Structural Organization of Cationic *O*-Ethylphosphatidylcholines and Their Lipoplexes

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Introduction

Because of their potential application in gene therapy and drug delivery applications [1, 2], positively charged lipids are now being intensively synthesized and studied. However, comprehensive knowledge of the organization of cationic lipid aggregates, the process of cationic lipid-DNA assembly, and the correlation between the structure and activity of these complexes is still deficient, and the synthesis of new compounds for transfection is mostly empirical. Among the numerous cationic "lipoids" synthesized and tested recently, phosphatidylcholine triesters (Fig. 1) [3, 4] are the only membrane lipid derivatives shown to be metabolized by cells. They are thus considered promising nonviral transfection agents. These compounds are chemically stable, hydrate well, and form liposomes, which readily fuse with anionic lipid vesicles [5]. Numerous parameters of the preparation protocols are shown to modulate the structure and morphology of their complexes with DNA [6], which, in turn, influence their transfection activity, but all the liquid crystalline members of this group exhibit high transfection efficiency provided proper attention is given to the mechanical aspects of lipoplex formation.

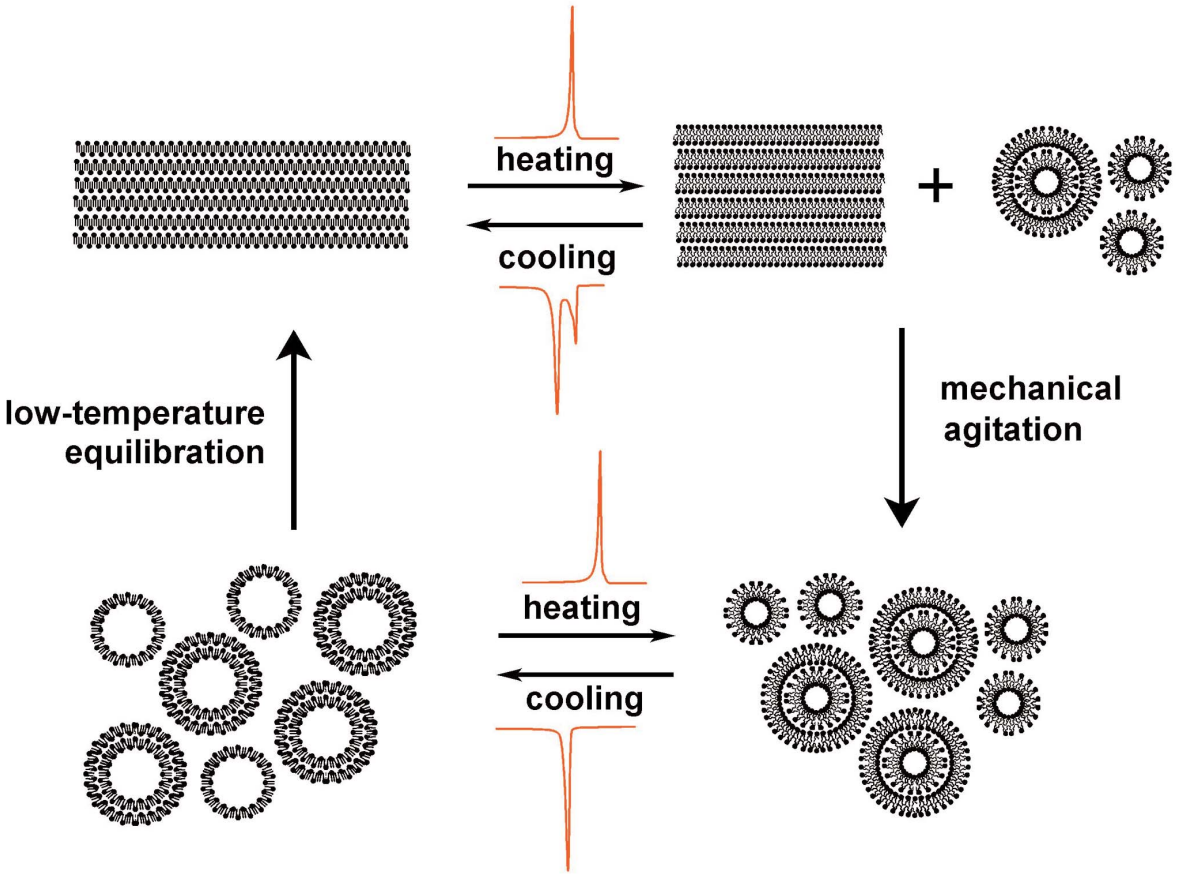

FIG. 1. Diagram of the morphological changes in EDPPC dispersions. The equilibrium low-temperature arrangement appears to be lamellar sheets, with chain interdigitation. Upon heating, liposomes and lamellar sheets (both noninterdigitated) coexist, which the mixture fully converts into liposomes (apparently the equilibrium liquid crystalline phase arrangement) only after mechanical treatment. Cooling back to the gel phase produces gel-phase liposomes, which convert back into lamellar sheets only after prolonged low-temperature exposure.

Methods and Materials

Triflate derivatives of dipalmitoyl ethylphosphatidylcholine (EDPPC), dimyristoyl ethylphosphatidylcholine (EDMPC), and dioleoyl ethylphosphatidylcholine (EDOPC) were synthesized as previously described [3, 4]. Herring sperm DNA (Invitrogen, Carlsbad, CA), 10 mg/mL solution in water, was used. High-sensitivity microcalorimetric measurements were performed by using a VP-DSC microcalorimeter (MicroCal Inc., Northampton, MA). Scans were performed at 0.2-0.5°C/min. For dry samples, a DSC 550 (Instrument Specialists Incorporated, Spring Grove, IL) heat-flow calorimeter was used. Small-angle x-ray diffraction (SAXD) measurements were performed at Bio-CAT beamline 18-ID and DND-CAT beamline 5-ID-D at the APS by using 12- to 15-keV x-rays. 2-D diffraction patterns were recorded by using high-sensitivity charge-coupled device (CCD) detectors. Sample-to-detector distance was 1.8-2 m. Silver behenate (DuPont, Wilmington, DE) was used as a calibrant (d_{001} = 58.376 Å). For temperature control, either a THMS600 thermal stage (Linkam Sci Instruments, Surrey, England) or a NESLAB programmable (Thermo NESLAB, Portsmouth, NH) water bath was used. Exposure times were typically 0.5-2 s. Diffraction intensity versus reciprocal space s plots were obtained by radial integration of the 2-D patterns done with the interactive data-evaluating program FIT2D [7]. Micrographs of lipid samples were taken by using a light microscope (Nikon Optiphot) that uses differential interference optics. Images were recorded with a video camera (MTI 65) connected to a personal computer by means of a Studio DC10 Plus (Pinnacle Systems, Inc., Mountain View, CA) video capturing system.

Results and Discussion

The saturated ethylphosphatidylcholines exhibit gel-liquid crystalline phase transitions at temperatures close to those of the parent phosphatidylcholines. Below the transition, they arrange into a gel phase with interdigitated hydrocarbon chains [8, 9]. Their liquid-crystalline phase is characterized by a significantly thinner water layer between the bilayers when compared with the phosphatidylcholines. Thermodynamic parameters of the gel-liquid crystalline phase transition of the hydrated EDPPC are virtually unchanged upon dehydration or in the presence of DNA. Thus, dry EDPPC exhibits a melting transition at the same temperature as when it is fully hydrated or involved in lipoplexes. This is taken to be a consequence of the reduced ability of ethyl-phosphatidylcholines to form hydrogen bonds. The interdigitated chain arrangement typical for the hydrated EDPPC gel bilayers is preserved and even probably enhanced in the dry state.

The cooling behavior of the hydrated EDMPC and EDPPC is sensitive to the sample history. It reflects the existence of two types of lipid aggregates — lamellar sheets and vesicles, the former being the equilibrium state for the gel phase, and the latter being the equilibrium state for the liquid crystalline phase (Fig. 1). The interconversion between these different morphologies is slow and probably kinetically hindered; thus, it does not correlate well with the much faster chain melting transition. Formation of lamellar sheets is promoted by low-temperature standing, while their conversion to vesicles is promoted by high-temperature mechanical agitation. High ionic strength facilitates the sheet-to-vesicle conversion. Similarly to its precursor DPPC, EDPPC exhibits a subtransition after a prolonged low-temperature exposure [10].

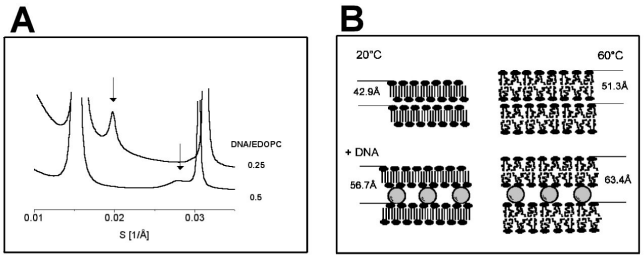

FIG. 2. A shows SAXD patterns of DNA/EDOPC samples at 1:2 and 1:4 molar ratio (arrows point at the peaks originating from the DNA-DNA in-plane correlation). B shows proposed molecular arrangement.

In the EDOPC/DNA complexes, DNA is ordered in a 1-D lattice sandwiched between the lipid bilayers. The interaxial spacing of DNA decreases from 50 Å at a DNA/EDOPC 1:4 charge ratio to 32 Å in the 1:1 and 2:1 samples. When added to EDPPC, DNA incorporates into the interbilayer space in both the gel phase and the liquid crystalline phase, and it increases the lamellar repeat distance by 12-15 Å. The melting transition temperature is not changed by the addition of an isoelectric amount of DNA. In the gel phase, the lipid chain interdigitation seems to be preserved, and the limiting DNA interaxial separation is ~30 Å. Thus, the interdigitated gel phase tolerates the inclusion of DNA between the bilayers (Fig. 2). Such complexes of DNA sandwiched between interdigitated gel lipid bilayers are reported for the first time [10]. In compositions containing excess DNA, another population of lipid/DNA aggregates of different organization possibly forms.

Acknowledgments

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