Photoinduced Fusion of Liposomes

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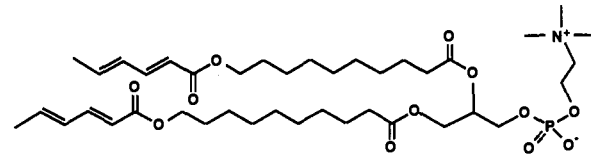
Processes that trigger the phase separation of phosphatidylethanolamines (PE) from other lipids yield enriched domains of PE and modify its local phase behavior. Thus the addition of selected ions, protons, or peptides to appropriately designed liposomes are known to initiate fusion between liposomes (liposome fusion).[1-5] Since PEs are significantly less hydrated than phosphatidylcholines (PC),[6,7] the formation of domains of PE in bilayer membranes facilitates the close approach of these regions of the bilayer surfaces. Contact between bilayers is a prerequisite for bilayer destabilization and liposome fusion.[1] The potential of these phenomena for drug delivery, gene transfer, and immunodiagnostic applications has been recently reviewed.[8] Here we describe the use of light to initiate liposome fusion.

The polymerization of bi- or multicomponent liposomes has been usefully employed to form domains of enriched lipids for the insertion of transmembrane proteins into partially polymerized liposomes,[9] for the efficient photoinduced lysis of oligolamellar liposomes,[10,11] and for the enhancement of energy transfer between membrane-bound dyes.[12] In each of these cases crosslinking of the polymerizable lipids separates the lipids into polymeric and monomeric domains.[13] If both components are lipids that prefer lamellar structures, e.g., PC, the polymerization proceeds with retention of liposome integrity.[9-12] However, if the unreactive lipid can form nonlamellar structures, then the liposomes are destabilized.[10,11] In the case of oligolamellar liposomes, where intraliposomal bilayer–bilayer contact is possible, this leads to leakage, whereas with unilamellar liposomes, where only interliposomal contact is possible, fusion is observed at concentrations that permit a high frequency of such contact.

Liposomes were composed of dioleoylPE (DOPE) and bis-SorbPC in a 3:1 molar ratio. Predominantly unilamellar liposome populations (LUV) were prepared by repeated freeze–thaw extrusion using 0.1 μm Nuclepore membranes.[14] The LUV mean diameter was estimated to be 120 ± 10 nm by quasi-elastic laser light scattering.[15]

Liposome fusion was examined using established fluorimetric assays for both lipid mixing and aqueous contents mixing between



bis-SorbPC

different LUV populations.[16,17] Lipid mixing was measured by the probe dilution assay of Düzgünes et al.[18] which utilizes the fluorescently labeled lipid, NBD-PE, and its quencher, N-(lissamine rhodamine B sulfonyl)dioleoyl PE, Rh-PE. LUV were prepared in a pH 9.5 buffer (115 mM NaCl, 10 mM glycine, 0.1 mM EDTA), where they were negatively charged due to deprotonation of the PE ammonium group. The residual fluorescence of labeled LUV containing 1.0 mol % of both the probes was the 0% value. The stirred unlabeled LUV were irradiated with a low-pressure mercury pen lamp and were then combined 9:1 with the labeled LUV to give a lipid concentration of 300 μM. Bilayer contact was initiated by neutralizing the LUV surface charge by adding small aliquots of H^+ or Mg^{2+} solutions.[19] Dark assays were performed in the same manner but without photolysis. Lipid mixing is reported as increased fluorescence from NBD-PE (530 nm) due to diminished energy transfer efficiency to Rh-PE as the probes are diluted. The assay is insensitive to liposome aggregation.[17] The value for 100% fusion was set by subjecting a 1:9 mixture of the labeled and unlabeled LUV to repeated cycles of freeze–thawing and sonication to randomize the lipids.[20]

Lipid mixing occurred at a higher rate and to a greater extent, at all initiation conditions, in photolyzed samples than in dark samples. Further, greater extents of photoconversion effected higher rates of lipid mixing as shown in Figure 1 for LUV at 37 °C and either pH 4.5 (panel A) or pH 9.5/20 mM Mg^{2+} (panel B). Lipid mixing was greater at 37 °C than at 20 °C (data not shown).

The ANTS/DPX [1-aminonaphthalene-3,6,8-trisulfonate and *N,N'-p*-xylenebis(pyridinium bromide)] fusion and leakage assays were performed according to the methods of Ellens et al.[1,21] All solutions were buffered with 10 mM glycine, pH 9.5, and were isoosmotic to the buffers used for gel permeation chromatography and for the subsequent assays. Mixing of aqueous contents between populations of LUV containing either ANTS or DPX decreases ANTS fluorescence due to DPX quenching. The ANTS and DPX LUV were combined in a 1:9 ratio at 300 μM lipid. The fluorescence scale was calibrated by taking the intensity of a mixture of ANTS and DPX LUV in glycine buffer as 0% fusion, and the intensity of a separate LUV population containing coencapsulated ANTS/DPX as 100% fusion. For the leakage assay, 0% leakage was set with the coencapsulated ANTS/DPX liposomes, and 100% leakage was set with LUV that had been lysed by addition of TX100. Since 254 nm photolysis bleached ANTS fluorescence to a limited but significant extent, the 100% fluorescence value was determined for each sample. Contents mixing and leakage assays were initiated by photolyzing the LUV and then neutralizing the surface charge by addition of small aliquots of H^+ or Mg^{2+} stock solutions.

Figure 2 illustrates the dependence of aqueous contents mixing (fusion) and leakage on the extent of photolysis at 37 °C and either pH 4.5 (panels A and C) or pH 9.5/20 mM Mg^{2+} (panels

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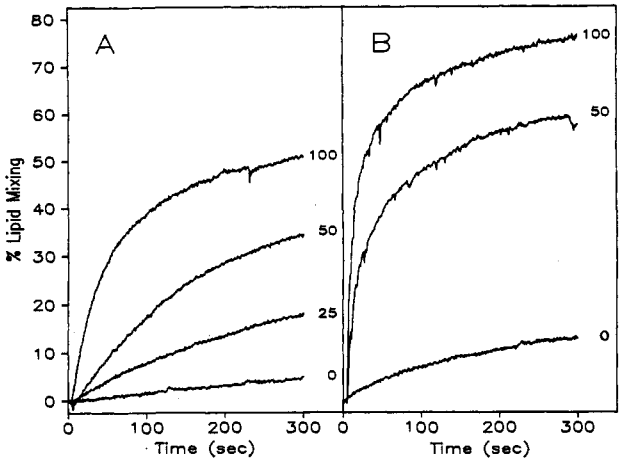

Figure 1. The effect of the extent of photopolymerization on the rate of lipid mixing at 37 °C for LUV composed of DOPE/bis-SorbPC (3:1). The unlabeled LUV were photolyzed to achieve different extents of polymerization and then combined with the dark labeled LUV in a 9:1 ratio at pH 9.5. The emission was measured over time after the surface charge was neutralized by the addition of small volumes of concentrated H^+ or Mg^{2+} to adjust the pH to 4.5 (panel A), or to pH 9.5/20 mM Mg^{2+} (panel B).

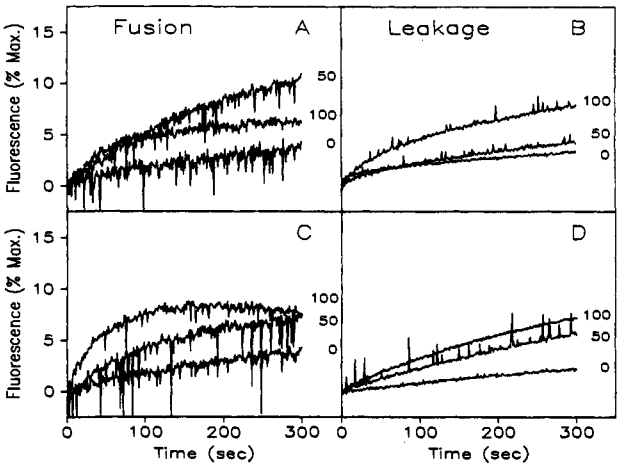

Figure 2. Dependence of liposome fusion and leakage at 37 °C and 300 μM lipid on the extent of photopolymerization of LUV composed of DOPE/bis-SorbPC (3:1). Fusion assays were performed between DPX-containing LUV, photolyzed to the extents shown, and dark ANTS LUV. The aqueous contents mixing assay for fusion was initiated by combining the DPX and ANTS LUV in a 9:1 ratio at pH 9.5 and adding small volumes of concentrated H^+ or Mg^{2+} solutions to achieve pH 4.5 (panel A) or pH 9.5/20 mM Mg^{2+} (panel C). The leakage assays with ANTS/DPX-containing LUV were initiated as described above at either pH 4.5 (panel B) or pH 9.5/20 mM Mg^{2+} (panel D).

B and D). Under all initiation conditions, fusion was enhanced by either partial or complete photopolymerization. At pH 4.5, fusion may occur slightly faster for 50% than for 100% photopolymerization. The lipid mixing assays demonstrate that this difference was not due to greater liposome contact at 50% than 100% polymerization. Rather, the observed fusion is controlled by the concurrent leakage. The data in panel B indicate that leakage, like lipid mixing, increases in rate with increased photolysis. At pH 9.5, fusion occurred faster at 100% than at 50% photopolymerization (panel C), despite the slightly faster leakage kinetics with greater photolysis (panel D). Although fusion for 100% photopolymerization was initially rapid, it reached a maximum in about 2 min and afterward was overcome by leakage. Fusion and leakage were both temperature dependent and occurred to a significantly greater extent at 37 °C than at 20 °C under the initiation conditions.

By controlling the extent of photolysis under certain conditions, the extent of liposome destabilization and the character of the resulting fusion event can be controlled. Apparently, leaky fusion occurs in systems that have been too greatly destabilized, whereas nonleaky fusion is favored in systems that are destabilized to a critical, but limited extent. Photoinduced fusion was dependent on the extent of polymerization, temperature, and the fusion initiation conditions, including pH and the presence of Mg^{2+} ions. These characteristics are consistent with previously proposed mechanisms of bilayer fusion, as well as the ^{31}P NMR and X-ray diffraction studies on extended bilayers of the DOPE/bis-SorbPC which indicate that lipid polymerization aids in the formation of isotropically symmetric structures between the lamellae of apposed bilayers.[22] These initial studies into the photoinduced fusion of liposomes open the possibility of using both the temporal and spatial characeristics of light to deliver reagents from liposomes to other bilayer bounded structures.

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