Photoinduced Destabilization of Sterically Stabilized Liposomes

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The encapsulation characteristics and biocompatibility of liposomes recommend them as carriers for therapeutic agents. A considerable research effort has been devoted to the development of liposomes for the delivery and buffering of drugs in the body. The delivery of liposomes to desired sites depends in part on long circulation times in the body, which can only be accomplished by reducing the uptake of liposomes by the mononuclear phagocytic system (MPS). In recent years several means have been described to sterically stabilize liposomes to increase their period of circulation.[1,2] A frequently used method is the incorporation of a poly(ethylene glycol)-conjugated phospatidylethanolamine (PEG-PE) into the liposome. In addition to requiring extended circulation times, the successful delivery of liposomes to tumor sites requires the liposomes to enter the interstitium. Several research groups have reported the increased localization of sterically stabilized liposomes (PEG-liposomes) at tumor sites.[3−7] The increased permeability of the vasculature at tumor sites allows PEG-liposomes to escape the capillaries to reach the tumor interstitial space. However, once the PEG-liposomes are at the tumor site the PEG groups can interfere with rapid release of the encapsulated reagents. Consequently, it continues to be important to find methods for the triggered release of reagents from PEG-liposomes.

The photoinduced destabilization of liposomes offers an attractive means to couple the spatial and temporal control of radiation to reagent delivery. Liposomes may be made photosensitive by the use of uniquely designed lipids that can alter the liposome properties via photoisomerization, photocleavage, or photopolymerization.[8,9] A particularly useful characteristic of the latter is the multiplicative nature of the polymerization process. Moreover, bis-substituted lipids, e.g., bis-SorbPC, react to form cross-linked polymer networks that significantly alter bilayer properties.[10] Both the Ringsdorf and O'Brien groups found that cross-linking polymerization of bis-substituted lipids in the presence of unreactive lipids causes the formation of lipid domains.[11] Here we use this same phenomenum to destabilize PEG-substituted liposomes.

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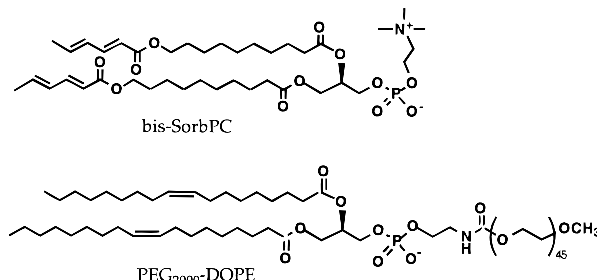
Photosensitive PEG-substituted LUV (100 nm diameter, large unilamellar liposomes) composed of PEG_{2000}-dioleoylPE, cholesterol, dioleoylPC, and bis-SorbPC (molar ratio: 15/40/15/30) were prepared by conventional extrusion procedures in the presence of buffer containing the water-soluble fluorescent marker, 25 mM 8-aminonaphthalene-1,3,6-trisulfonic acid (ANTS), and its collisional quencher, 90 mM *p*-xylene-bis-pyridinium bromide (DPX).[12] The resulting ANTS/DPX containing PEG-LUV were



eluted through a Sephadex G75 column with an isoosmotic pH 7, sodium phosphate, sodium chloride buffer. The extent of unencapsulated ANTS/DPX was determined from the sample fluorescence for 60 s prior to photolysis. Immediately after sample photolysis at 37 °C the loss of monomeric bis-SorbPC was determined by UV absorbance with a diode array spectrophotometer, and the sample fluorescence was measured continuously over several minutes to attain equilibrium. Finally the sample was lyzed with an aliquot of Triton X-100 solution (5% v/v) to determine the 100% leakage after correcting for the bleaching of ANTS during photolysis and the dilution factor due to the Triton X-100. The percent ANTS bleaching was determined by comparing the fluorescence measurement after Triton X-100 treatment of a photolyzed sample to a similar measurement performed on a sample of nonphotolyzed liposomes that was of the same total lipid concentration.

The percent leakage at time t is given by the following expression:

$$\% \text{ leakage} = (I_t - bI_0)/(1.16\, I_{100} - bI_0) \quad (1)$$

where I_t is the fluorescence intensity at time t, I_0 is the fluorescence intensity prior to photolysis, I_{100} is the fluorescence intensity after addition of Triton X-100, and b is the bleaching factor. Because the initial change in concentration inside the liposomes is relatively small, the initial leakage is pseudo-zero-order, and the plot is linear. The rate of leakage reported in Figure 1 was calculated from this linear region by using a least-squares fit. The fluorescence of a solution of ANTS-DPX is linear over the concentration range used in this experiment. Therefore, it was possible to determine both the amount of ANTS originally encapsulated, and the amount that leaked at any time directly from the equation.

The data in Figure 1 show both the extent of photoconversion of bis-SorbPC and the log of the rate of release of ANTS from the PEG-LUV at 37 °C as a function of the sample irradiation time. In this experiment a 2 min exposure caused a 90% loss of monomer and a nearly 50-fold increase in the rate of ANTS release from the liposomes. A longer photolysis of the PEG-LUV sample increased the rate of ANTS release from the photolyzed liposomes to greater than 220 times that of an unexposed sample. The photolysis of liposomes having similar compositions showed a similar dependence of liposome leakage on the exposure of the sample. However, if the PEG-LUV did not contain bis-SorbPC,

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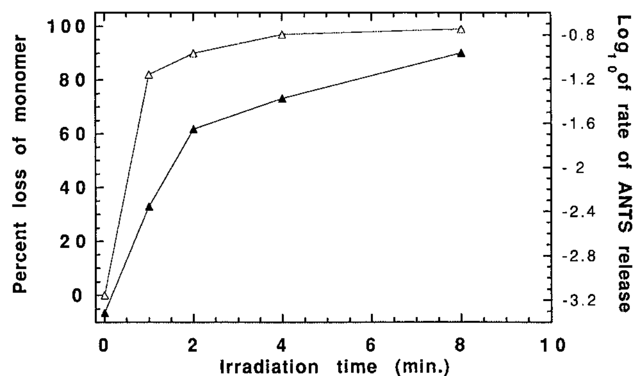

Figure 1. Effect of the photolysis of PEG-liposomes (pH 7 buffer) composed of PEG_{2000}-dioleoylPE, cholesterol, dioleoylPC, and bis-SorbPC (molar ratio: 15/40/15/30). Both the percent loss of monomeric bis-SorbPC (△, left axis) and the log of the percent ANTS released per sec from the liposomes (▲, right axis) are shown as a function of the sample exposure time at 37 °C.

they were not photosensitive. Moreover, if they did not contain a sufficient mole fraction of cholesterol, the rate of ANTS leakage prior to photolysis was too great to effectively determine the effect of sample photolysis.

Cross-linking polymerizations of liposomes in water composed of neutral polymerizable PCs and the anionic dioleolyl phosphatidic acid (DOPA) reduced the average distance of separation of the DOPA, as shown by the increased efficiency of electronic energy transfer between a pair of cationic dyes that were electrostatically associated with the liposomes after the polymerization was completed.[13] Using an analysis of fluorescence energy transfer at bilayer surfaces,[14] this change in energy transfer efficiency indicates that the average distance of separation of charged lipids was reduced by nearly an order of magnitude by the polymerization. Therefore, the lipid cross-linking served to substantially concentrate charged lipids in hydrated bilayer membranes. The work accomplished by the polymerization in a charged lipid system compensated for the potential energy due to repulsion of the lipids which is proportional to $(1/d)$, where d is the average distance of separation.

The steric repulsion of tethered polymer chains on a surface was treated by Alexander as a competition of the interaction energy per chain vs the elastic free energy.[15] At high densities of tethered chains the interaction energy is high and the chains extend to yield a greater layer thickness, L. The equilibrium state of the tethered chains may be obtained by minimizing the total free energy, F, relative to L, giving a dependence of $(1/d)^{5/3}$. When d is small, the polymer chains are in an extended "brush" conformation, whereas when d is large, the polymer chains may assume a tethered random coil, i.e., "mushroom" conformation. The distance dependence in the above expression suggests that bilayer polymerization could increase the local concentration of PEG-lipids by a factor of 3 to 5. At high PEG-lipid concentrations polymer-grafted micelles are formed in equilibrium with the bilayer.[16] Kenworthy et al. found that in mixtures of less than 82 mol % distearoylPC and more than 18 mol % PEG_{2000}-distearoylPE the hydrated lipids exist in a mixed micellar−lamellar phase, which became more micellar as the proportion of PEG-lipid increased.[17]

These observations suggest that polymerization of bis-SorbPC results in an increased local concentration of the PEG-lipid accompanied by the possible loss of PEG-lipid from the liposomes to mixed micelles. This process may cause increased bilayer membrane permeability either by increased disorder in the bilayer at the interface between PEG-lipids and other lipids or at the interface between poly(lipid) and other lipids. The borders of these domains may be disordered in a manner similar to the domain edges in a bilayer of pure lipid at the main phase transition temperature, which also results in an ca. 10^2 increase in bilayer permeability.[18]

Although the underlying mechanism of the photoinduced increase in PEG-LUV permeability remains to be clarified, it is clear that properly designed sterically stabilized liposomes can be destabilized via the photopolymerization of reactive lipids. Since we have already shown that bilayer polymerizations can be sensitized to green or red light,[19] it is reasonable to expect that selectively applied long wavelength light could be used to increase the rate of therapeutic agent release from PEG-LUV sequestered at tumor sites. Both the mechanism and application of the photodestabilization of PEG-LUV are being actively investigated and will be described in due course.

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