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Thermally Gated Liposomes

Wen-Hua Chen and Steven L. Regen*

Department of Chemistry, Lehigh University, Bethlehem, Pennsylvania 18015

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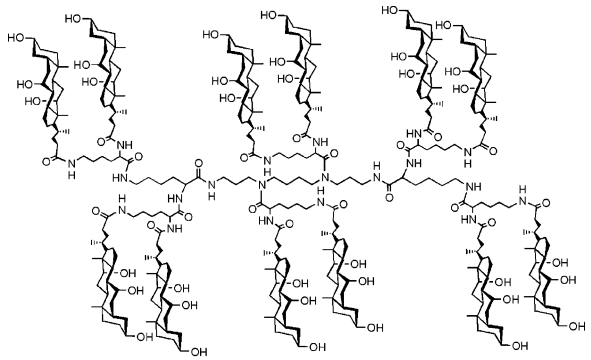
This paper describes a new approach to the temperature-controlled release of liposome-encapsulated agents. Specifically, we report the combined use of a pore-forming amphiphile (**1**) and thermally sensitive liposomes made from 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) to produce what we term "thermally gated liposomes" (Chart 1). At temperatures that lie above the gel to liquid−crystalline phase transition temperature of this lipid (i.e., 41 °C), **1** creates pores within the membrane through which entrapped aqueous solutes (e.g., carboxyfluorescein, CF) can readily pass. Below this temperature, efflux rates are greatly reduced. The potential of thermally gated liposomes (TGLs) as devices for the targeted delivery of therapeutic agents is briefly discussed.

The use of temperature-sensitive liposomes for the targeted delivery of drugs is well-established.[1] Recently, there has been a resurgence of interest in this technique, especially in the area of cancer chemotherapy.[2−4] Classically, temperature-sensitive liposomes take advantage of increased bilayer permeability that accompanies the conversion from the gel to the liquid−crystalline phase.[5] Finding ways to increase release rates (i.e., to produce "burst" kinetics) is a major objective that bears, directly, on the utility of this approach, for example, delivering anticancer drugs, selectively, to solid tumor tissue.[4,6]

In previous studies, we have shown that DPPC can "squeeze out" a sterol-based ion conductor when the bilayer is converted from the fluid to the gel phase.[7] On the basis of this observation, it occurred to us that certain pore-forming amphiphiles might behave similarly, and that one might be able to exploit such behavior from a drug delivery standpoint. Specifically, we reasoned that facially amphiphilic, pore-forming conjugates would allow the release of entrapped solutes from fluid phase liposomes, but when in the gel state, the "gates" would be closed; that is, strong hydrophobic interactions between neighboring DPPC molecules would drive these conjugates either partially or completely out of the bilayer, thereby sealing the membrane.

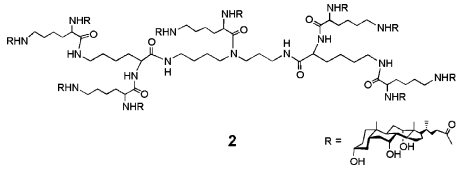
With this rationale in mind, we sought a conjugate that (i) exhibited high pore-forming activity, (ii) was derived from naturally occurring materials (to increase the likelihood of being biodegradable), and (iii) could be readily prepared. On the basis of these criteria, we chose conjugates **1** and **2** as synthetic targets (Chart 2). Previous studies have shown that analogous conjugates made from cholic acid, lysine, spermidine, and spermine have Na^+ transport activities that increase *exponentially* on going from 4 to 6 to 8 choloyl groups per conjugate.[8] If this trend were to continue with **1** and **2**, then even higher activities would be possible. Additionally, the synthesis of **1** and **2** seemed reasonably straightforward. Thus, acylation of the terminal amino groups of spermine with $N_\alpha N_\epsilon$-di-Boc-L-lysine hydroxylsuccinimide ester, followed by deprotection with trifluoroacetic acid and complete acylation with lysine−dicholamide was expected to yield **1** (Scheme 1). An analogous scheme, based on the use of spermidine as the polyamine backbone, was expected to afford **2**. Using procedures that are described in the Supporting Information section, both conjugates were readily prepared by such a strategy.

Chart 1



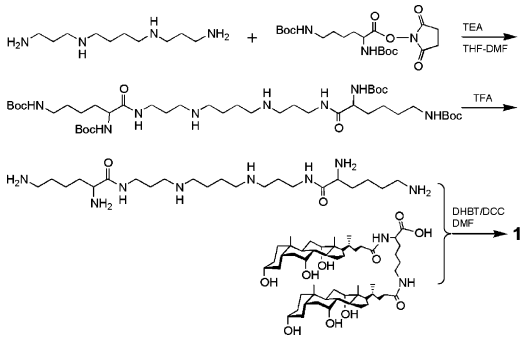
1

Chart 2



2

Scheme 1



The ion transport properties of **1** and **2** were then characterized by measuring their ability to promote Na^+ transport across POPC liposomes (200 nm, extrusion) using $^{23}Na^+$ NMR methods similar to those previously described.[8] Plots made of the pseudo-first-order rate constants, k_{obsd}, for Na^+ transport versus (mol % of conjugate)^2 for **1** and **2** were found to be linear, indicating that *transport-active dimers* are involved (Figure 1). Here, it is assumed that only a small fraction of the conjugate is in the dimer form, where it can be shown that $k_{obsd} = k_2[\text{monomer}]^2/K$, where K is the equilibrium constant for dissociation of the dimer, k_2 is the rate constant for ion transport, and [monomer] is the analytical concentration of the conjugate that is present in the dispersion.[7] A plot of ion transport activity (expressed as k_2/K) versus the number of choloyl groups per conjugate for **1** and **2**, along with data previously obtained for

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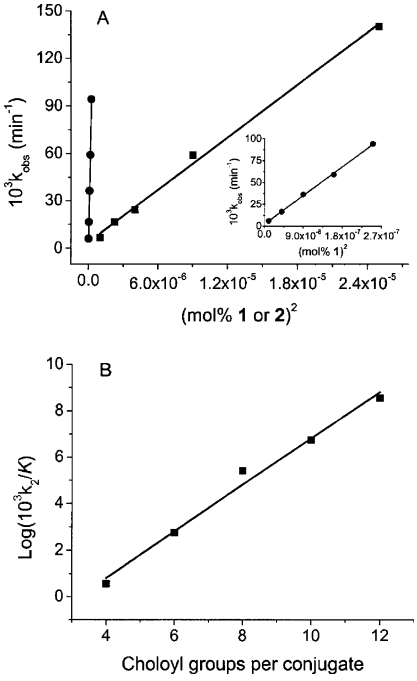

Figure 1. (A) Plot of k_{obsd} versus (mol % conjugate)^2 in POPC liposomes at 35 °C for **1** (●) and **2** (■). (B) Semilogarithmic plot of k_2/K versus the number of choloyl groups per conjugate.[8]

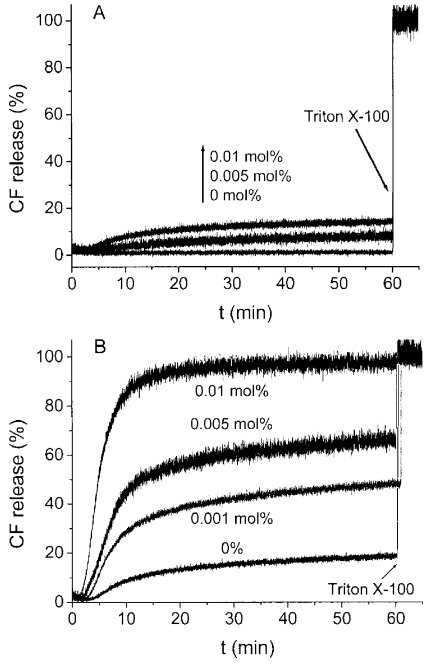

Figure 2. Plot of percentage of CF release from liposomes made from DPPC containing 0, 0.001, 0.005, and 0.01 mol % of **1** as a function of time at (A) 37 °C and (B) 43 °C.

analogues bearing 4, 6, and 8 choloyl groups, shows that this exponential relationship extends from 4 to 12 sterol units (Figure 1).[8]

To test the feasibility of thermal gating, liposomes (200 nm, extrusion) were prepared from DPPC plus varying mole percentages of the more active conjugate (**1**) at 60 °C, using an aqueous solution that was 50 mM in CF and 10 mM in HEPES buffer (pH 7.4). After cooling the dispersion to room temperature, the nonentrapped CF was removed via gel filtration. Addition of 200 μL of this dispersion to 1.8 mL of a 10 mM HEPES buffer (pH 7.4), which was maintained at either 37 or 43 °C, produced efflux profiles that are shown in Figure 2. Such measurements are based on the fact that the entrapped CF exhibits negligible fluorescence due to efficient self-quenching, and that the released CF is fluorescent due to its dilution in the bulk aqueous phase. The 100% release value in each case was obtained by destroying the liposomes with Triton X-100. From this figure, it is clear that the presence of **1** results in greatly enhanced release of CF at 43 °C, and that this release is dependent on the mol % of **1** that has been included in the membrane.[9] The fact that **1** also enhances CF release at 37 °C, albeit to a much lesser extent, implies that **1** is, at least, partially retained within DPPC bilayers at this reduced temperature.

The present findings clearly demonstrate that **1** provides an effective means for amplifying the release of CF from thermally sensitive liposomes. They strongly suggest, therefore, that thermally gated liposomes may be exploitable from a drug delivery standpoint.

Studies that are currently in progress are aimed at designing thermally gated liposomes that are completely impermeable toward CF at 37 °C and at characterizing pore sizes and lifetimes in the fluid phase.[10] The results of these studies will be reported in due course.

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Supporting Information Available: Procedures for synthesizing **1** and **2**, measuring Na^+ transport and CF release; CF release profiles using liposomes made from DPPC/DSPC (9/1, w/w) plus **1** and from DPPC plus **2** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(9) The less than 100% CF release that is apparent, when lower concentrations of **1** are used, is a likely consequence of a nonuniform distribution of **1** among the liposomes in the dispersion.
(10) Liposomes made from DPPC/1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC) (9/1, w/w) release ca. 6% of entrapped CF at 37 °C in the presence of 0.01 mol % of **1** after 60 min. This is significantly less than the 15% release that is observed under the same conditions for liposomes made from 100% DPPC. When 0.01 mol % of **2** is used in DPPC liposomes, 5.0% of the entrapped CF is released after 60 min at 37 °C. In the absence of any conjugate, ca. 1% of CF is released (see Supporting Information for details).

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