CHEMISTRY LETTERS, pp. 433-436, 1989.

Liposomes That Can Be Disintegrated by Photo-Irradiation

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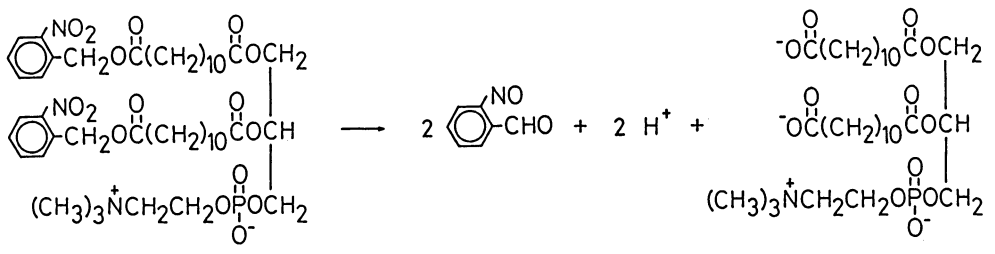
Photo-labile phosphatidylcholine (PLPC) was synthesized which contains 2-nitrobenzyl esters at the terminals of both alkyl chains. Photolysis of the esters generates carboxylate ions in the hydrophobic region of the PLPC membrane, leading to instant disintegration of PLPC liposomes as observed by the phase contrast microscopy. Upon UV irradiation, entrapped macromolecules (45-kdalton ovalbumin) were released from PLPC liposomes.

Photosensitive artificial membranes and liposomes have been a subject of intensive studies in recent years.[1,2] Liposomes that show increased release of entrapped solutes when exposed to light are of particular interest for the purpose of introducing reagents into living cells and designing selective drug delivery systems.[2] In the present study, we have developed liposomes that can be disintegrated by photo-irradiation. These liposomes are more advantageous than the photosensitive liposomes published previously because destruction of liposomes warrants fast and complete release of the entrapped materials even if they are *macromolecules*.

Photo-labile phosphatidylcholine (PLPC) was synthesized by introducing the light-cleavable 2-nitrobenzyl ester moieties[3,4] at the terminals of both alkyl chains. Briefly, one equivalent of 2-nitrobenzyl alcohol was allowed to react with dodecanedioyl dichloride[5] followed by hydrolysis of the remaining acid chloride. The formed mono-2-nitrobenzyl ester of dodecanedioic acid was purified by silica gel column chromatography (eluted with hexane/ethyl acetate = 4:1 to 2:1, v/v). Condensation of the mono ester and *sn*-glycero-3-phosphocholine cadmium chloride complex by 1,3-dicyclohexylcarbodiimide in the presence of 4-dimethylaminopyridine



PLPC

Scheme 1.

afforded PLPC according to the reported method for synthesis of phosphatidylcholine derivatives.[6] The structure of PLPC was confirmed by elemental analysis and NMR (UV (ethanol) λ_{max} = 257 nm, ε = 9800 M^{-1} cm^{-1}). It is thought that photolysis of the esters generates carboxylate ions at the hydrophobic terminals of PLPC as shown in Scheme 1[4], causing large perturbation to the liposome structure.

The PLPC liposomes formed by sonication, and purified by gel filtration[6] (60% PLPC recovered in the void volume) were examined by transmission electron microscopy, after negative staining with uranyl acetate (data not shown). The diameter of the vesicles ranged between 16 and 30 nm.

To study the molecular organization of liposomes made of PLPC, stearic acid spin labels (5-, 12-, and 16-doxyl stearic acid spin labels) were incorporated into PLPC liposomes formed by vortexing[7] and their motional freedom was investigated by using ESR spectroscopy. As the doxyl group is placed toward the methyl terminal of the stearic acid, the overall splitting values decreased (50.2 G, 45.3 G, and 33.1 G for 5-, 12-, and 16-doxyl stearic acid spin labels, respectively, at 37 °C), indicating that the motional freedom of the nitroxide group increases. This result implies that PLPC molecules are organized in the form of usual lipid bilayers[7] and not of micelles. No phase transition was detected by the spin labeling method when the temperature was varied from 0 °C to 60 °C.

When translucent PLPC vesicle suspension (absorption λ_{max}= 264 nm), prepared by sonication and purified by gel filtration, was extensively irradiated with UV-light from a high-pressure mercury lamp for 30 min, it turned into a clear yellow solution (λ_{max}= 308 nm). Thin layer chromatography indicated complete decomposition of PLPC.

Morphological changes of PLPC liposomes by brief light irradiation were observed by using the phase-contrast microscopy. PLPC liposomes were prepared by vortex mixing,[7] placed under the phase-contrast microscope, and examined before and after photo-irradiation with an epi-fluorescence excitation system through the same objective lens.[8] One of the giant liposomes, which were frequently found with a large number of smaller liposomes, is shown in Fig. 1A. Morphological change of the identical giant liposome after light irradiation for 10 s is displayed in Fig. 1B: the giant liposome is disintegrated. It is fractured into smaller pieces, the structures of which are yet to be determined. No observable changes took place when liposomes made of 1,2-dimyristoyl-3-*sn*-phosphatidylcholine, which does not contain any photo-cleavable group, were irradiated under the microscope under the same conditions. In agreement with these observations, 90°-light scattering of the same liposome sample monitored at 500 nm with a fluorimeter instantaneously decreased from 69 to 34 after UV irradiation for 10 s (22 after 1-min irradiation and 1.2 with buffer only, in an arbitrary unit), indicating a substantial decrease of liposomes in the average size and/or number (of intact liposomes) after photo-irradiation.

Light-triggered release of ovalbumin, a protein whose molecular weight is appproximately 45 kdalton, entrapped in the PLPC liposome was studied. The liposomes containing ovalbumin in the inner aqueous volume were prepared by the reverse-phase evaporation method[9] as detailed in the legend to Fig. 2. Entrapped ovalbumin was released from the PLPC liposomes upon UV irradiation with an HBO 100

lamp (Fig. 2). After 20 s light irradiation, 75% of the entrapped ovalbumin leaked out. Phospholipid analysis of this sample indicated that less than 5% of PLPC was decomposed on the basis of quantification of PLPC collected from its spot on the thin layer chromatogram.[6] These results suggest that decomposition of small fractions of PLPC leads to disintegration of liposomes and leakage of the entrapped macromolecules. No light-induced release of ovalbumin was found for 1,2-dimyristoyl-3-*sn*-phosphatidylcholine liposomes.

The methods of rapid mixing and stopped flow have made significant contributions to studies of kinetics and elementary processes of biochemical

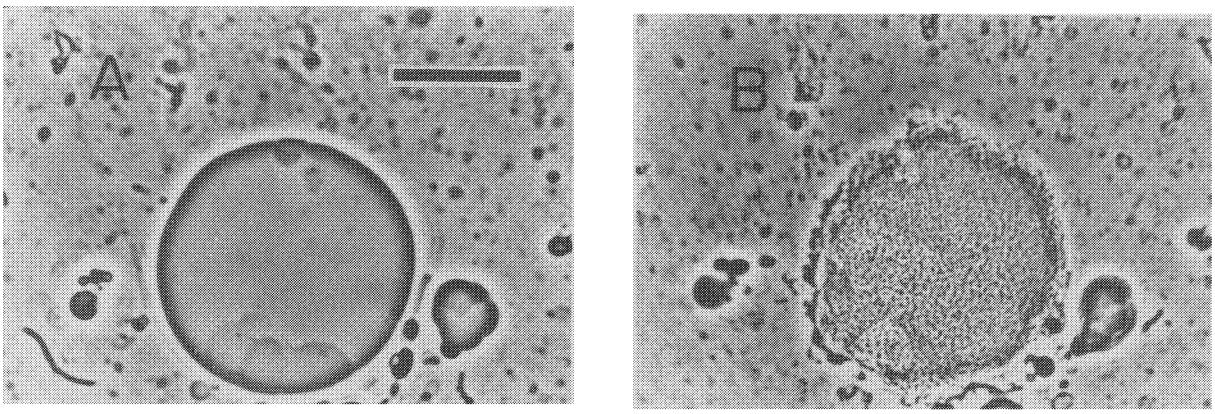
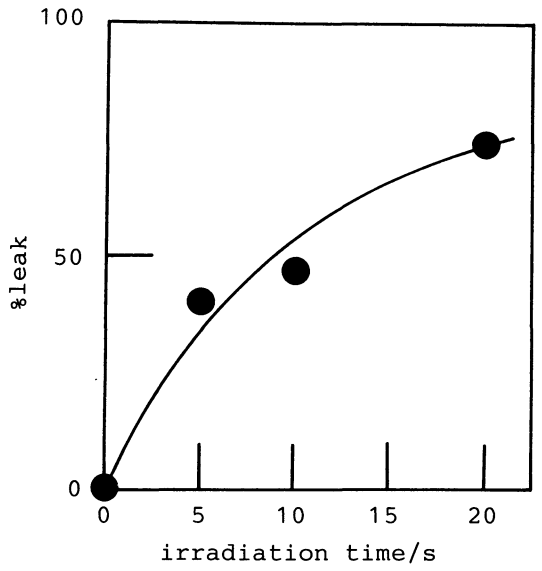


Fig. 1. Phase-contrast micrographs of multilamellar dispersion of PLPC prepared as reported previously[7] (in 5 mM 1,4-piperazinediethanesulfonic acid (PIPES), pH 7.2) before (A) and after (B) the light irradiation for 10 sec. Note that the identical field under the microscope is displayed in (A) and (B), showing the same giant liposome before and after the light irradiation. A Zeiss universal microscope was used with a Zeiss 40 x objective lens (0.75 numerical aperture). Light irradiation was carried out by using a Zeiss epi-fluorescence excitation system (an HBO 100 W lamp, a short cut filter at 350 nm, a dichroic mirror at 500 nm). The bar indicates 30 μm.

Fig. 2. Light-induced release of entrapped ovalbumin from PLPC liposomes. Ovalbumin (40 mg/ml) was entrapped in PLPC vesicles formed by the reverse-phase evaporation method.[9] Untrapped ovalbumin was removed by repeated centrifugation and resuspension in 5 mM PIPES, pH 7.2. After the final resuspension, the (average) concentrations of ovalbumin and PLPC were 15.3 mM and 890 mM, respectively, as determined after the lysis of the liposome with 0.1% (v/v) Triton X-100. The suspended liposomes were transferred to a quartz cuvette and irradiated with light from an HBO 100 lamp followed by centrifugation at 100 000 x g for 2 hours. No leakage was detected for the unirradiated sample after the 2h centrifugation. The concentration of ovalbumin in the supernatant was determined by the method of Bradford.[10]



reactions.[11] Since, with PLPC liposomes, the mixing reaction can be initiated by light irradiation and even under the microscope, photo-degradable liposomes have opened up the possibilities of carrying out kinetic experiments in living systems such as cultured cells. The PLPC liposomes could also be used as a drug carrier *in vivo*, releasing drugs only at a selected site at a selected time by photo-irradiation, through optical fibers combined with an endoscope.

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8) Because phase contrast objectives made of quartz glass are not available on the market, we were unable to irradiate the sample under the microscope at the wavelengths around the absorption maximum of PLPC. The wavelengths of the irradiated light were between 340 and 500 nm. Since the long tail of the absorption spectrum of PLPC can be detected up to 360 nm, and since the light intensity per unit area is greatly increased by the objective lens, it appears that a small fraction of the incident light with wavelengths a little below 360 nm was sufficient to induce photolysis of PLPC. The possibility of heat effect is excluded because liposomes made of 1,2-dimyristoyl-3-*sn*-phosphatidylcholine were not affected by the light irradiation.
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(Received December 10, 1988)