrophobic tail is incorporated in the lipid bilayer and forms an integral part thereof. Our results show that *in vitro* DOX release profiles can be modulated by the electrostatic interactions between the drug molecule and the charged LCP used. We demonstrate that monitoring shifts of the voltammetry peak potentials gives a unique opportunity of following even subtle changes of pH inside the aqueous channels of the cubic mesophase, while measurement of the peak current allows establishing the release profile of the drug from the dopant-modified mesophase. Small angle X-ray scattering (SAXS) data confirm that the behavior of DOX in the mesophase was not dependent on the LCP symmetry, as it remains unchanged, but rather on the charges delineating the water channel compartment.

1. Introduction

Novel drug delivery systems (DDS) based on the lipidic mesophases have attracted increasing interest in recent years. Ideally, the DDS should have a high drug loading capacity so as to minimize the quantity of matrix material needed, should be stable and biocompatible, and should enable a controlled release of the drug, thus achieving the correct dose. Specifically for anticancer drugs, encapsulation into the DDS is used to reduce side effects caused by the drug, which is made available only when released from the carrier.

Lipidic mesophases such as cubic and hexagonal phases are advantageous materials for drug delivery since they are stable in excess of water and can accommodate relatively large amount of drug due to the high surface area of ca. 400 m^2 g^{-1} [1]. The most well-studied mesophase system is based on monoacylglycerols, which form various phases with distinct material properties upon hydration, the most complex one being is the inverse bicontinuous lipidic cubic phase (LCP) [2]. This highly structured phase exhibits a combination of material properties that render it interesting for applications in various areas of science and technology, ranging from matrices for membrane protein crystallization [3] to emerging vehicles for drug delivery [1,4,5].

Molecularly, LCPs exhibit liquid crystalline structures composed of highly curved lipid bilayers that are surrounded by two identical, yet non-intersecting aqueous channels, which yield an inner structure with a very large interfacial surface and enable diffusion in both lipidic and aqueous compartments. Macroscopically, LCPs based on monoacylglycerols are highly viscous biomaterials that are stable in excess water and are malleable, transparent, biocompatible and non-toxic. Because of their internal structure, LCPs can incorporate a large amount of guest molecules of virtually any polarity or charge. These hydrophilic, amphiphilic or hydrophobic molecules can diffuse in the respective compartments or at their interface [6–8].

LCPs have therefore been extensively investigated as vehicles for the sustained release of bioactive molecules of various size and molecular weight. Location of the drug is an important parameter influencing the diffusion and the release rate [9,10–12]. Partitioning between the lipidic and aqueous compartments, which is related to the hydrophobicity of the drug, largely determines the release kinetics. Whereas hydrophobic drugs tend to partition into the bilayer, hydrophilic drugs reside preferentially in the aqueous channels. Additional factors that

☆ In memory of Roger Parsons.

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influence the diffusion of an incorporated drug from the LCP are the size and polarity of the molecules. Drug release from bicontinuous cubic phase is significantly faster than from the other mesophases [13–15].

A diverse range of compounds may be added to functionalize lipidic mesophases. Functionalization may change the curvature of lipid bilayer, which results in changing the structure or diameter of the aqueous channel. Modulating the size of the aqueous channel of the hosting cubic phase can be used to regulate its rate of transport [4,15–17]. Release of the incorporated guest molecules from LCP depends also on the composition and structure of the lipid bilayer and their interactions with guest molecule, and can be tuned by applying lipids with different acyl chains to change the charge delineating the aqueous channels [4,18–21]. Incorporation of cationic surfactants into the bicontinuous LCP was shown to slow down the release rate of negatively charged water-soluble ketoprofen due to the increasing attraction and loading of materials [22].

We have shown that judicious synthesis of additives and their incorporation within LCP, cubosome and hexagonal systems yields biomaterials with remarkable structural and functional properties [23–28]; these range from tailored LCPs containing oleolipid-nucleic acid conjugate guest molecules as an alternative semipermeable protocell model [24] to new stimuli-responsive nanomaterials that exhibit efficient pH- and light-induced binding, release and sequestration of hydrophilic dyes. Significantly, these processes can be performed sequentially, demonstrating temporal and dosage control [26].

Recently we have shown that location of the drug is an important parameter affecting the release rate [25,27]. Thus the pH-dependent behavior of the model anticancer drug DOX in three distinct LCPs composed of monoolein (MO), monolinolein (ML), and phytantriol (PT) was investigated, leading to their potential capacity to trigger release of the drug preferentially in the environment of the cancer cells. This system was found to be pH-dependent, which may have implications in triggering release in the vicinity of cancer cells.

In the present study we show that release of the positively charged drug DOX can be controlled by introduction of appropriate charged dopants into the LCP structure, thereby modulating electrostatic interactions. Negatively or positively charged LCP were obtained by incorporating small amounts of designed derivatives of monoolein. The charged hydrophilic head group of the dopant is exposed to the aqueous channels, whereas the hydrophobic tail is incorporated in the lipid bilayer. The resulting negatively charged lipid (doped with deprotonated AL) was expected to lower the release rate of an oppositely charged DOX, whereas introduction of a positively charged lipid (protonated CL) to enhance drug release due to the electrostatic repulsion.

2. Materials and methods

2.1. Chemicals and substrates

Monoolein (1-oleoyl-rac-glycerol) purity ≥ 99% (MO), 2-(*N*-morpholino) ethanesulfonic acid (MES), and doxorubicin hydrochloride (DOX) were purchased from Sigma. The lipid additives *N*-Oleoyl-glycine (AL) and *N*-(2-aminoethyl) oleamide (CL) were synthesized according to the recently published procedure [23]. All solutions were prepared with Milli-Q water (18.2 MΩ cm^{-1}), Millipore, Bedford, MA, USA. To prepare MES buffer 0.1 M 2-(*N*-morpholino) ethanesulfonic acid or was titrated with 0.1 M NaOH to obtain pH 5.5.

2.2. LCP preparation

Non doped LCP was obtained by mixing melted monoolein (MO) with buffer solution. The amount of water added was chosen on the basis of the phase diagram [29]. To prepare the doped LCP, MO and the respective lipid additive were first dissolved in chloroform. Chloroform was then evaporated under reduced pressure and left overnight to remove traces of chloroform before hydration. The final compositions of LCPs were 62.1/37.9 and 60.9/1.2/37.9 wt% for the MO/MES buffer or and MO/lipid additive/MES buffer, respectively. To prepare DOX-loaded LCP, DOX dissolved in MES buffer was initially added to the lipid mixture and further mixed with buffer. The final compositions of LCPs doped with DOX were 62.1/0.6/37.3 or 60.9/1.2/0.6/37.3 wt% for MO/DOX/MES buffer or MO/lipid additive/DOX/MES buffer, respectively, to obtain the same amount of total lipid in all LCP formulations. Cubic phases were prepared by mixing in a desktop centrifuge for 30 min, and were left in dark at 25 °C for few days to equilibrate.

2.3. Small angle X-ray scattering (SAXS)

SAXS was used to determine the type of cubic phase and its structural parameters using a Bruker Nanostar system working with CuKα radiation, equipped with Vantec 2000 area detector. Samples were loaded into 1.5 mm capillaries and left to equilibrate for 12 h. Before measurement samples were equilibrated for 20 min at 25 °C. Data were then collected for 30 min.

The crystallographic lattice parameter (a) was obtained by fitting the X-ray pattern to the structure with Pn3m symmetry. To determine the aqueous channel radius, the water volume fraction was estimated using the equation [30]:

$$\phi_{water} = \frac{C_W}{C_W + (1 - C_W)\frac{\rho_{water}}{\rho_{lipid}}} \quad (1)$$

where φ_{water} - water volume fraction, C_w - water weight fraction, ρ_{water} - density of water (ρ_{water} = 0.997 g ml^{-1}), ρ_{lipid} - density of lipid (ρ_{MO} = 0.942 g ml^{-1}).

Next, the lipid length was obtained by solving Eq. (2):

$$\phi_{lipid} = 2A_0\left(\frac{l}{a}\right) + \frac{4}{3}\pi\chi\left(\frac{l}{a}\right)^3 \quad (2)$$

where: φ_{lipid} is lipid volume fraction ($\varphi_{lipid} = 1 - \varphi_{water}$), l - lipid chain length/monolayer thickness, a – is lattice parameter of corresponding phase, A_0 and χ are the ratio of the area of the minimal surface in a unit cell to (unit cell volume)$^{2/3}$ and the Euler–Poincaré characteristic, respectively (for *Pn3m*: A_0 = 1.919 and $\chi = -2$). The aqueous channel radius was obtained as

$$r_w = \left(-\frac{A_0}{2\pi\chi}\right)^{1/2} a - l \quad (3)$$

These equations were also used to determine parameters of doped with charged lipids and solubilized the drug LCPs.

2.4. Electrochemistry

Electrochemical measurements were recorded using a CHI bipotentiostat with a standard three-electrode arrangement in buffered solution. Ag/AgCl was used as the reference electrode and a platinum foil as the counter electrode. The working electrode was glassy carbon electrode (GCE) modified with the LCP film. Before the experiments, the working electrode was polished on alumina of decreasing size (from 0.3 to 0.05 μm) on a polishing cloth. The electrodes were subsequently sonicated to remove adhered alumina particles, rinsed with ethanol and water and left to dry. The surface area of GCE was 7.01 mm^2. The entire setup was placed in a Faraday cage to minimize external disturbances. Cyclic voltammetry was used to study the influence of lipid additive on the DOX electrode reaction. Differential pulse voltammetry was used to determine the DOX release from the matrix. For each type of LCP formulation triplicate experiments were done.