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POLYMERIZED PHOSPHATIDYL CHOLINE VESICLES. STABILIZED AND CONTROLLABLE TIME-RELEASE CARRIERS*

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SUMMARY

Polymerized phosphatidyl choline vesicles (diameters ranging between 350 and 1400 Å) have been synthesized from lipids bearing one or two methacrylate groups per monomer. Compared to nonpolymeric analogues, these vesicles exhibited improved stability and controllable time-release properties.

INTRODUCTION

We have recently introduced the concept of polymerized vesicles, and have suggested that phosphatidyl choline derivatives might constitute a new and attractive class of drug carriers (1). In principle, they should be intrinsically more stable than liposomes (vesicles derived from naturally occurring phospholipids) and, with the incorporation of a suitable cross-linking agent, have controllable permeability (2-15). Preliminary studies carried out with ammonium-based vesicles revealed that while stability was improved through polymerization, the leakage rate of entrapped sucrose remained too high to be determined. In the following report, we describe the synthesis and characterization of polymerized phosphatidyl choline vesicles (16-18). Results obtained with these systems clearly demonstrate that polymerization not only improves carrier stability but also provides a means for reducing and controlling leakage.

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MATERIALS AND METHODS

12-Hydroxydodecanoic acid and methacryloyl chloride were purchased from Aldrich Chemical Company and were used without further purification. L-α-lysophosphatidyl choline palmitoyl was obtained from Sigma Chemical Company; L-α-glycerophosphoryl choline was prepared from egg lecithin. [^{14}C] Sucrose was obtained as a 20% ethanol solution (ICN Laboratories) with a specific activity of 360μCi/μmol. Vesicles were prepared in 10-mL spectroscopic cells equipped with a septum, and were degassed by purging with nitrogen (water or D_2O was distilled and stored in a nitrogen atmosphere). The sonication was monitored by the measurement of turbidity at 400 nm; transmittance was at least 70% for the final sonicated sample. All permeability experiments employed 10 mg of lipid/2mL of water. Radioactivity in dialysis bags was measured by liquid scintillation counting. Dialysis procedures used were similar to those previously described (7). Samples for electron microscopy were prepared by (1) placing one drop of the vesicle solution onto a carbon-coated copper grid, (2) allowing the grid to remain in contact with excess liquid for 30 s, (3) blotting the grid and (4) applying a staining solution (2% uranyl acetate) in an analogous fashion and (5) drying the grid overnight in a desiccator. Turbidity measurements were made at 400 nm. ^{1}H NMR spectra were recorded on a JEOL FX 60Q instrument. Photopolymerization reactions were carried out in a Rayonet Photochemical Reactor (Southern N.E. Ultraviolet Co., Middleton, Conn.) equipped with 16 2537 Å Rayonet Photochemical Reactor Lamps.

RESULTS AND DISCUSSION

Synthetic routes used for the preparation of phosphatidyl cholines bearing one and two methacrylate groups, respectively, are outlined in Figure 1. Esterification of 12-hydroxydodecanoic acid with methacryloyl chloride followed by chromatographic purification (silica gel, ethyl acetate--hexane) afforded a 54% yield of 1; subsequent anhydride formation ($ClCO_2C_2H_5/(C_2H_5)_3N$) was quantitative (19). L-α-lysophosphatidyl choline palmitoyl (0.05 g, 0.10 mmol) was esterified (20) with 2 (0.084 g, 0.152 mmol), and the resulting lipid (3), was purified by passage through an AG-50-X8 resin, followed by column chromatography (silica gel, $CHCl_3$--CH_3OH); the isolated yield was 0.063 g (82%). Similar esterification of L-α-glycerophosphoryl choline produced a 29% yield of 4. Lipids 3 and 4 gave the expected TLC behavior, ir and ^{1}H NMR spectra plus correct elemental analyses.

Vesicles derived from 3 were prepared by sonicating 6 mg of the lipid in 3 mL of $D_2O(H_2O)$ for 5 min. at 50°C (Heat Systems Model W-225 R bath type, 155 watts), manually shaking the suspension for 5 min., and continued sonication for an additional 1 h. A Fourier Transform ^{1}H NMR spectrum of the re-

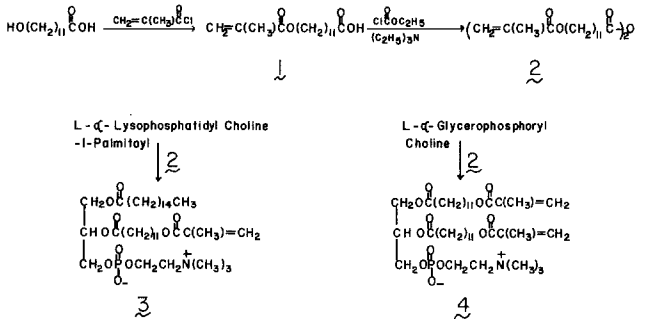

Figure 1. Syntheses of phosphatidyl cholines bearing one or two methacrylate groups.

sulting clear solution showed the presence of the intact vinylidene group. Electron micrographs recorded on a Philips 400 TEM microscope indicated the formation of closed vesicles having diameters ranging between 350 and 1400 Å. In order to confirm that no significant polymerization or lipid decomposition occurred during sonication, an aqueous preparation was extracted with chloroform; recovery of 3 was quantitative.

Vesicle polymerization reactions were carried out by irradiating samples at 254 nm, and were complete after 1 h, as evidenced by ^{1}H NMR spectroscopy. Electron micrographs established that the resulting vesicles retained their spherical shape and approximate size (Figure 2). Attempted extraction with chloroform failed to remove any lipid monomer, oligomer or polymer present in the aqueous phase. Removal of water under reduced pressure (0.1 mm, 20° C) followed by ir analysis of the residue (Nujol) indicated the complete disappearance of monomer. Improved vesicle stability was demonstrated by their response to the addition of ethanol (1) and also their shelf life. When subjected to dilution with 25% ethanol (v/v), the turbidity of the polymerized vesicle suspension remained unchanged. Similar experiments performed with nonpolymerized vesicles resulted in a 62% decrease in turbidity. While vesicles derived from 3 precipitated on standing after 48 h, polymerized preparations were stable for more than two weeks.

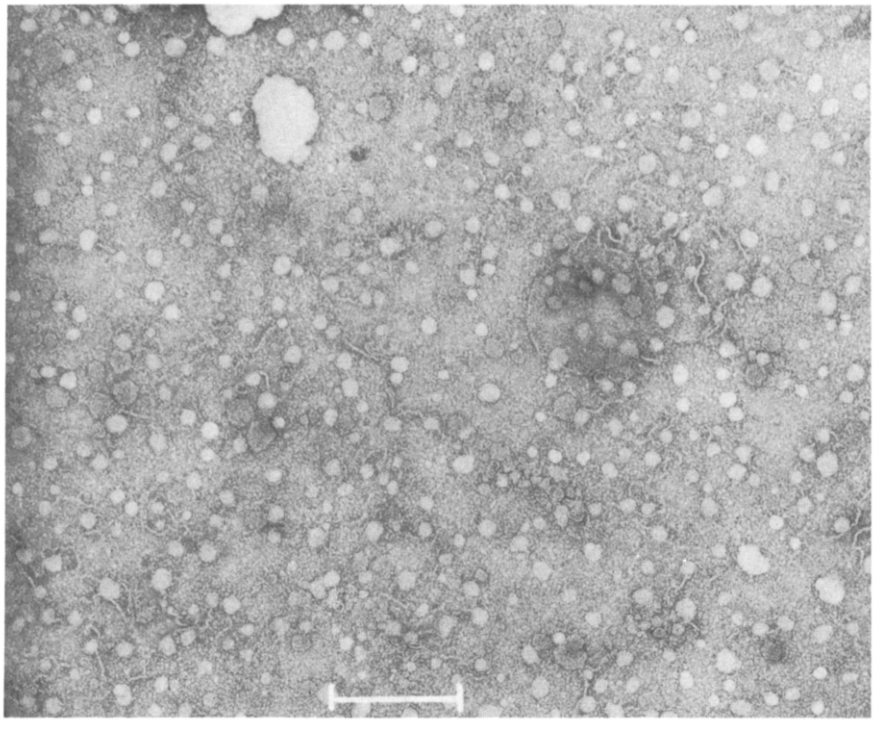

Figure 2. Electron micrograph of polymerized vesicles derived from 3. Bar represents 3000 Å.

Entrapment and permeability experiments provided additional evidence that these polymeric vesicles are sealed and established their controllable time-release action. After sonic dispersal of 3 in water containing 1 μCi of [^{14}C] sucrose, and after polymerization, nonentrapped sucrose was removed by gel filtration on Sephadex G-50 with pure water as the eluent. Vesicles

TABLE 1. RETENTION OF [^{14}C] SUCROSE

Vesicle	Retention after 4 h, %	Retention after 8 h, %
3 (nonpolymerized)	29	16
3 (polymerized)	65	48
80% 3 + 20% 4 (polymerized)	79	69

recovered in the void volume of the column contained ca. 0.4% of the sucrose. Within experimental error, nonpolymerized vesicles of 3 and copolymerized vesicles of 3 and 4 (80 mol% of 3) entrapped the same percentage of sucrose. Immediately after gel filtration, 1-mL aliquots of the vesicle suspension were added to pre-soaked seamless cellulose bags and placed in beakers containing 50 mL of distilled water (22°C). Dialysis data presented in Table 1 show that homopolymerization and copolymerization reduce and control leakage rates.

Further characterization of these and related phosphatidyl choline vesicles are now in progress. Complete details of these studies plus results of *in vitro* and *in vivo* drug delivery experiments will be reported in due course.

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