

Photoinduced Formation of Bilayer Vesicles

Andrea Veronese, Nathalie Berclaz, and Pier Luigi Luisi*

Institut für Polymere, ETH Zentrum, Universitätsstrasse 6, CH-8092 Zürich, Switzerland

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A photochemical reaction, which generates surfactant molecules which then spontaneously assemble into vesicles, is described. Upon irradiation of a dispersion of the photocleavable water-insoluble precursor didecyl-2-methoxy-5-nitrophenyl phosphate, the phosphate–phenolate bond was selectively hydrolyzed and didecyl phosphate was released in the medium. Light microscopy was used to assess the presence of vesicles, which were generally in the range of 1–10 μm . This light-induced formation of vesicles is of general interest in the field of liposomes technology and may be seen as an additional contribution to the prebiotic chemistry of compartmented bilayer formation.

Introduction

Light-induced reactions leading to the formation of vesicles are of great interest as they connect self-aggregation phenomena with the most dispensable energy source, namely light.

Different effects of light on bilayer vesicles have been studied: reorganization¹ and polymerization² of bilayer membranes, release of vesicle content,³ or a model of the photosynthetic apparatus which upon irradiation transports protons across a lipid bilayer.⁴ Morowitz et al.⁵ first proposed that light-dependent formation of alkyl phosphates might be a plausible prebiotic synthesis that would produce membrane. However, not much is known about experiments describing light-induced vesicle formation except for an older communication by Folsome and Morowitz.⁶ In the present letter, we describe a simple and effective method to accomplish this. Thus, this finding can be considered of theoretical relevance for prebiotic chemistry⁷ as well as of general interest in the field of liposome technology.

The sensitivity of some chemical bonds to light has been known for a long time.⁸ Recent studies have shown that phosphate ester linkages derived from 2-methoxy-5-nitrophenol can be rapidly and quantitatively photohydrolyzed using a simple and inexpensive photoreactor.⁹

Considering this chemistry, we devised conditions under which a water-insoluble phosphotriester could be photolytically converted into a dialkyl phosphate that spontaneously self-assembles into vesicles.

Dialkyl phosphates were first found to build bilayer membranes in the late 1970s¹⁰ and have been used since then as models of biological membranes. Dialkyl phosphates form vesicles under basic conditions. One important characteristic of vesicles is the transition temperature, T_c , associated with a gel to liquid crystalline transition. T_c of dialkyl phosphate decreases with decreasing alkyl chain length¹¹ and is dependent on the counterion.¹² The shortest dialkyl phosphate actually known to build vesicles is didecyl phosphate.

Therefore, the following phosphotriester, didecyl-2-methoxy-5-nitrophenyl phosphate (**1**), was chosen as model compound for this study. **1** was first synthesized, and then its photohydrolysis was investigated using a KOH 0.2 M aqueous solution.¹³ The photohydrolysis of **1** produced 2-methoxy-5-nitrophenolate (**3**) and didecyl phosphate (**2**) that, under the chosen conditions,

formed vesicles (Scheme 1). The kinetics of the reaction were monitored by TLC densitometry, which permitted the simultaneous quantification of precursor **1** and product **2** present in the reaction mixture.

Experimental Section

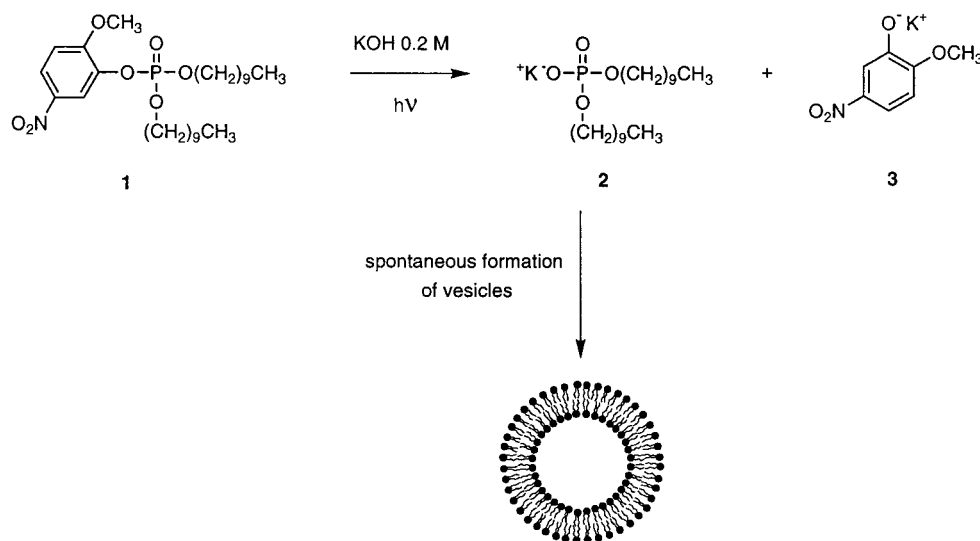
General Procedures. All the reagents were purchased from Fluka and were of the highest grade available. Densitometry analyses were performed using a Shimadzu Flying Spot Scanning CS-9301PC Densitometer. Light micrographs were obtained using an Axioplan microscope from Zeiss.

Synthesis of Didecyl-2-methoxy-5-nitrophenyl Phosphate (1).¹⁴ Phosphoryl chloride (1 g, 6.52 mmol) was dissolved in CH_2Cl_2 (40 mL, stored over molecular sieves) in a three-necked round-bottom flask and then cooled to -18°C using an acetone/dry ice bath. Redistilled decanol (2.06 g, 13.01 mmol) and Et_3N (1.32 g, 13.04 mmol) in CH_2Cl_2 (10 mL) were added dropwise, while stirring, to the precooled phosphoryl mixture over a period of 15 min. The reaction mixture was then stirred at room temperature for 5 h and cooled again to -5°C using a salt/ice bath. A CH_2Cl_2 solution containing 2-methoxy-5-nitrophenol (0.999 g, 6.52 mmol) and another equivalent of Et_3N (0.66 g, 6.52 mmol) was then added dropwise to the reaction mixture while stirring. As soon as the addition was complete, the ice bath was removed and the reaction mixture was refluxed for 8 h under argon. The reaction solution was then washed with saturated aqueous NaHCO_3 solution (6×20 mL). The organic phase was dried over Na_2SO_4 and concentrated using a rotary evaporator. The crude product was then purified by silica-gel column chromatography using CHCl_3 to yield **1** (0.8 g, 23%) as an orange oil.

R_f (CHCl_3): 0.26. ^1H NMR (CDCl_3): δ 8.16 (m, 1H, arom H6), 8.1 (m, 1H, arom H4), 7.01 (d, $J = 9$ Hz, 1H, arom H3), 4.2 (q, $J = 6.9$ Hz, 4H, OCH_2CH_2-), 3.97 (s, 3H, OCH_3), 1.72 (m, 4H, OCH_2CH_2-), 1.26 (m, 28H, alkyl chain). ^{13}C NMR (CDCl_3): δ 156.3 (arom C), 140.9 (arom C), 139.63 (arom C), 122.2 (arom C), 117.4 (arom C), 111.5 (arom C), 69 (OCH_2CH_2-), 56.6 (OCH_3), 31.9–14.1 (CH_2 , chain). ^{31}P NMR (CDCl_3): δ -5.43 . MS (m/z): 530.4 ($M + 1$, 57%), 1059.8 ($2M + 1$, 35%).

Hydrolysis of Didecyl-2-methoxy-5-nitrophenyl Phosphate in 0.2 M KOH Using UV Light. General Procedure.

SCHEME 1



Didecyl-2-methoxy-5-nitrophenyl phosphate was weighted in a 20 mL Pyrex vessel (light cutoff at 300 nm), and a 0.2 M KOH aqueous solution was then added to it. The mixture was hand-shaken, vortexed, and finally sonicated until a fine emulsion of oil droplets was obtained. The emulsion was then immersed in a thermostatic bath (25 °C) in front of a 125 W low-pressure Hg lamp.

With Continuous Irradiation. The suspension was irradiated continuously for 19 h.

With Intermittent Irradiation. The emulsion was first kept in the dark for 4 h and then exposed to UV light for 1 h. The lamp was then turned off for 2 h and again turned on until the reaction was complete.

Monitoring of the Reaction. The reaction was followed by densitometry. Samples (200 μ L) were taken from the reaction mixture during the reaction progress. CHCl_3 (200 μ L) and 1 M HCl (100 μ L) were then added to the samples and the mixtures were vortexed for 5 min. A 10 μ L amount of the CHCl_3 phase was loaded on a TLC plate, which was first developed in CHCl_3 :EtOAc (40:1). The UV-active precursor **1** was then directly quantified by measuring its absorption at 260 nm. The TLC plate was developed a second time using CHCl_3 : CH_3OH :AcOH (70:15:2.5). Surfactant **2** was then visualized using a Dittmer–Lester reagent¹⁵ and quantified by measuring the absorption of the molybdenum–phosphor complex at 560 nm.

Results and Discussion

Analogous to what was already reported by other authors for 2-methoxy-5-nitrophenyl phosphate,⁹ **1** was stable for several hours in a 2:1 CH_3CN :0.2 M NaOH solvent mixture in the absence of light but was photohydrolyzed within a few minutes when irradiated with a UV light (not reported).

In our case, the photohydrolysis had to be carried out in an aqueous solution to permit the formation of vesicles. Phosphate triester **1** is highly insoluble in water; a fine emulsion was obtained by vortexing and sonicating a dispersion of **1** in a 0.2 M KOH solution. If this emulsion was kept in the dark, no chemical modification was observed over a time span of few days (apart from a slow phase separation due to the formation of small oil droplets at the water surface). However, upon exposure of the emulsion to the light of a Hg lamp, the reaction started, and after few minutes of irradiation, the color of the

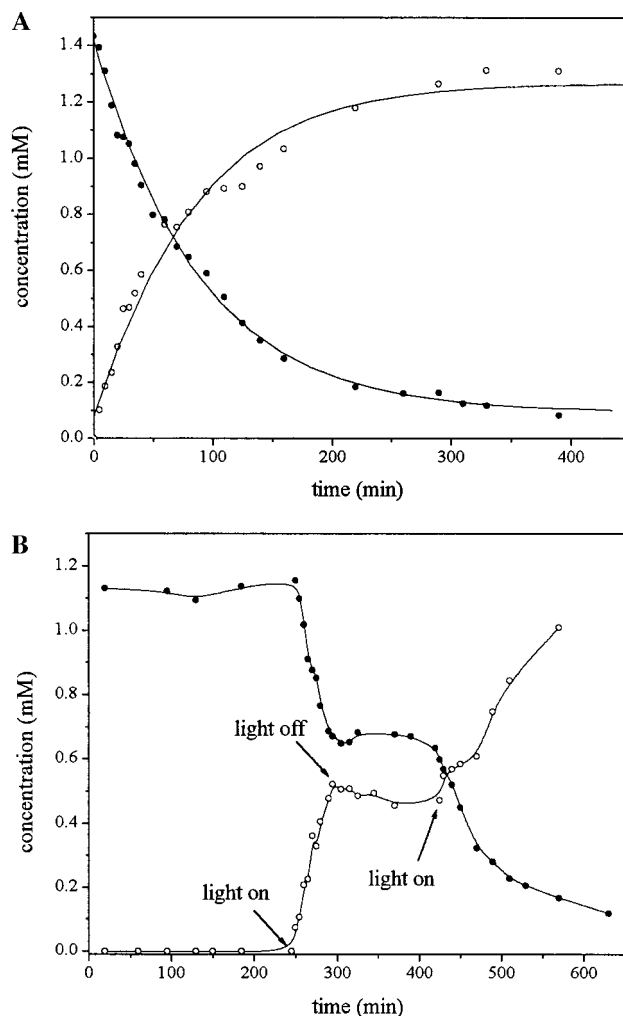


Figure 1. Time-dependent changes of precursor **1** (●) and product **2** (○) concentration measured by TLC densitometry during photohydrolysis. (A) The reaction was carried out by exposing an emulsion of **1** (1.4 mM) in a 0.2 M KOH solution, at $T = 25$ °C, to the light of a low-pressure Hg lamp. (B) In this case, the lamp of the photoreactor was switched off for the first 4 h and then turned on and off as indicated by the arrows. The reaction was started from an emulsion of **1** (1.15 mM) in a 0.2 M KOH solution at $T = 25$ °C.

reaction mixture began to turn orange. The color intensity increased to reach a maximum and then slowly decreased. After

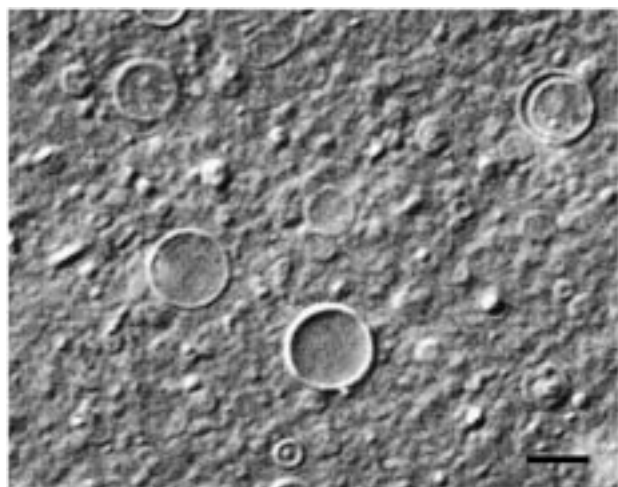


Figure 2. Light microscopy picture of the reaction mixture at $T = 25$ °C after 19 h of irradiation: liposomes are observed. The length of the bar corresponds to 5 μm .

20 h, the initial emulsion was transformed into a pale yellow, opalescent suspension.

A typical reaction profile is shown in Figure 1A. It shows that the reaction proceeded efficiently, leading to a quantitative conversion of precursor **1** into surfactant **2**.¹⁶ Compound **2**, once formed, was very stable and did not undergo subsequent hydrolysis or other degradation processes. However, as already described in the literature,^{17,18} the second reaction product, 2-methoxy-5-nitrophenolate (**3**), after being released in solution (appearance of the orange color), was then photochemically degraded (decrease of the orange color with longer irradiation times). Figure 1B shows the dependence of the chemical conversion on irradiation: the reaction took place only when the light was turned on.

Light microscopy analyses of the reaction mixture clearly showed that surfactant generation was accompanied by vesicle formation (Figure 2). In particular, large vesicles, with dimensions up to 10 μm , were visible by light microscopy. Together with oil droplets, large vesicles were already present after 1 h of irradiation and their number increased with irradiation time. After 19 h, the reaction seemed to be complete. No evidence of liposome formation could be detected if the starting emulsion was maintained in the dark for several days. Once the photoconversion had been brought to completion, the vesicular suspension remained stable for several weeks. No precipitation or phase separation was observed.

In conclusion, we have described a new method by which a vesicular suspension can be obtained simply by irradiating a water emulsion of an insoluble precursor. In general, this type of photochemical reaction may be interesting for the origin of life as it indicates that membrane bilayers can indeed be easily formed by light-induced processes. This work may also be of interest in liposome technology, including pharmaceutical formulations: in fact this is a field where the preparation of liposomes without the need of organic solvents is still a problem of great relevance.

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References and Notes

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