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Visible light-induced destabilization of endocytosed liposomes

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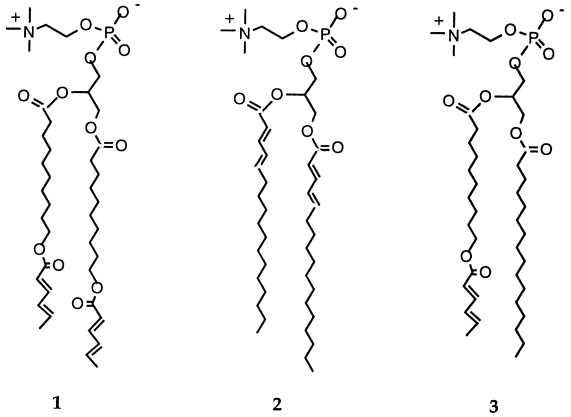
Abstract The potential biomedical utility of the photoinduced destabilization of liposomes depends in part on the use of green to near infrared light with its inherent therapeutic advantages. The polymerization of bilayers can be sensitized to green light by associating selected amphiphilic cyanine dyes, i.e. the cationic 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine (DiI), or the corresponding anionic disulfonated DiI (DiI-DS), with the lipid bilayer. The DiI sensitization of the polymerization of 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine/1,2-bis[10-(2′,4′-hexadienoyloxy)-decanoyl]-*sn*-glycero-3-phosphocholine liposomes caused liposome destabilization with release of encapsulated aqueous markers. In separate experiments, similar photosensitive liposomes were endocytosed by cultured HeLa cells. Exposure of the cells and liposomes to 550 nm light caused a net movement of the liposome-encapsulated 8-hydroxypyrene-1,3,6-trisulfonic acid (HPTS) from low pH compartment(s) to higher pH compartment(s). This suggests that photolysis of DiI-labelled liposomes results in delivery of the contents of the endocytosed liposomes to the cytoplasm. The release of HPTS into the cytoplasm appears to require the photoactivated fusion of the labelled liposomes with the endosomal membrane. These studies aid in the design of visible light sensitive liposomes for the delivery of liposome-encapsulated reagents to the cytoplasm.

Key words: Liposome; Photoinduced destabilization; Membrane fusion

1. Introduction

A variety of methods to modulate the phase behavior of hydrated lipid membranes and cause their destabilization rely on the polymorphism of lipids, such as phosphoethanolamines (PE). These methods include the addition of ions, protons, peptides, and more recently *light*. Several pH sensitive lipid systems have been proposed for the delivery of encapsulated molecules into cells [1–4]. Each system illustrates the development of new fusion protocols based on the evolving understanding of the nature of membrane reorganization. Interest in these methods is generated by the desire to introduce a variety of encapsulated hydrophilic molecules into cells, either in culture or in vivo. The spatial and temporal characteristics of light could be used to increased the specificity of reagent delivery to cells, if liposomes could be rendered sufficiently sensitive to light of suitable wavelengths [5]. Several methods to perturb lipid bilayers by the use of light have been reported and reviewed [6]. These methods include UV light-activated photoisomerizations of lipids to achieve moderate changes in bilayer membrane permeability. More substantial reorganization of phospholipid bilayers with accompanying loss of encapsulated aqueous contents has been demonstrated by the photoinduced cleavage of plasmalogen lipids [7,8], by the photogeneration of acid to initiate polyelectrolyte lysis of liposomes [9], and by the photopolymerization of appropriately designed liposomes [10–12]. In the first two cases, the lipid bilayers appear to be destabilized by the progressive transformation to mixed micelles of lipid and lysolipid [7,8], or lipid and polymeric surfactant [13]. The photoinduced enhancement of both liposome fusion and leakage was demonstrated by the photo-crosslinking of mixed lipid bilayers to form lipid domains enriched in lipids such as PE [12,14].

The linear chain polymerization of reactive lipids in a liposome composed of several thousand lipid molecules creates a structure that contains several poly(lipid) chains [15,16]. Lipids, such as phosphocholines (PC), that contain a reactive group in each hydrophobic tail, e.g. 1,2-bis[10-(2′,4′-hexadienoyloxy)-decanoyl]-*sn*-glycero-3-phosphocholine (bis-SorbPC) (**1**) and 1,2-bis-[2,4-octadecadienoyl]-*sn*-glycero-3-phosphocholine (**2**), react to form crosslinked polymer networks within a bilayer, whereas mono-substituted lipids, e.g. lipid **3**, yield linear polymers [17]. A host of reactive moieties has been successfully utilized to confer lipid photoreactivity, including dienoyl, sorbyl and styryl, which are maximally sensitive to short wavelength UV light [16].



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The potential biomedical utility of the photoinduced destabilization of lipid bilayers depends in part on the use of green

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Abbreviations: ANTS, 8-aminonaphthalene-1,3,6-trisulfonic acid; bis-SorbPC, 1,2-bis[10-(2′,4′-hexadienoyloxy)-decanoyl]-*sn*-glycero-3-phosphocholine; DiI, 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate; DiI-DS, 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine-5,5′-disulfonic acid; DOPE, 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine; DPX, *p*-xylene-bis-pyridinium bromide; HPTS, 8-hydroxypyrene-1,3,6-trisulfonic acid; LUV, large unilamellar liposome; MEM, modified Eagle's medium; PC, *sn*-glycero-3-phosphocholine; PE, *sn*-glycero-3-phosphoethanolamine

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to near infrared light with its inherent therapeutic advantages. We have demonstrated that the polymerization of liposomes composed of lipids such as **1**–**3** can be sensitized to green or red light by associating selected cyanine dyes with the lipid bilayer [18]. This sensitization chemistry offers the potential to use visible light for the release of reagents from liposomes or for the enhancement of liposome fusion. Here, we describe the use of ballasted cyanine dyes, i.e. the cationic 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (DiI), and the corresponding anionic disulfonated DiI (DiI-DS), to sensitize the visible light destabilization of liposomes.

2. Materials and methods

2.1. General

Polymerizable lipids were synthesized as described previously [9]. The other lipids were purchased from Avanti Polar Lipids. 8-Aminonaphthalene-1,3,6-trisulfonic acid (ANTS), 8-hydroxypyrene-1,3,6-trisulfonic acid (HPTS) and *p*-xylene-bis-pyridinium bromide (DPX) were obtained from Molecular Probes. The osmolarity of each buffer was determined with an Osmette S Osmometer. Phospholipid concentrations were determined colorimetrically by the method of Stewart [19].

2.2. Liposome preparation

Large unilamellar liposomes (LUV, ca. 100 nm diameter) were prepared by freeze/thaw and extrusion [20]. Liposome size distributions were measured using dynamic light scattering (Brookhaven BI-8000AT correlator with a 5 mW He-Ne polarized laser source). LUV were examined at a total lipid concentration of 100 μM at angles of 60, 90 and 120°. Two fitting methods, non-negative least squares and CONTIN, were used to extract the set of exponential functions that made up the autocorrelation functions [21].

When DiI was included in the LUV membrane, it was added from a methanolic stock to preformed LUV since the cationic DiI is partially removed by anionic Nuclepore membranes. The volume of methanol was less than 1% of the volume of buffer to prevent disruption of LUV. If DiI-DS was included in the liposomal membrane, it could be added from a methanolic stock to the lipid mixture prior to solvent evaporation and freeze/thaw and extrusion.

2.3. Photosensitized destabilization of liposomes

Two populations of LUV were prepared. A carrier population encapsulating ANTS and DPX was prepared by hydration of 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE)/**1** (molar ratio 3:1) with 12.5 mM ANTS, 45 mM DPX, 31 mM NaCl, 10 mM glycine, followed by freeze/thaw and extrusion. The encapsulated ANTS/DPX was separated from unencapsulated compounds on Sephadex G-75 gel-filtration columns (1.6×20 cm) with 115 mM NaCl, 10 mM glycine buffer, pH 9, as eluent. The two buffers were isoosmotic at 230 mOs. An 'empty' LUV population composed of DOPE:**1**:DiI (molar ratio, 15:5:1) was hydrated with 115 mM NaCl, 10 mM glycine buffer, pH 9, then extruded as before. The dye addition to LUV is described above.

The empty DiI-labelled LUV were irradiated in a cuvette with green light produced by the filtered (>470 nm) output of a 200 W Hg(Xe) arc lamp. The extent of photoconversion of lipid **1** to poly-**1** in the LUV was monitored by the decrease in absorbance of **1** as described previously [18]. After the desired degree of conversion was attained, the photolyzed LUV were combined with the carrier unphotolyzed LUV in a ratio of 9:1 empty (photolyzed) to carrier (unphotolyzed). The sample was placed in a fluorimeter to monitor the emission of ANTS and the sample pH was decreased (by addition of an aliquot of acid) to facilitate LUV interactions. The change in ANTS emission was used to calculate the percent ANTS leakage using the protocol described by Bennett and O'Brien [12].

2.4. Cell-liposome incubations

HeLa cells, purchased from the American Type Culture Collection, were maintained in monolayer in modified Eagle's medium (MEM), supplemented with 5% fetal bovine serum. Cells were routinely seeded at 2×10^6 cells in 75 cm^2 flasks (Costar) at 37°C and 5% CO_2 and passaged every 2 days. The cells that were used in the fluorescence spectroscopy experiments were seeded at 5×10^5 cells and grown in 25 cm^2 cell culture flasks (Costar) for 2 days. Cells were incubated at 37°C and 5% CO_2 in MEM media supplemented with 5% fetal bovine serum. Cells were counted with a Coulter Counter. Media were removed from the cells and the cells were washed three times in phosphate-buffered saline (PBS)-CMG buffer (137 mM NaCl, 2.7 mM KCl, 1.5 mM KH_2PO_4, 8.1 mM Na_2HPO_4, 0.4 mM $CaCl_2$, 0.4 mM $MgCl_2$, 5 mM glucose, pH 7.4).

LUV were prepared from dried lipid films that were hydrated in HPTS buffer (35 mM HPTS, 10 mM glycine, 45 mM NaCl, pH 9.5) to a concentration of about 13 mM before freeze/thaw and extrusion. Encapsulated material was separated from unencapsulated material on Sephadex G-75 gel-filtration columns (1.6×20 cm) with glycine buffer (141 mM NaCl, 10 mM glycine, 0.1 mM EDTA, pH 9.5) as eluent. LUV were diluted to 300 μM in PBS-CMG buffer and sterilized with Nalgene 0.2 μm pore size syringe filters.

The LUV were incubated with the adherent HeLa cells for 2 h, then the cells were washed with PBS-CMG buffer to remove excess LUV from the cells. Next, the adherent cells were dislodged for examination by fluorescence spectroscopy. The cells were dislodged by treatment with 1.5 ml PBS-EDTA buffer (137 mM NaCl, 2.7 mM KCl, 1.5 mM KH_2PO_4, 8.1 mM Na_2HPO_4, 10 mM EDTA, pH 7.4) for 10 min at 37°C. Cells were diluted with 1.5 ml PBS buffer (137 mM NaCl, 2.7 mM KCl, 1.5 mM KH_2PO_4, 8.1 mM Na_2HPO_4, pH 7.4) in a fluorescence cuvette. The fluorescence spectra were obtained with a SPEX FluoroLog 2 fluorometer. Fluorescence emission for binding and endocytosis measurements using HPTS (λ_{ex} 380–480 nm) was obtained at 510 nm with continuous stirring at 37°C. The excitation intensities at λ_{ex} 403, 413 and 450 nm were measured. The intensity at 413 nm, the isosbestic point, is pH-independent and serves as a measure of the total number of liposomes associated with the cells regardless of their location along the endocytotic pathway. The ratio of excitation intensities at 450 nm/413 nm was used to determine the pH sampled by the HPTS [23]. A low pH indicates that some of the HPTS is localized in a low pH compartment such as the endosome, whereas a pH of 7.4 shows that the HPTS is either outside the cell and/or in the cytoplasm.

2.5. Irradiation of cells incubated with liposomes

Irradiation of cells in the fluorimeter was performed after normal fluorescence spectroscopy. Following observation at slit widths of 2 mm for excitation and 4 mm for emission, the slits were opened to 8 mm for excitation and the sample was irradiated at 550 nm for 30 min with stirring at 37°C. Following irradiation, the sample emission and excitation spectra were redetermined using the original slit widths.

2.6. Microscopy of cells with liposomes

Adherent cells, that had been incubated for 4 h with DOPE/**1**/DiI-DS (molar ratio 16:4:1) liposomes encapsulating acridine homodimer (3 mg/ml), were washed three times with PBS-CMG buffer to remove excess liposomes. They were then viewed by epifluorescence with a Zeiss Axioplan Universal microscope. The acridine homodimer emission was observed using a 395–440 nm excitation filter, and the sensitizing dye emission was observed with a 510–560 nm excitation filter.

3. Results and discussion

Previous studies found that DOPE/**1** LUV could be photodestabilized by UV photolysis of **1** [10,12,14]. The photoactivated reaction of **1** yields crosslinked domains of poly-**1** and enriched domains of DOPE in lipid bilayers. Consequently, the photolyzed LUV are less stable, and conditions that favor bilayer contact between photolyzed LUV and other LUV promote liposome fusion as well as leakage of the aqueous contents [10,12]. The temperature for the onset of rapid liposome fusion, i.e. the critical fusion temperature, can be decreased by ca. 20° by UV exposure of DOPE/**1** LUV [12,14]. Here, we extend the previous studies to more practical liposome compositions that are rendered sensitive to longer wavelength light. The polymerization of reactive lipids in liposomes can

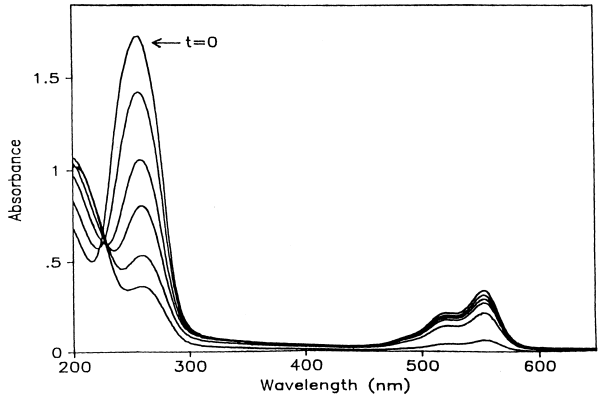

Fig. 1. Absorption spectra of a suspension of LUV composed of bis-SorbPC and DiI at various exposures (4 min intervals) to filtered light (>470 nm) from a Hg(Xe) high pressure lamp.

be made sensitive to visible light by the inclusion of a ballasted sensitizing dye in the lipid bilayer [18]. This chemistry can be employed with various buffers in the presence of oxygen, and at physiological temperatures. Thus, if LUV composed of bis-SorbPC (**1**) and DiI (molar ratio 20:1) are exposed to green light, which is only absorbed by the DiI, then the concentration of monomeric lipid decreases (Fig. 1). The loss of monomeric **1** is accompanied by the formation of poly-**1**.

LUV composed of DOPE/**1**/DiI (15:5:1) were prepared and then irradiated with green light. After 35% conversion of monomeric **1** to poly-**1**, these LUV were combined with a population of dark and undyed DOPE/**1** (3:1) LUV with an encapsulated fluorophore, ANTS, and its collisional quencher, DPX, at pH 9 and 37°C. There was no apparent loss of entrapped dye over several minutes. Repetition of the experiment at pH 7.4 or 6.3 showed only a small amount of ANTS leakage (Fig. 2). However, when an aliquot of the photolyzed LUV was combined with the dark LUV at pH 4.5 and 37°C, there was a rapid increase in fluorescence signifying the release and dilution of the ANTS and DPX upon mixing of the two populations of LUV. The photopolymerized LUV samples were stable in the presence of unpolymerized target LUV at or below room temperature and above pH 6. In the absence of the sensitizing dye (DiI) during the photolysis, ANTS release was not observed. In a separate control, the ANTS/DPX was encapsulated in the photolyzed DOPE/**1**/DiI LUV then combined with empty dark DOPE/**1** LUV. Again, similar results were observed, thereby showing that it is the destabilizing interaction of the two LUV populations that leads to release of the ANTS and DPX. The number of molecules leaked/s was calculated based on the number of ANTS molecules encapsulated per liposome, i.e. 2900 ANTS/LUV at a concentration of 12.5 mM. The leakage rate corresponds to ca. 30 ANTS molecules/s.

The effect of temperature and % conversion of **1** to poly-**1** on the initial rate of ANTS leakage at pH 4.5 is shown in Fig. 3. Unexposed liposome showed little or no ANTS leakage from 11 to 37°C. There may be a small increase in leakage at higher temperatures (44°C). When the photoconversion of **1** to poly-**1** was 35%, the liposomes begin to show significant leakage at 37°C, but not at lower temperatures. At higher photoconversions of 47% or greater, a substantial enhancement in the initial rate of leakage was observed at 37°C

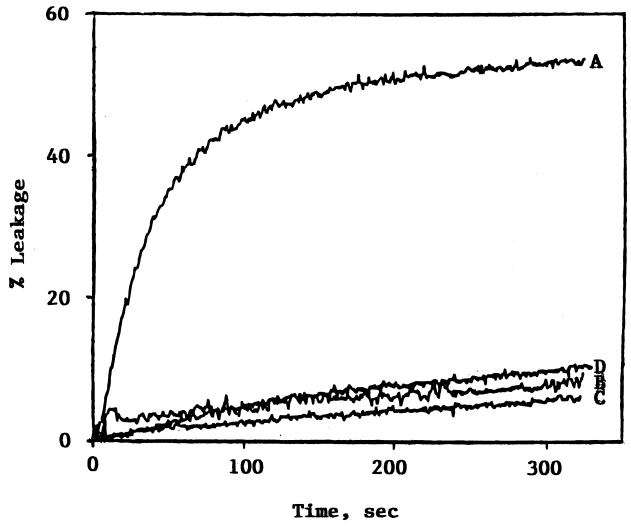

Fig. 2. The leakage vs. time of encapsulated ANTS and DPX from carrier LUV in the presence of empty photolyzed LUV. The sample pH for each experiment shown was (A) 4.5; (B) 8.5; (C) 7.4; and (D) 6.3. The LUV composition (300 μM lipid) was nine parts empty LUV composed of DOPE:**1**:DiI (molar ratio 15:5:1) and one part carrier LUV composed of DOPE:**1** (molar ratio 3:1). Zero time is the sample acidification time.

(Fig. 3). These results are consistent with the prior studies of the UV-induced fusion of DOPE/**1** liposomes, which showed that increasing the extent of poly-**1** in the liposome decreased the temperature for the onset of rapid liposome destabilization and fusion which was accompanied by the formation of non-lamellar lipid structures [11,12,14].

Previously, Miller et al. reported that HeLa cells endocytose positively charged LUV to a greater extent than either neutral or anionic LUV [22]. The inclusion of either a cationic lipid, 1,2-dioleoyl-3-dimethylammonium propanediol, or a cationic cyanine dye, DiI, into 1,2-dioleoyl-*sn*-glycero-3-phosphocholine LUV had a similar effect on the extent of endocytosis over 2–4 h at 37°C. Here, the cationic DiI was included in photoreactive LUV to render them photosensitive to green

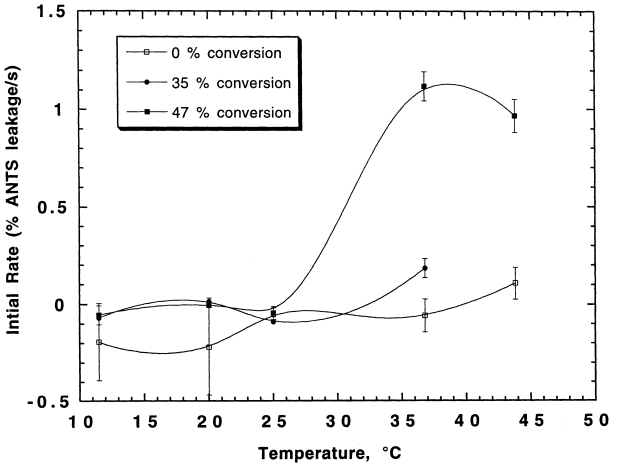

Fig. 3. Initial rate of ANTS leakage from liposomes at pH 4.5 vs. temperature for various photoconversions of **1** to poly-**1** for a ratio of 9:1 empty LUV to carrier LUV (300 μM total lipid). Empty LUV were DOPE:**1**:DiI (molar ratio 15:5:1) and carrier LUV were DOPE:**1** (molar ratio 3:1).

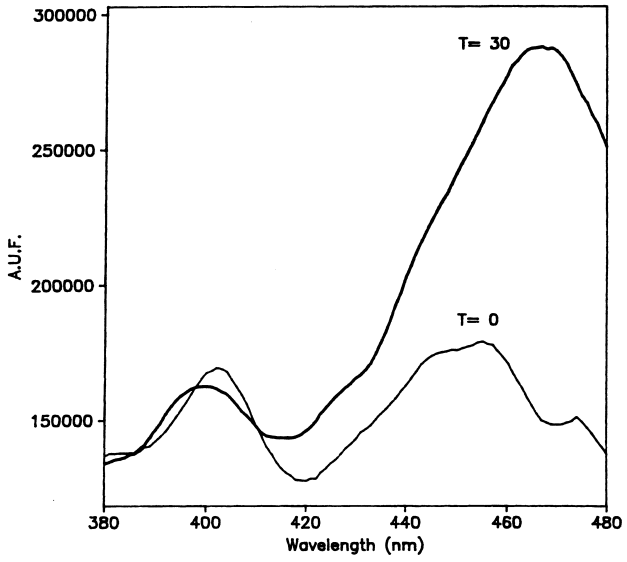

Fig. 4. HPTS excitation spectra before ($T=0$) and after irradiation ($T=30$) of cell-associated LUV with 550 nm light. HeLa cells were allowed to endocytose DOPE/**1**/DiI (molar ratio 16:4:1) LUV for 2 h, then the adherent cells were washed three times in PBS-CMG buffer and then dislodged into a cuvette by washing with PBS-EDTA. An excitation spectrum (380–480 nm) was determined for the cell suspension (λ_{em} 510) at $T=0$. The 450 nm/413 nm ratio indicates the average pH sampled by the HPTS is 6.8. The cells were then irradiated in the fluorometer at 37°C with 550 nm light for 30 min in a stirred cuvette, and the excitation spectrum was redetermined. Following irradiation of the cell-associated DiI-labelled LUV, the new 450 nm/413 nm ratio at $T=30$ indicates the measured pH in the vicinity of HPTS is 7.4.

light as well as to facilitate their endocytosis by HeLa cells. HeLa cells were incubated with DOPE/**1**/DiI (16:4:1) liposomes for 2 h at 37°C and 5% CO_2 in culture flasks. The LUV were prepared in the presence of PBS-CMG buffer that contained HPTS, and the HPTS was removed from the LUV exterior by gel permeation chromatography. Immediately following the incubation of LUV with the HeLa cells, the cells were washed three times in PBS-CMG buffer to remove LUV that were loosely associated with the cells. The adherent cells were then dislodged using PBS-EDTA buffer and were transferred to a fluorescence cuvette. The excitation spectra of HPTS were obtained at 37°C (λ_{ex} 380–480, λ_{em} 510) (Fig. 4). The ratio of excitation intensities at 450/413 nm was used to determine the global pH of the HPTS in the cell suspension. The measured pH was 6.8, indicating a significant fraction of the LUV were endocytosed. In the absence of endocytosis, the pH would be 7.4. The cell suspension was then irradiated with 550 nm light (the λ_{max} for DiI) for 30 min in a stirred cuvette at 37°C in the fluorometer by opening the excitation slits to 8 mm. After the sample photolysis, the slits were returned to normal and the excitation spectrum redetermined. The observed 450/413 nm ratio after green light exposure indicated that the global pH was now 7.4. These data indicate that the sample irradiation caused a net movement of the HPTS from low pH compartment(s) to higher pH compartment(s), either the cytoplasm or the extracellular medium. Delivery to the cytoplasm appears to be more likely because it would result from destabilizing interaction of the photolyzed liposomes with the endosomal membrane. A process that is similar to that described above for photolyzed and dark LUV. In contrast, a much more complicated sequence of transformations would need to occur to facilitate HPTS release first from the liposome, then the endosome, and finally the cell to reach the extracellular medium.

The ability to photodestabilize liposomes upon exposure to green light is not limited to the inclusion of the cationic DiI in the bilayer. Similar experiments were performed with the analogous anionic DiI-DS. Inclusion of this dye in the liposomes makes them anionic. Our studies with HeLa cells indicated that although anionic liposomes are less readily endocytosed than cationic liposomes, there is still a substantial fraction endocytosed [22]. Consequently, in those circumstances where anionic liposomes are preferred over cationic liposomes, it is possible to sensitize the photoinduced destabilization of the liposomes.

Photopolymerization of liposomes composed of reactive and unreactive lipids can increase the phase separation of the lipids into enriched domains. When the unreactive lipid is polymorphic, e.g. DOPE, this process destabilizes the liposome and lowers the fusion temperature [12,14]. Liposome destabilization is indicated by aqueous contents mixing (fusion) and loss of encapsulated contents (leakage) in liposome-liposome studies. Such a process is sometimes termed leaky fusion. If liposomes localized in endosomes are rendered less stable, then their interaction with the endosomal bilayer, whether via fusion and/or leaky fusion, will result in delivery of the liposomal contents to the cytoplasm of the cell. The fluorescence spectroscopy experiments with LUV-encapsulated HPTS provide clear evidence for this hypothesis. Further evidence was sought from fluorescence microscopy. However, HPTS was a less effective probe for microscopy, because its emission is weak and it was difficult to distinguish it from the emission of the sensitizing dyes when using the broad band filter sets of the microscope. Consequently, a water soluble membrane impermeant fluorescent probe, acridine homodimer, was used to test whether a DOPE/**1**/DiI-DS liposome could be photodestabilized within the endosome and release its contents to the cytoplasm. Following 4 h incubation of the liposomes with HeLa cells, the cells were washed as before, then examined by fluorescence microscopy. The cells showed both puctuate DiI-DS emission, indicating cellular localization of the dye and the liposomes, as well as intense blue–green fluorescence from the acridine homodimer. The latter emission signifies dye-nucleic acid binding, which occurs if the dye is released from the liposomes and endosomes into the cytoplasm and/or the nucleus, where it fluoresces on binding to AT-rich regions of RNA and/or DNA.

These data illustrate new ways to modify liposome properties in a manner that could enhance the delivery of reagents to cells. While these liposomes described here are not necessarily optimal for in vivo use, it is clear that dye sensitization makes it possible to destabilize liposomes of suitable composition with visible light. These findings present new opportunities for the design of liposomal carriers for drug delivery.

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References

[1] Düzgunes, N., Straubinger, R.M., Baldwin, P.A., Friend, D.S. and Papahadjopoulos, D. (1985) Biochemistry 24, 3091–3098.

[2] Ellens, H., Bentz, J. and Szoka, F.C. (1985) Biochemistry 24, 3099–3106.
[3] Parente, R.A., Nadasdi, L., Subbarao, N.K. and Szoka, F.C. (1990) Biochemistry 29, 8713–8719.
[4] Litzinger, D.C. and Huang, L. (1992) Biochim. Biophys. Acta 1113, 201–227.
[5] Chowdhardy, R.K., Green, C.A. and Morgan, C.G. (1993) Photochem. Photobiol. 58, 362–366.
[6] O'Brien, D.F. and Tirrell, D.A. (1993) Bioorganic Photochemistry (Morrison, H., Ed.), Vol. 2, J. Wiley and Sons, New York, pp. 111–168.
[7] Anderson, V.C. and Thompson, D.H. (1992) Biochem. Biophys. Acta 1109, 33–42.
[8] Thompson, D.H., Gerasimov, O.V., Wheeler, J.J., Rui, Y. and Anderson, V.C. (1996) Biochim. Biophys. Acta 1279, 25–34.
[9] You, H. and Tirrell, D.A. (1991) J. Am. Chem. Soc. 113, 4022–4023.
[10] Lamparski, H., Liman, U., Frankel, D.A., Barry, J.A., Ramaswami, V., Brown, M.F. and O'Brien, D.F. (1992) Biochemistry 31, 685–694.
[11] Barry, J.A., Lamparski, H., Shyamsunder, E., Osterberg, F., Cerne, J., Brown, M.F. and O'Brien, D.F. (1992) Biochemistry 31, 10114–10120.
[12] Bennett, D.E. and O'Brien, D.F. (1995) Biochemistry 34, 3102–3113.
[13] Thomas, J.L. and Tirrell, D.A. (1992) Acc. Chem. Res. 25, 336–342.
[14] Miller, C.R., Bennett, D.E., Chang, D.Y. and O'Brien, D.F. (1996) Biochemistry 35, 11782–11790.
[15] Lamparski, H. and O'Brien, D.F. (1995) Macromolecules 28, 1786–1794.
[16] O'Brien, D.F., Armitage, B., Benedicto, A., Bennett, D.E., Lamparski, H.G., Lee, Y.-S., Srisiri, W. and Sisson, T.M. (1998) Acc. Chem. Res. 31, 861–868.
[17] Sisson, T.M., Lamparski, H.G., Kölchens, S., Elyadi, A. and O'Brien, D.F. (1996) Macromolecules 29, 8321–8329.
[18] Clapp, P.J., Armitage, B.A. and O'Brien, D.F. (1997) Macromolecules 29, 32–41.
[19] Stewart, J.C.M. (1980) Anal. Biochem. 104, 10–14.
[20] Hope, M.J., Bally, M.B., Webb, G. and Cullis, P.R. (1985) Biochem. Biophys. Acta 812, 55–65.
[21] Kölchens, S., Ramaswami, V., Birgenheier, J., Nett, L. and O'Brien, D.F. (1993) Chem. Phys. Lipids 65, 1–10.
[22] Miller, C.R., Bondurant, B., McLean, S.D., McGovern, K.A. and O'Brien, D.F. (1998) Biochemistry 37, 12875–12883.
[23] Daleke, D.L., Hong, K. and Papahadjopoulos, D. (1990) Biochim. Biophys. Acta 1024, 352–366.