Self-Assembling DNA-Lipid Particles for Gene Transfer

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Purpose. We have demonstrated that a heteromolecular complex consisting of cationic lipids and DNA can be prepared and isolated (1). Cationic lipids bind DNA through electrostatic interactions. However, when sufficient lipids are bound to DNA the physical and chemical properties of the complex are governed by hydrophobic effects. Here we describe an approach where this hydrophobic complex is used as an intermediate in the preparation of lipid-DNA particles (LDPs).

Methods. The approach relies on the generation of mixed micelles containing the detergent, *n*-octyl β-D-glucopyranoside (OGP), the cationic lipid, N-N-dioleoyl-N, N-dimethylammonium chloride (DODAC), and selected zwitterionic lipids, 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) or egg sphingomyelin (SM).

Results. When these micelles were prepared at low detergent concentrations (20 mM OGP) and combined with pCMVβ DNA, LDPs spontaneously formed. The mean diameter of these particles as measured by quasielastic light scattering was 55–70 nm, a result that was confirmed by negative stain electron microscopy. Further characterization of these LDPs showed that DNA within the particles was inaccessible to the small fluorochrome TO-PRO-1 and protected against DNase I degradation. LDPs could also be prepared in high concentrations of OGP (100 mM), however particles formed only after removal of OGP by dialysis. Particles formed in this manner were large (>2000nm) and mediated efficient transfection of Chinese hamster ovary cells. Transfection activity was greater when the lipid composition used consisted of SM/DODAC. Small particles (<100nm) prepared of SM/DODAC were, however, inefficient transfecting agents.

Conclusions. We believe that LDP formation is a consequence of the molecular forces that promote optimal hydrocarbon-hydrocarbon interactions and elimination of the hydrocarbon-water interface.

KEY WORDS: gene transfer; cationic lipid; DNA complexes; hydrophobic effect.

INTRODUCTION

In an effort to develop better defined lipid-based carriers for gene transfer, we have characterized the binding of cationic lipids added in monomer or micellar form to DNA (1,2). The complex that results from this binding reaction is hydrophobic, a conclusion supported by data demonstrating that the complex can be isolated in organic solvents. The fact that lipid-polynucleotide binding results in the formation of a hydrophobic complex is surprising and we believe that the binding reaction is of general interest in terms of developing a better understanding of the chemical and physical attributes of a novel self-assembling structure. The lipid-DNA complex can serve as a self-assembling hydrophobic intermediate which, in the absence of stabilizing factors, will form particles. In this study we form lipid-DNA complexes using a method that consists of preparing mixed micelles containing a neutral detergent and cationic lipids.

MATERIALS AND METHODS

Materials

Egg sphingomyelin (SM) and 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) were purchased from Avanti Polar Lipids (Alabaster, AL). N-N-dioleoyl-N, N-dimethylammonium chloride (DODAC) was synthesized and supplied by Steven Ansell of INEX Pharmaceuticals Corp. (Vancouver, B.C.). TO-PRO-1 was purchased from Molecular Probes Inc. (Eugene, OR). Dialysis membrane (SPECTRA/POR, mwco:12,000–14,000) was purchased from Fisher Scientific (Ottawa, ON). DNase I (from bovine pancreas) and *n*-octyl β-D-glucopyranoside (OGP) were purchased from Sigma Chemical Company (St. Louis, MO). All other chemicals used were reagent grade and all solvents used were HPLC grade. The 7.2 kb pCMVβ (GenBank Accession No. U02451) encoding *Escherichia coli* β-galactosidase was obtained from CLONTECH Laboratories Inc. (Palo Alto, CA) and was isolated and purified using standard techniques (1,3).

Preparation of Lipid DNA Particles (LDPs)

The method for the preparation of LDPs was based on detergent dialysis. The protocol involves the preparation of solutions of pCMVβ DNA in OGP and lipid-detergent mixed micelles. DODAC and the neutral lipid were dissolved in the same concentration of OGP used to dilute the DNA. To ensure that the lipids were completely dissolved, the mixtures were heated to 50°C for 5 min and vortexed vigorously. Individual solutions were prepared with or without neutral lipid. When there was no neutral lipid involved, the DNA was added to the DODAC solution followed by gentle vortexing and then incubated at room temperature for 30 min. When the neutral lipid was present, the detergent solution containing DNA was mixed with the detergent solution containing the neutral lipid. This mixture was incubated for 30 min at room temperature and then added to detergent solution containing the cationic lipid DODAC. To remove detergent, mixtures were transferred to dialysis bags and dialyzed against six changes of sterile water over 72 hrs. The volume of each sample was less than 1 mL.

Measurement of LDP Formation, Particle Size and Liposome Dissolution

LDP formation was evaluated by measuring changes in 90° light scattering intensity at 600 nm. This technique was

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ABBREVIATIONS: LDP, Lipid-DNA particle; β-gal, β-galactosidase; DODAC, N-N-dioleoyl-N,N-dimethylammonium chloride; DOPE, 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine; SM, egg sphingomyelin, OGP, n-octyl β-D-glucopyranoside; DNase I, deoxyribonuclease I; CHO, Chinese hamster ovary; TO-PRO-1, thiazole orange monomer; QELS, quasielastic light scattering, CMC, critical micelle concentration.

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also used to assess the ability of OGP to solubilize pre-formed liposomes of DODAC or SM. Multilamellar liposomes were prepared at a final lipid concentration of 1.0 mM by hydrating powdered lipid in distilled water at 60°C. The lipid suspensions were sonicated for 5 min (100 watts, 90% duty cycle, at 20kHz) using a probe sonicator (Sonifier Cell Disrupter 350, Branson Sonic Power Co., Danbury, CN) to produce a homogeneous suspension. For the lipid dissolution measurement, an aliquot of the lipid suspension was diluted with distilled water to a final lipid concentration of 0.2–1.0 mM. This lipid suspension was titrated with 200 mM OGP and mixed well prior to measuring light scattering intensity at room temperature using a Luminescence Spectrometer 50B (Perkin Elmer). The size distribution of LDPs was measured using quasielastic light scattering (QELS) determined with NICOMP Submicron Particle Sizer (model 270, Pacific Scientific, Santa Barbara, CA). QELS measurements were made at a photopulse rate of 300–400 kHz at room temperature using 'solid particle' analysis. The size distribution was analyzed using Gaussian distribution for direct comparison since the goodness-of-fit parameter χ^2 was less than 5 for most samples prepared using 20 mM OGP.

Evaluation of DNA Condensation and Stability Against DNase I

The fluorochrome TO-PRO-1 was used to evaluate DNA condensation as described elsewhere (1,4). LDP formulations containing 1 μg DNA were diluted to 0.6 mL in water or OGP (final concentration of 25–30 mM). TO-PRO-1 was added to a final concentration of 1×10^{-6} M and the fluorescence intensity was measured after 5 min at room temperature using a Luminescence Spectrometer 50B (Perkin Elmer) with excitation and emission wavelengths of 509 nm and 533 nm, respectively. The condensation index was calculated by $(I_o - I)/I_o$, where I refers to the fluorescence intensity of the LDP formulation and I_o refers to the flourescence intensity obtained from a reference water solution containing a DNA concentration equivalent to that in the LDPs.

To evaluate the protective effect of lipids on DNA, 100 μl of the formulations containing 1 μg pCMVβ DNA were incubated with 0.67 unit of DNase I at 37°C for 20 min in the presence of buffer (0.05 M Tris-HCl pH 8.0, 0.01 M $MgSO_4$, 0.1 mM dithiothreitol) or 20 mM OGP. The enzymatic reactions were stopped by the addition of 5 μl of 0.5 M EDTA. DNA was extracted in the presence of added NaCl using a modified Bligh and Dyer extraction procedure (1). DNA was precipitated and subjected to electrophoresis on a 0.8% agarose gel in TBE buffer (89 mM Tris-Borate, 2 mM EDTA, pH 8.0) as described previously (1,5).

Electron Microscopy

LDPs were evaluated by electron microscopy (EM) using two methods. First, the samples were prepared for negative stain EM by placing a drop of a concentrated LDP formulation (3 mM lipid) onto a formvar coated nickel grid. After 1 min the sample was carefully drawn away using filter paper and stained with a 2.5% ammonium molybdate solution. The stained samples were immediately examined and photographed using a Carl Zeiss EM10CR electron microscope operated at 80 Kv. Second, LDPs were prepared for freeze-fracture EM, where a sample of concentrated LDP formulation (15 mM lipid) was mixed with glycerol (25% v/v), frozen in a freon slush, and subjected to freeze-fracture employing a Balzers BAF 400D apparatus. Micrographs were obtained using a JEOL Model JEM-1200EX electron microscope.

In Vitro Cell Transfection

CHO cells (American Type Collection Culture, Rockville, MD) were plated at 2×10^4 cells per well in a 96 well culture plate (Costar, Cambridge, MA) in αMEM supplemented with 10% Fetal Bovine Serum (FBS). The cells were grown for 24 hrs in a 37°C 5% CO_2 incubator and were 40–50% confluent at the time of transfection. Media was removed from cells prior to addition of 100 μl of diluted LDP formulations prepared from 25 μl LDP formulation containing 0.3–1.2 μg DNA and 75 μl of αMEM supplemented with 10% FBS. Cells were incubated at 37°C for 4 hrs, prior to the addition of 100 μl of αMEM (10% FBS) and a further incubation at 37°C for 2 days. β-galactosidase activity was assayed as described elsewhere using chlorophenol red galactopyranoside (CPRG, 1 mg/mL in 60 mM Na_2HPO_4, 1 mM $MgSO_4$, 10 mM KCl, 50 mM β-mercaptoethanol) as a substrate (5). All assays were evaluated in at least 3 wells per plate and the values are reported as means ± standard deviations.

RESULTS AND DISCUSSION

In this study lipid-DNA complexes were formed using a method that consisted of preparing mixed micelles containing a non-ionic detergent and cationic lipids. When these micelles were combined with plasmid DNA, particles formed either spontaneously or after detergent removal. Evidence of LDP

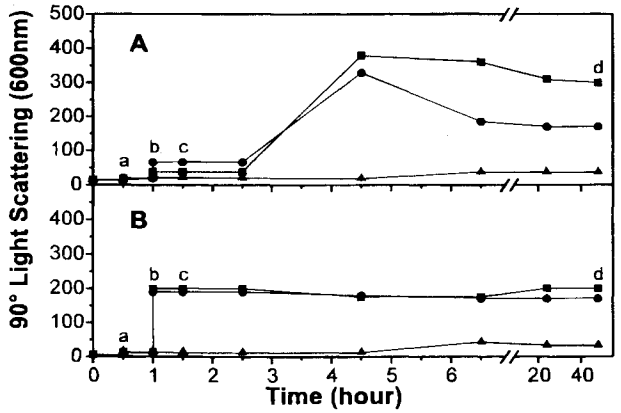

Fig. 1. Changes in sample turbidity measured by 90° light scattering at 600 nm during the preparation of DNA-lipid particles in the presence of 100 mM (A) or 20 mM (B) *n*-octyl β-D-glucopyranoside (OGP). Samples were prepared in steps as indicated below (a–d). Three samples consisting of 200 μl of 20 μg pCMVβ, 400 μl of 0.8 mM SM and 400 μl of 0.8 mM DODAC were prepared in OGP. Step a: The DNA solution was mixed with SM (squares) or 400 μl of OGP (circles) and the mixture was left to equilibrate at 25°C for 30 min. Step b: The DNA-SM-OGP or DNA-OGP mixture was then added to the DODAC solution. For the lipid control 400 μl SM was added to 400 μl DODAC (triangles). Step c: The final mixture was dialyzed against a 500-fold excess of distilled H_2O which was changed every 12 hrs for 2 days. Step d: The dialysis procedure was stopped after 2 days.

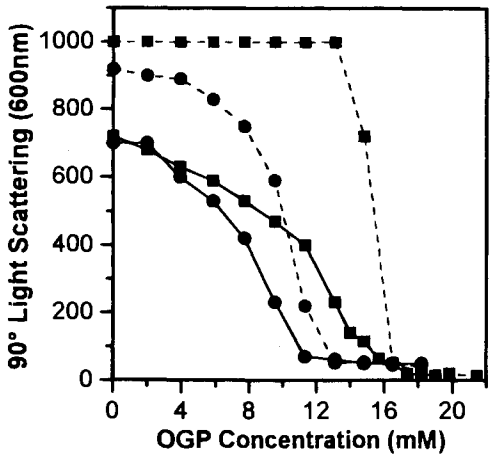

Fig. 2. Solubilization of pre-formed DODAC (●) and SM (■) vesicles in OGP as measured by 90° light scattering. Liposomes were prepared at a final lipid concentration of 0.2 mM (solid lines) and 1.0 mM (broken lines) by hydrating dried lipid in distilled water at 60°C. Subsequently, OGP was titrated into the lipid solutions at room temperature using a 200 mM stock solution. The light scattering measurements were performed at room temperature using a Luminescence Spectrometer 50B (Perkin Elmer) with excitation and emission wavelength of 600 nm (slit width of 2.5 nm).

formation is provided in Figure 1, where changes in light scattering at 600 nm were measured following the addition of detergent solution of DNA (pCMVβ) to mixed micelles of DODAC and OGP. An additional variable that was evaluated was the effect of the neutral lipid sphingomyelin (SM) on particle formation. SM was used as an alternative to the zwitterionic non-bilayer forming lipid DOPE, which is typically used as a helper lipid in the preparation of cationic liposomes used for gene transfer. Importantly, SM has a choline phosphate group linked to ceramide, a lipid which has hydrocarbon chains linked *via* an amide bond which is stable to acid hydrolysis. Further, in contrast to DOPE the solubility of the bilayer forming SM in OGP was readily achieved at the critical micelle concentration (CMC) of OGP (see below). Lipids and DNA were pre-dissolved in OGP at a concentration of either 100 mM (Figure 1A) or 20 mM (Figure 1B). In 100 mM OGP there were no significant changes in solution turbidity observed when DNA was added to DODAC/OGP mixed micelle solution in the presence and absence of SM. After 3 hrs of dialysis the solutions became turbid and light scattering increased, a reflection of increased particle size and/or aggregation. After 4.5 hrs, a decrease in light scattering was observed for systems prepared in the absence of SM, a result of the formation of larger visible aggregates. When the samples were prepared in 20 mM OGP (Figure 1B), a concentration close to the CMC of OGP in the absence of added lipids (6,7), light scattering increased at the time when DNA was added to DODAC/OGP micelles. This increase in turbidity is indicative of spontaneous particle formation in the presence of detergent.

To establish that particle formation was not due to the initial formation of cationic lipid vesicles and subsequent cationic vesicle-mediated DNA aggregation, the concentrations of OGP required to solubilize pre-formed liposomes of DODAC and SM were determined. The results, shown in Figure 2, were obtained by measuring light scattering at 600 nm as pre-formed DODAC or SM liposomes were mixed with increasing concentrations of OGP. The light scattering intensities decreased with increasing OGP concentration and became negligible above 12 mM and 16 mM OGP for DODAC and SM vesicles, respectively. The decrease in light scattering is associated with dissolution of the pre-formed liposomes and the formation of

Table 1. Characteristics of Lipid-DNA Particles Formed with pCMVβ and DODAC/SM (1:1 Mole Ratio) Prepared Using 20 mM and 100 mM OGP Before and After Dialysis

	Mean diameter ± SD(nm)[b]			
(Cation/anion[a])	Before dialysis	After dialysis	Aggregation state[c]	Condensation index[d]
100 mM OGP				
1:1	ND[e]	>2000	++	0.759
2:1	ND	>2000	+	0.927
4:1	ND	>2000	+	0.974
8:1	ND	>2000	++	0.991
20 mM OGP				
1:1	71.2 ± 37.0	192 ± 110	-	0.875
1.5:1	63.1 ± 33.8	119 ± 76	--	0.985
2:1	60.8 ± 33.3	58.6 ± 37.8	--	0.991
4:1	56.7 ± 32.0	55.9 ± 32.6	--	0.994
8:1	64.6 ± 33.4	66.4 ± 35.4	--	0.989

[a] The charge ratio of cationic lipids to DNA phosphate groups.
[b] Mean diameter was measured using QELS techniques as described in the Methods. The instrument used to evaluate particle size is accurate only under conditions where the mean particle size is less than 1.0 μm.
[c] The aggregation state of the formulations after dialysis was evaluated qualitatively through visual inspection of the samples and scored as follows: ++ large aggregates that settle out of solution within 5 min after sample mixing; + small to medium aggregates present but the solution retains a uniform "milky" appearance; -no obvious aggregates unless viewed by microscopy; --no aggregates and homogeneous as assessed by QELS.
[d] DNA condensation index, a reflection of TO-PRO-1 binding to DNA in the presence and absence of lipid binding, was determined as described in the Methods.
[e] ND: not detectable because particles were not formed.

spheroidal mixed micelles (7,8). Dissolution of structures formed following hydration of DOPE were dependent on a variety of factors including temperature, detergent/lipid ratio and detergent type (F. Wong, unpublished observations). The results shown in Figure 2 demonstrate that it is unlikely that lipid vesicles form under conditions where the detergent concentration is equal to or greater than 20 mM. LDP formation in the presence of 20 mM OGP is therefore not due to DNA-mediated aggregation of cationic liposomes.

There are several parameters that we believe are critical in defining the characteristics of the particles formed using the techniques described here. The detergent concentration, and in particular, the lipid/detergent ratio will be a critical parameter. The CMC of OGP in the absence of added lipid is reported to be 20 mM (6,7). The liposome dissolution studies shown in Figure 2 suggest that the effective CMC of OGP decreases to 12 mM and 16 mM in the presence of DODAC or SM, respectively. Therefore, when LDPs formed spontaneously under conditions where the detergent concentration was low (20 mM OGP), the lipid concentration per given mixed micelle was high in comparison with that at the high detergent concentration (100 mM OGP) where no spontaneous LDP formation was observed. It can be suggested that a high lipid/detergent ratio in the mixed micelles is important for facilitating the transfer of cationic lipids or the binding of the cationic mixed micelles to DNA. We cannot preclude the possibility that the micelle structure (9,10) may also have a significant impact on LDP formation. Under conditions where LDPs were formed following dialysis of the solutions containing all the components in high detergent concentration, the point at which LDPs form will likely be when a critical concentration of detergent is achieved. The physical characterization of LDPs formed under these conditions will be a consequence of the rate of particle formation. It is predicted that this, in turn, would be dependent on the rate of detergent removal, the type of lipids and detergent used as well as the detergent concentration and lipid/detergent ratio. A detailed analysis of these factors is now underway.

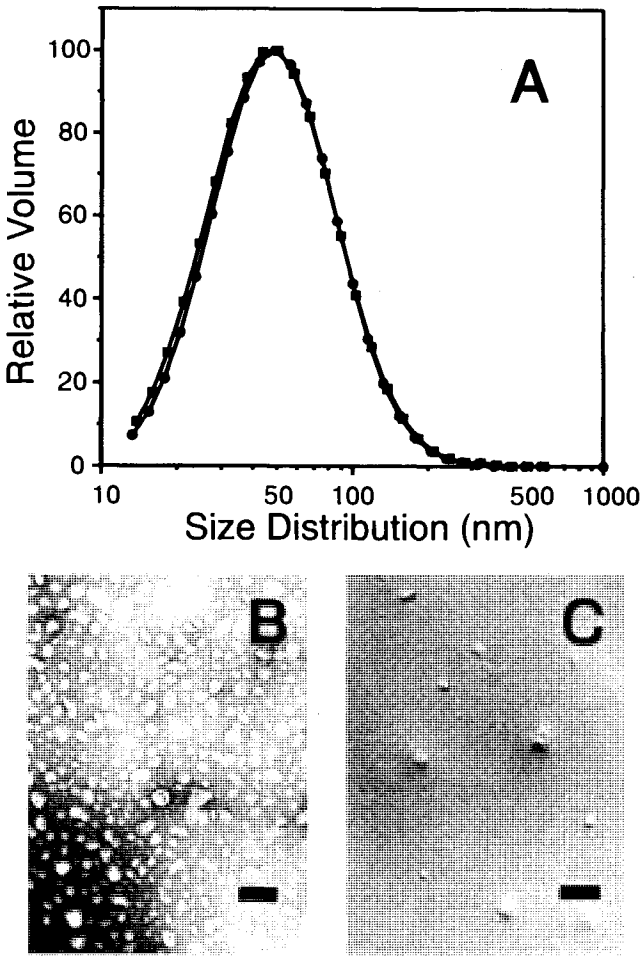

Fig. 3. Particle size distribution determined by QELS operating in solid particle analysis mode for a LDP formulation composed of pCMVβ and DODAC/SM (lipids were mixed at a 1:1 mole ratio and a charge ratio of 2:1 (+/−)) and prepared using 20 mM OGP before (●) and after (■) dialysis (A). The same LDP formulation after dialysis was also examined by electron microscopy (B, negative stain and C, freeze-fracture). Bar = 100 nm.

The physical characteristics of the LDPs, formed either spontaneously or following detergent removal, are summarized in Table 1 and Figure 3. The parameters evaluated include (i) particle size estimated by QELS and electron microscopy, (ii) the observed degree of aggregation/flocculation, and (iii) an assessment of TO-PRO-1 binding, an intercalating agent that fluoresces when bound to DNA (1,4). Since it is believed that LDP formation is dependent on cationic lipid binding to DNA, particle characteristics were assessed under conditions where the cationic lipid to anionic phosphate charge ratio was varied from 1:1 to 8:1. Under conditions where particle formation occurred following detergent removal (i.e. lipid and DNA mixtures prepared in 100 mM OGP) the resulting particles were large (>2000 nm) and aggregated (Table 1). This tendency to aggregate was dependent on the charge ratio. After LDP formation, the DNA assumed a structure that was not accessible to TO-PRO-1 intercalation. Accessibility to DNA intercalating dyes has been used as a measure of DNA condensation, a process that is associated with charge neutralization of DNA and formation of well defined toroid-like structures (11). In the context presented here, however, the condensation index is used simply to define whether DNA is in a form that can still bind the dye. This may be due to a structural change in DNA, the presence of DNA that is coated with lipid or, conversely, the presence of DNA electrostatically bound to lipid.

When low detergent concentrations (20 mM OGP) were used to promote spontaneous LDP formation there was, in general, no significant change in particle size or aggregation state as a function of detergent removal. Significant increases in particle size were observed when charge ratios of 1:1 and 1.5:1 were used (Table 1) and when DOPE was used as the neutral lipid component (data not shown). As shown in Figure 3A, QELS data indicated that for samples prepared using the 2:1 charge ratio, the particles were homogeneous and fit a Gaussian analysis with a mean diameter of 59 ± 38 nm. This result is comparable with observations made using negative stain electron microscopy (Figure 3B). Data obtained from freeze-fracture electron microscopic analysis of the particles (Figure 3C) was ambiguous. Regardless of sample concentration (up to 15 mM total lipid) there were only a few regions on the freeze-fracture replica that exhibited fracture surfaces typical of membrane bilayer structures. Instead, numerous bumps were detected on the replica consistent with the sugges-

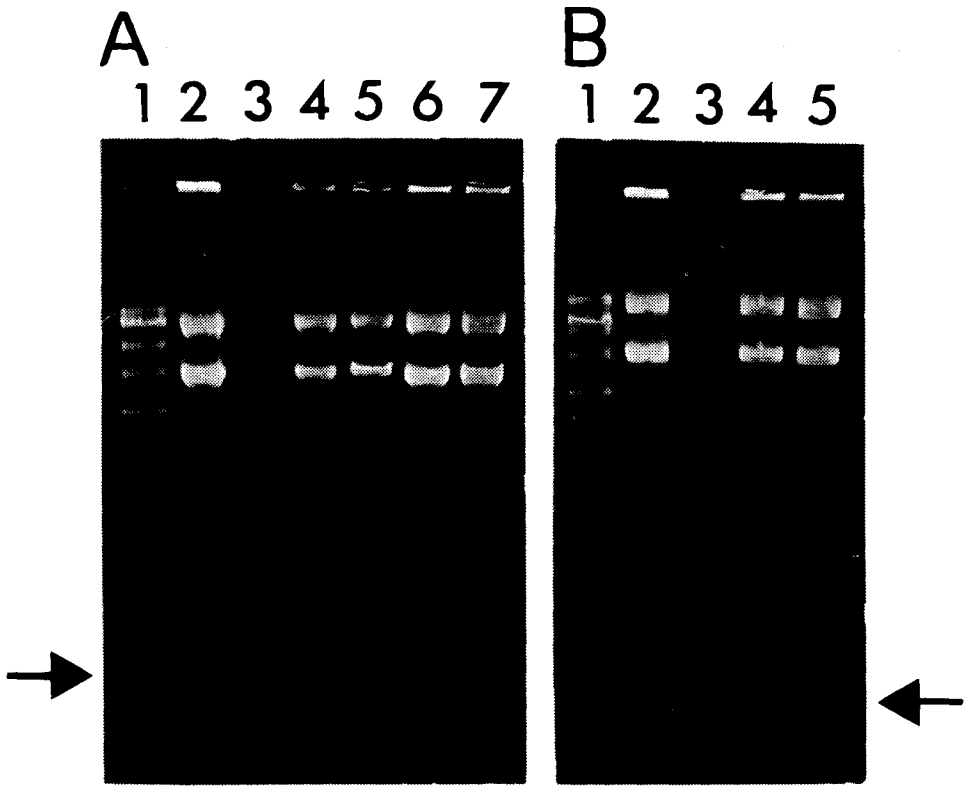

Fig. 4. Agarose gel electrophoresis of DNA isolated from formulations prepared in 100 mM and 20 mM OGP (charge ratio of 2:1 and SM/DODAC ratio of 1:1) and tested for DNase I sensitivity in the absence (A) and presence (B) of OGP. Panel A: molecular weight standards (lane 1), pCMVβ in the absence of added lipid or DNase I (lane 2), pCMVβ following incubation with DNase I (lane 3), DNA isolated from a dialyzed LDP formulation prepared using 100 mM OGP following incubations in the absence (lane 4) and presence (lane 5) of DNase I, and DNA isolated from particles prepared using 20 mM OGP and dialyzed following incubations in the absence (lane 6) and presence (lane 7) of DNase I. The first 3 lanes in panel B are identical to those in panel A except that pCMVβ was incubated in 20 mM OGP in the absence (lane 2) and presence (lane 3) of DNase I. DNA isolated from a formulation prepared in 20 mM OGP (prior to detergent removal) was incubated in the absence (lane 4) and presence (lane 5) of DNase I in 20 mM OGP. Arrow indicates degraded DNA.

tion that particles rather than liposomes were formed. DNA was accessible to TO-PRO-1 following spontaneous particle formation and condensation indices of less than 0.05 were typically measured prior to detergent removal. This result was unexpected and suggests that particle formation is not an indicator of whether DNA is condensed. After detergent removal, TO-PRO-1 intercalation was not observed and the resulting DNA condensation indices were high (≈1.0) (Table 1).

To further define the characteristics of the LDPs produced as a consequence of DNA-cationic lipid complex formation, we evaluated whether the DNA in the LDPs was protected against the endonuclease activity of DNase I. The results presented in Figure 4A show that after detergent removal, DNA within the particle remained intact in the presence of DNase I (lanes 5 and 7). Interestingly, DNA within particles that had formed spontaneously in the presence of 20 mM OGP remained intact in the presence of DNase I even in the absence of detergent removal (Figure 4B, lane 5).

These novel particulate systems were assessed for their use as plasmid delivery systems *in vitro*. Chinese hamster ovary (CHO) cell transfection studies using LDP formulations prepared using 100 mM OGP are presented in Figure 5 and are evaluated by enzyme production (β-galactosidase activity) as the end product of gene transfer. The transfection efficiency for these systems increased as the cationic lipid to DNA nucleotide phosphate (charge) ratio increased from 1:1 to 4:1 (Figure 5A). The decrease in transfection observed at the 8:1 ratio could not be explained by increased cytotoxicity, therefore it is suggested that this is due to some change in complex structure. Importantly, the transfection data presented in Figure 5 were obtained in the presence of fetal bovine serum (10%). This is in contrast to results obtained with pre-formed liposome/plasmid DNA aggregates which efficiently transfect only under conditions where serum is absent during the initial 4 hr incubation period. Particle-induced cell toxicity data are reflected by a reduction in enzyme activity/well observed with increasing amounts of LDP formulation prepared at the 4:1 charge ratio (Figure 5B). A significant difference between pre-formed liposome-DNA aggregates and the LDPs concerns the use of DOPE as a helper lipid required for optimal transfection (12–14). As shown in

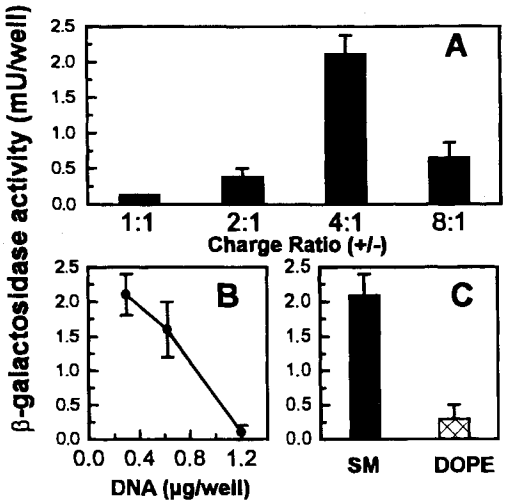

Fig. 5. *In vitro* Chinese hamster ovary (CHO) cell transfection using LDP formulations composed of pCMVβ and SM/DODAC (SM/DODAC mole ratio of 1:1 and charge ratio (+/−) of 1:1 to 8:1) prepared using 100 mM OGP followed by dialysis. (A) Influence of charge ratio (+/−) on β-galactosidase transfection. (B) Particle induced toxicity as measured by reduced β-galactosidase activity per well for formulations prepared using a charge ratio of 4:1. (C) β-galactosidase transfection achieved with large LDPs prepared using SM (solid bar) or DOPE (hatched bar) as the neutral lipid (charge ratio of 4:1 and DODAC to neutral lipid mole ratio of 1:1).

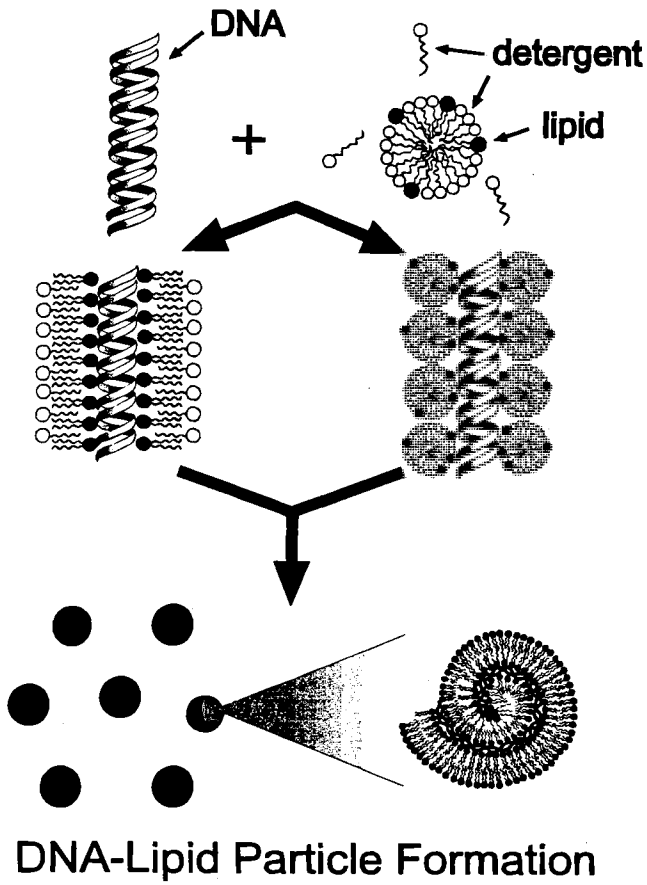

Fig. 6. Model describing the intermediates that may be involved in the generation of a novel lipid-DNA particle.

Figure 5C, large particles prepared using the detergent dialysis procedure with DODAC and SM were more effective in transfecting CHO cells *in vitro* than particles prepared using DODAC and DOPE.

The transfection activity of small LDPs prepared using DODAC and SM was negligible (data not shown) and because of difficulties in the preparation of DODAC/DOPE LDPs in 20 mM OGP we were unable to assess the activity of small (<100 nm) LDPs formed using this lipid composition. Preliminary results from our laboratory suggest, however, that the detergent concentration is more critical in terms of preparing small LDPs using DOPE as a second lipid component. We are presently characterizing the role of detergent type in solubilizing this non-bilayer forming lipid, assuming that incorporation of this lipid may be required in order to obtain effective gene transfer with small LDPs. Clearly, the influence of lipid composition on particle formation, particle characteristics, and transfection activity needs to be evaluated in greater detail.

In summary, we believe that LDP formation is a consequence of cationic lipid binding to DNA. A self-assembling system is envisioned where cationic lipid binding results in a significant change in the characteristics of DNA and is best illustrated by the fact that the complex can be isolated in organic solvents (1,2). In the presence of detergents, however, the mechanisms of self-assembly may not simply involve cationic lipid binding to DNA. As modeled in Figure 6, it is possible that cationic lipids solubilized in mixed lipid detergent micelles may serve as crosslinking structures capable of forming intra- and inter-molecular bridges within DNA strands and between DNA molecules. Alternatively, the cationic lipid binding reaction may be dependent on an equilibrium between cationic lipid associated with micelles (one phase) and that bound to DNA (an alternative phase). Regardless of how cationic lipids associate with DNA, LDP formation will likely be the result of the hydrophobic lipid-DNA complex adopting a structure that minimizes lipid acyl chain contact with water. The process whereby particles form is fascinating and is presumably a consequence of self-assembly processes mediated initially by electrostatic interactions and subsequently by hydrophobic effects. This approach will permit a level of control over physical (size and aggregation state) and chemical (lipid composition and particle charge) characteristics that is not feasible with previously described lipid-based carrier systems for DNA. We are presently assessing factors that influence LDP destabilization as well as methods that will facilitate binding of LDPs to internalized receptors overexpressed on the cell surface.

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