Lipid-DNA Complex Formation: Reorganization and Rupture of Lipid Vesicles in the Presence of DNA As Observed by Cryoelectron Microscopy

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ABSTRACT Cryoelectron microscopy has been used to study the reorganization of unilamellar cationic lipid vesicles upon the addition of DNA. Unilamellar DNA-coated vesicles, as well as multilamellar DNA lipid complexes, could be observed. Also, DNA induced fusion of unilamellar vesicles was found. DNA appears to adsorb to the oppositely charged lipid bilayer in a monolayer of parallel helices and can act as a molecular "glue" enforcing close apposition of neighboring vesicle membranes. In samples with relatively high DNA content, there is evidence for DNA-induced aggregation and flattening of unilamellar vesicles. In these samples, multilamellar complexes are rare and contain only a small number of lamellae. At lower DNA contents, large multilamellar CL-DNA complexes, often with >10 bilayers, are formed. The multilamellar complexes in both types of sample frequently exhibit partially open bilayer segments on their outside surfaces. DNA seems to accumulate or coil near the edges of such unusually terminated membranes. Multilamellar lipid-DNA complexes appear to form by a mechanism that involves the rupture of an approaching vesicle and subsequent adsorption of its membrane to a "template" vesicle or a lipid-DNA complex.

INTRODUCTION

Cationic lipid-DNA (CL-DNA) complexes are widely used for cell transfection in vitro. They are also promising candidates as nucleic acid delivery systems for in vivo gene therapy. CL-DNA complexes form spontaneously when DNA is combined with a suspension of lipid vesicles containing cationic lipids and zwitterionic "helper" lipids. Various formulations for in vitro use are commercially available and many more have been reported in the literature (Lasic, 1997, and references therein). While it has been shown that cationic lipids are essential for mediating DNA transfer into the target cell, to date, the precise nature and outcome of DNA interactions with lipids in such formulations are incompletely understood. Despite the wealth of empirical data available, it has proven surprisingly difficult to obtain an insight into the underlying interactions and mechanisms. This is unfortunate, since a better comprehension on a fundamental level could provide a starting point for the development of more efficient artificial gene delivery systems.

In this article we examine the structural characteristics of CL-DNA complexes by cryoelectron microscopy. We discuss the formation of two basic types of CL-DNA complex by elucidating the nature of structures formed from vesicles of well-defined size. We also argue that a plethora of intermediate stages exists, the propensity for their formation depending on the precise conditions under which complex formation is induced.

Current models for the structure of CL-DNA complexes

The present knowledge of complex morphology is based largely on studies of the final structure of CL-DNA associates by electron microscopy (EM), small angle x-ray scattering (SAXS), and atomic force microscopy (AFM). By using these and other techniques, a variety of structures was observed with a number of different CL preparations. Several models have been proposed to explain the findings.

An early model (Gershon et al., 1993) suggested that DNA is encapsulated in the interior of large unilamellar liposomes that form by liposome fusion in the presence of DNA. The finding that after combination of DNA and cationic liposomes, digestive enzymes and intercalating agents become unable to interact with the DNA has initially supported this notion.

Cryo and freeze-fracture electron microscopy, which revealed globular particles with attached fibers (Sternberg et al., 1994), gave rise to a different picture. Evidence was shown to suggest that the globular particles corresponded to aggregated liposomes while the fibers were held to be DNA surrounded by a lipid bilayer. Moreover, a structure in which DNA is entrapped in the water channels between lipids in the inverted hexagonal lipid phase was recently derived from SAXS data (Koltover et al., 1998).

AFM studies of DNA condensed on a supported cationic bilayer showed aligned and parallel DNA helices with an interaxial spacing of ~4 nm (Fang and Yang, 1997). This indicates that, in the presence of a lipid matrix, DNA can form well-ordered two-dimensional structures.

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Multilamellar lipid-DNA aggregates

There is increasing evidence that some CL formulations can form multilamellar complexes when combined with DNA. A popular model advocated in the pertinent literature describes these complexes as multilamellar structures of lipid bilayers alternating with monolayers of parallel DNA helices (Lasic and Templeton, 1996; Lasic et al., 1997; Rädler et al., 1997; Battersby et al., 1998).

In addition to the orientational order within each DNA layer, long-range orientational correlation between DNA in different layers has been predicted (Golubovic and Golubovic, 1998; O'Hern and Lubensky, 1998; Podgornik and Žekš, 1998). The conclusion was that DNA helices in different layers should essentially be aligned. This does not necessarily imply a *positional* correlation of the DNA helices, as found, for example, in the columnar phase. Rather, a new "sliding columnar" phase and a nematic lamellar phase have been proposed.

SAXS on multilamellar CL-DNA complexes showed a long periodicity of 6.5 nm (Lasic et al., 1997; Rädler et al., 1997). This periodicity was attributed to a 4-nm-thick lipid bilayer and an adjacent 2.5-nm-thick monolayer of adsorbed hydrated DNA. Moreover, a second, shorter periodicity of ~3.5–3.7 nm was observed by SAXS and identified with the interaxial separation of DNA helices in the DNA monolayer.

Consistent with the above-mentioned SAXS data, cryo-EM studies of different CL systems showed multilamellar structures as well. These often comprised 10 or more lamellae. The interlamellar spacing found in these studies ranged from 6.5 nm to 7.8 nm, depending on the lipids used (Gustaffson et al., 1995; Lasic et al., 1997; Lasic and Templeton, 1996). The second, shorter periodicity, attributed to parallel helices in the DNA monolayer, was observed by cryo-EM as well. Lasic et al. (1997) found a periodicity of 3.5 nm, while our group observed a periodicity of 4 nm for the system dimyristoyl phosphatidylcholine/dimethylaminoethane-carbamoyl-cholesterol/DNA (DMPC/DC-Chol/DNA) (Battersby et al., 1998).

Moreover, in DNA complexes with the lipids DC-Chol and DMPC, cryoelectron microscopy provided evidence for long-range orientational correlation between the polymer strands in different layers (Battersby et al., 1998). In addition to the strong short-range interaction between DNA helices in one layer and between DNA and the adjacent lipids, there was evidence of a long-range interaction across the bilayer stacks in the multilamellar complex, leading to the alignment of DNA between different DNA monolayers.

In this study, the interaction between DNA and a homogeneous suspension of small unilamellar vesicles composed of DMPC and DC-Chol was investigated. It was found that the morphology of CL-DNA structures is largely influenced by the initial amount of DNA in the sample. In the CL-DNA complexes, partially open bilayer segments were often observed. To our knowledge, these segments have not been previously reported.

MATERIALS AND METHODS

The cationic lipid 3β[*N*-(*N*′,*N*′-dimethylaminoethane)-carbamoyl] cholesterol (DC-Chol) was obtained from Bachem Biochemica (Heidelberg, Germany) and the zwitterionic lipid 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) was purchased from Avanti Polar Lipids (Alabaster, AL). Small unilamellar vesicles consisting of DMPC/DC-Chol in a molar ratio of 3:2 were prepared by repeated sequential extrusion (Mayer et al., 1986) through 400- to 50-nm polycarbonate filters. The preparation of small vesicles and all experiments were performed in 50 mM triethanolamine, 0.54 mM EDTA buffer adjusted to pH 7.4 with hydrochloric acid. Total lipid concentration before the addition of DNA was 4.8 mM.

Linear DNA was prepared by digesting calf thymus DNA (Sigma Chemical Co., St. Louis, MO) with the restriction endonuclease *Eco*RV (Strategene, La Jolla, CA) into fragments with an average length of 6000 bp. The DNA was purified by repeated phenol/chloroform extraction (Maniatis et al., 1989). The buffer was exchanged by exhaustive dialysis against the appropriate buffer with a molecular weight cutoff of 3. DNA concentration was determined by measuring the absorbance at a wavelength of 260 nm.

CL-DNA complexes were formed by combining the aqueous solution of DNA and a suspension of the extruded DMPC/DC-Chol small unilamellar vesicles at a nominal DNA/lipid charge ratio of either 0.2 or 0.9. The final lipid concentration was 1.6 mM. Images of the CL-DNA complexes and of the original liposome suspension without DNA were taken no earlier than 24 h and no later than 32 h after sample preparation.

Specimens for cryoelectron microscopy were prepared in the usual manner by vitrification in liquid ethane. A Philips CM 120 Biofilter microscope (de Jong et al., 1996), equipped with a postcolumn energy filter, was used at an acceleration voltage of 120 kV and a defocus of 1 μm. Magnification of 55,000 allowed a pixel width of 0.46 nm. Images were collected under low dose conditions (Grimm et al., 1998), with the dose being <0.1 electrons/nm^2.

Vesicle diameters and membrane thicknesses were directly measured on the magnified electron micrographs. Periodicities of adsorbed DNA helices were obtained by both direct measurement and Fourier transformation of the EM images. Size distribution was determined by manually measuring, and counting, individual CL-DNA complexes. Only quasi-spherical vesicles were considered, since the surface area of such vesicles can be estimated most easily and accurately from the radius.

RESULTS

The suspension without DNA contains only unilamellar vesicles with a narrow size distribution

As revealed by the dynamic light scattering and EM measurements (Fig. 1), the extruded suspension without added DNA contains a homogeneous population of small unilamellar vesicles with diameters of 65 nm ($\pm$18 nm). No multilamellar structures are observed before DNA addition. The bilayer thickness is 5.1 $\pm$ 0.4 nm, as measured from the electron micrographs.

Multilamellar complexes are more abundant in samples with low nominal DNA/lipid charge ratio than in samples with higher charge ratio

Combination of the small unilamellar vesicles with the polymer at a DNA/lipid charge ratio of 0.2 induces a drastic change in the vesicle's morphology. This produces a wealth of large, mainly multilamellar, complexes (see, e.g., Fig. 2). In agreement with previous findings (Battersby et al., 1998),

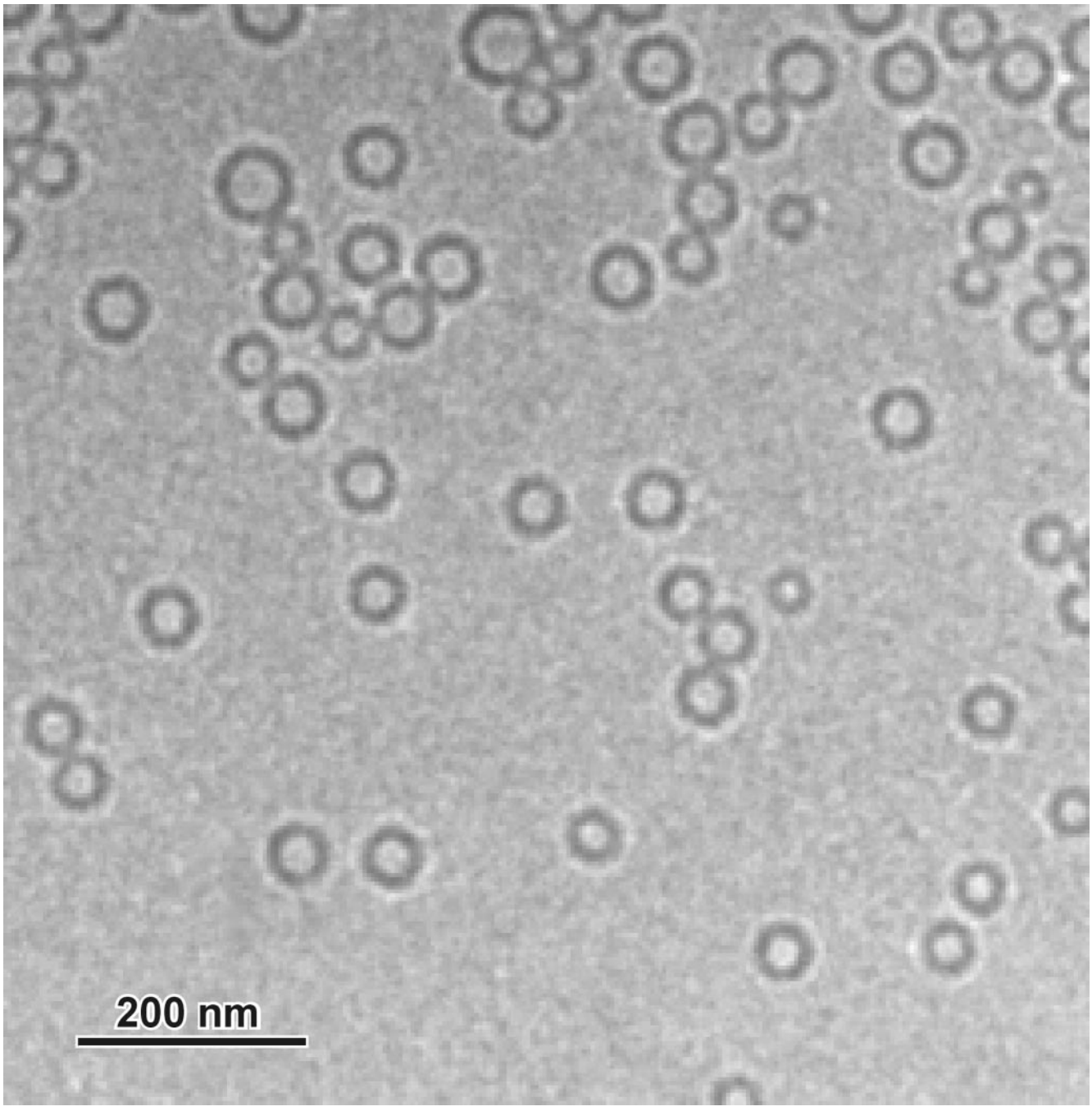

FIGURE 1 Cryoelectron microscopy of extruded cationic lipid vesicles. The unilamellar vesicles (1.6 mM total lipid) consist of the zwitterionic phospholipid, DMPC, and the cationic lipid, DC-Chol, in a molar ratio of 3:2.

such complexes are superimposed with a clear fingerprint-like pattern showing a periodicity of 3.8 nm ($\pm$0.2 nm). Multilamellar CL-DNA complexes are abundant in samples where the DNA/lipid charge ratio is 0.2 (Fig. 2), but are much rarer when the charge ratio is 0.9 (Fig. 3). The complexes in the former samples usually possess a large number of lamellae, while the latter complexes often exhibit less than five lamellae. There are multilamellar complexes with an even number of lamellae as well as such with an odd number of lamellae, with no preference for one or the other kind. In most cases, the innermost bilayer is completely closed with a diameter similar to or greater than that of an original 65-nm vesicle. At the lower nominal charge ratio (i.e., 0.2), aggregation of the multilamellar complexes is evident, sometimes with one or more outer bilayers being shared between two or more complexes in an aggregate.

Unilamellar vesicles are coated with DNA

In samples where the nominal DNA/lipid charge ratio is 0.2, the majority of lipid seems to be bound in multilamellar

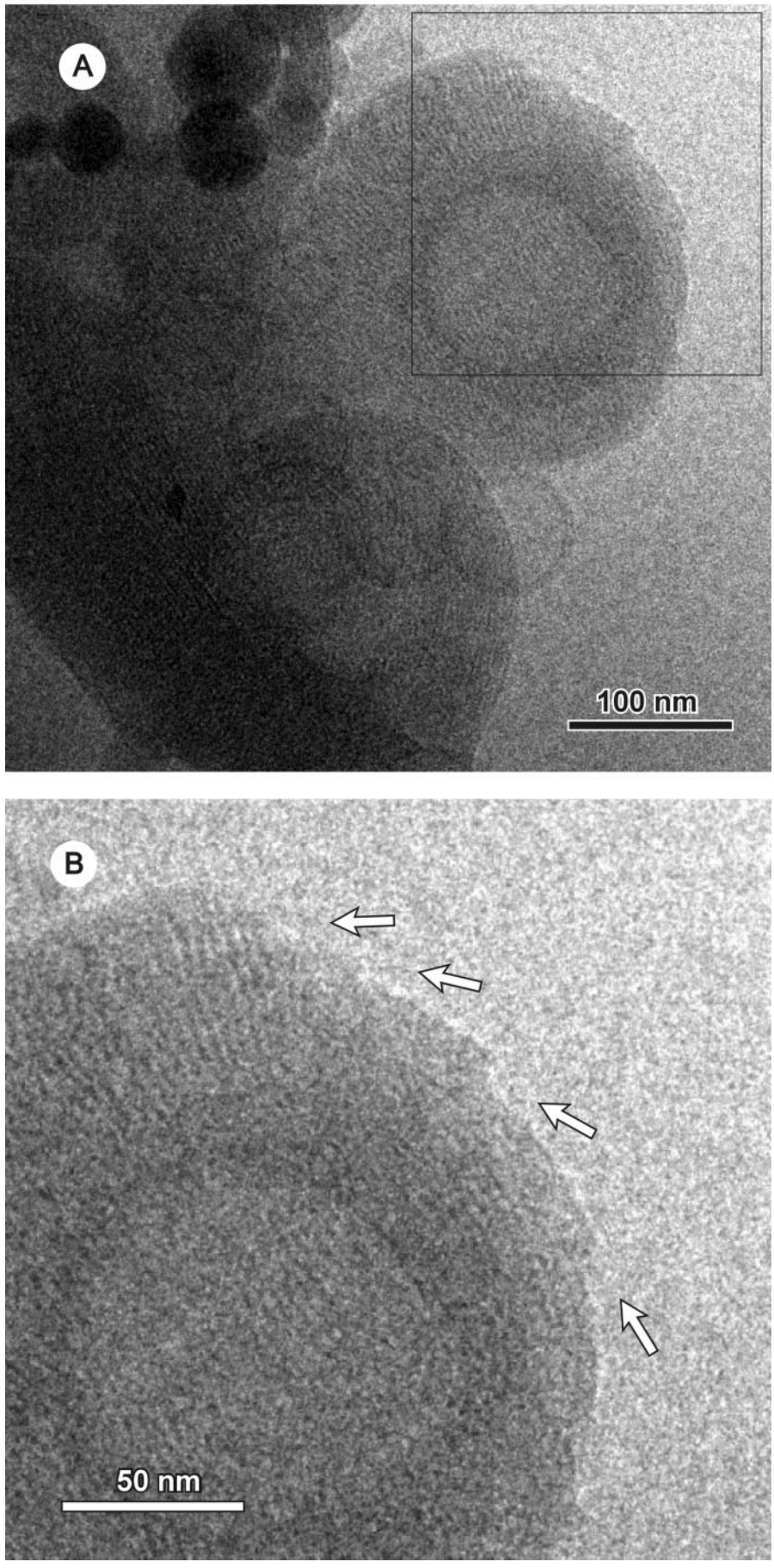

FIGURE 2 (*A*) A few representative large cationic lipid-DNA complexes embedded in vitreous ice and imaged by cryoelectron microscopy. Such complexes are formed by combining DNA with unilamellar cationic liposomes (DMPC/DC-Chol molar ratio = 3:2, DNA/lipid charge ratio = 0.2). They consist of aggregated, spontaneously formed, multilamellar liposomes in which DNA is intercalated between the lipid bilayers. Superimposed on the complex is a fingerprint-like pattern, caused by DNA organized into parallel helices, which are correlated between individual layers. (*B*) Detail from (*A*). The white arrows indicate the edges of unclosed bilayers.

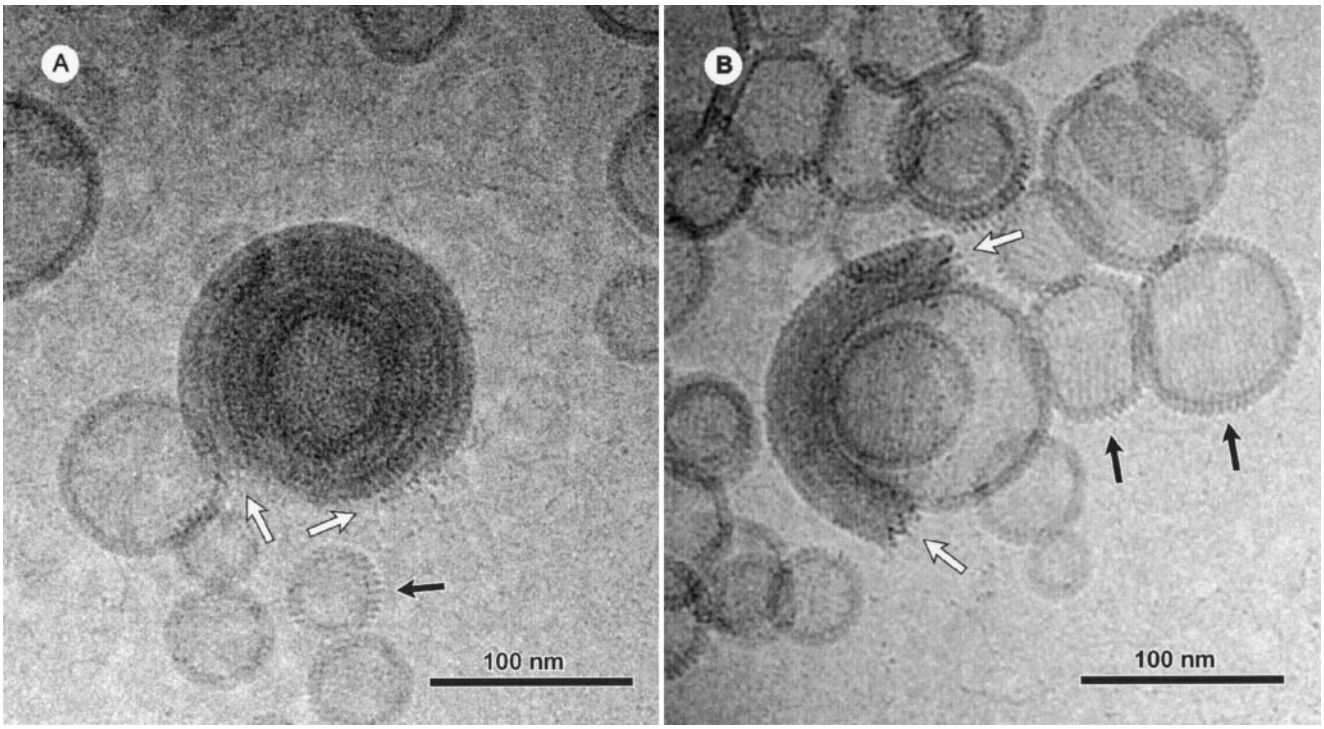

FIGURE 3 An abundance of DNA-coated unilamellar vesicles are evident in samples with higher DNA/lipid charge ratio = 0.9. Multilamellar complexes are present in low amounts. These complexes often possess unclosed outer bilayers. The white arrows indicate the edges of unclosed bilayers. The dark spots near the edges in (*B*) are believed to be caused by DNA accumulating or coiling at the edges. Black arrows indicate unilamellar vesicles on which the pattern caused by the parallel DNA helices is particularly well-visible.

structures (Fig. 2). At a higher DNA content (DNA/lipid charge ratio = 0.9), small unilamellar vesicles prevail (Figs. 3 and 4). Such vesicles show a clear fingerprint-like pattern, which resembles the pattern observed in the multilamellar complexes and in the AFM studies of DNA on supported bilayers, which indicates the existence of a DNA coating. The pattern is best visible on or near the outline of a vesicle (*black arrows* in Figs. 3 and 4) and has a periodicity of 4.1 ($\pm$0.2) nm. These DNA-coated vesicles often form clusters of 10 to 100 and are greatly deformed or flattened in the contact regions. The thickness of the contact regions of two unilamellar DNA-coated vesicles is 13.5 nm ($\pm$0.4 nm).

DNA can induce vesicle fusion

In all samples, the unilamellar DNA-coated vesicles have diameters of 65 nm or larger. This corresponds to the size of the vesicles in the original suspension or to several fused vesicles, respectively (Fig. 5). Taken that the original suspension without DNA contained no large vesicles, there can be no doubt that the vesicle's enlargement was induced by the addition of the polymer.

Fig. 6 shows the final size distribution for unilamellar vesicles as deduced from EM images. (The sizes are given as surface area relative to the average surface area of the original 65-nm vesicles before addition of DNA.) Population is seen to decrease with increasing size. Peaks at dual and triple area of that of the original vesicles are found. Larger unilamellar DNA-coated vesicles usually are too deformed to be included in the analysis.

The outer lamellae of multilamellar complexes often comprise bilayers with open rims

Multilamellar complexes formed at both charge ratios (0.2 and 0.9) exhibit outer bilayer spheres that are incompletely closed; that is, large areas of bilayer are missing (Fig. 3 and *white arrows* in Fig. 2 *B*). The estimated surface area of these open bilayers is in all cases equal to or greater than the surface area of one original 65-nm vesicle. Frequently, dark dots are observed near the open bilayer edges, implying high electron density in this region (*white arrows* in Fig. 3, *A* and *B*). As discussed later, we suggest that such high electron density may be caused by locally accumulated DNA.

DISCUSSION

The presence of DNA-coated unilamellar vesicles in samples with a relatively high DNA content (i.e., DNA/CL charge ratio = 0.9; Figs. 3 and 4), is evidence that the DNA molecules first adapt to the template provided by the lipid bilayer, and not vice versa. It appears that if a sufficient quantity of DNA is present in the suspension, the growth of multilamellar complexes is impeded. The unilamellar vesi-

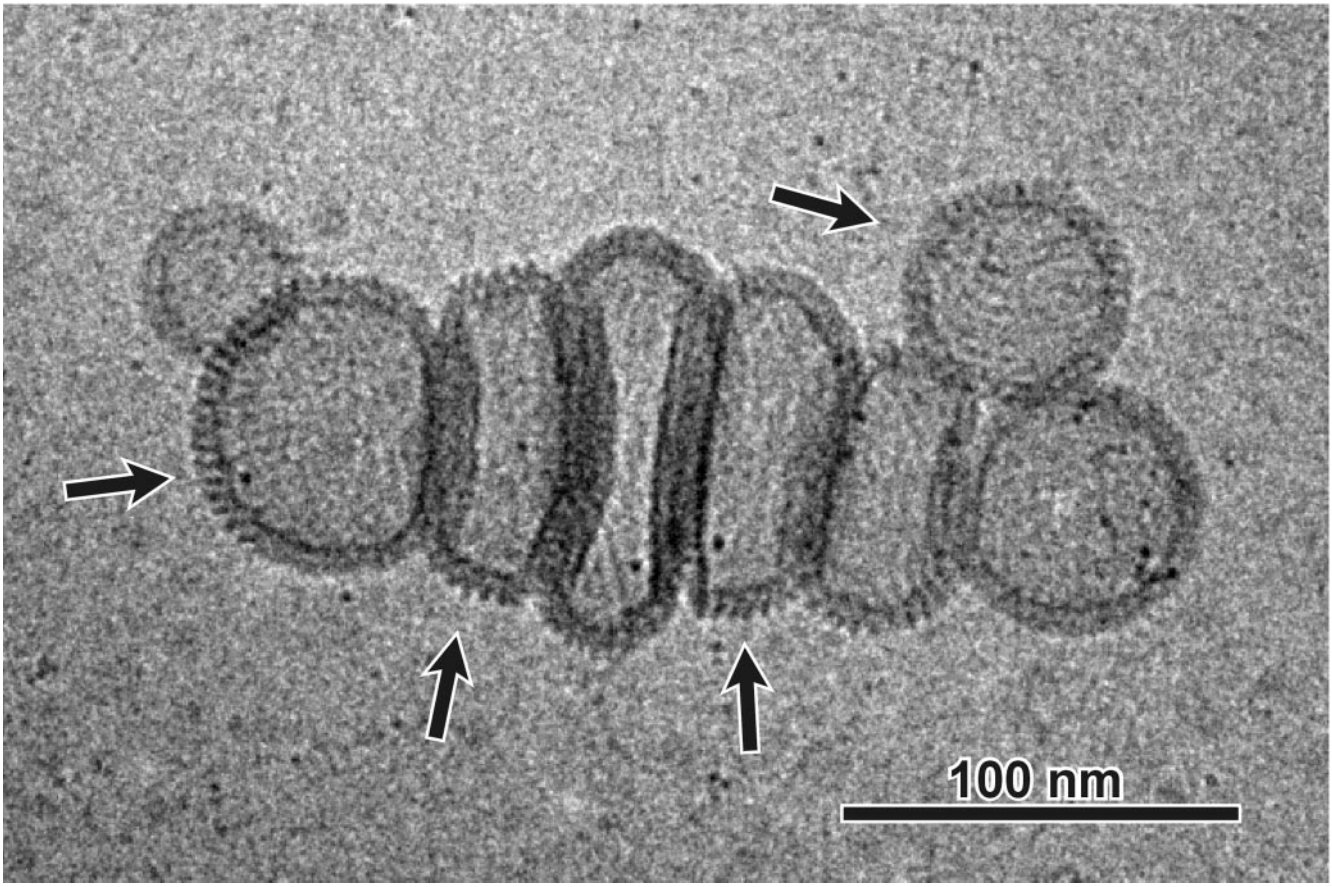

FIGURE 4 Clusters of DNA-coated unilamellar vesicles at DNA/lipid charge ratio = 0.9. As in Fig. 3, the black arrows indicate locations where the pattern caused by adsorbed DNA is best visible. Flattening of the bilayers at the contact regions of adjacent vesicles is evident.

cles remain, typically coated with one monolayer of aligned DNA. Often, they are adsorbed to each other, forming clusters.

In said clusters, the vesicles are deformed at the regions of contact with adjacent vesicles, but do not rupture. We believe that such structures resemble intermediates in the formation of multilamellar complexes. The fact that in the samples with a high relative amount of DNA the clusters survive for at least 24 h indicates that the excess DNA prevents—or eliminates the need for—further steps in the formation of multilamellar complexes. As seen in Fig. 2, multilamellar complexes grow readily when there is an abundance of lipid.

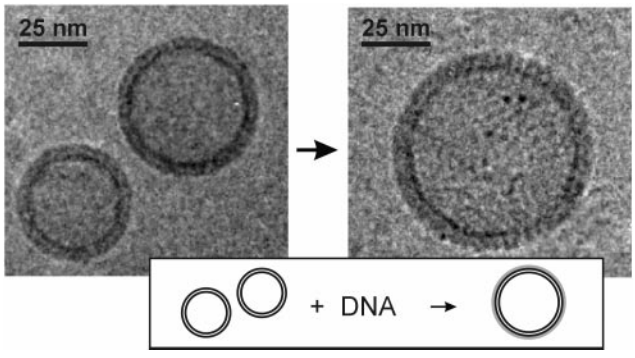

FIGURE 5 Two unilamellar vesicles in the vesicle suspension without DNA and one large DNA-coated unilamellar vesicle in the suspension after addition of DNA.

At vesicle contact regions, the DNA from each unilamellar vesicle is effectively sandwiched between two bilayers. The thickness of the contact regions (13.5 $\pm$ 0.4 nm) is sufficient to allow for two lipid bilayers (5.1 $\pm$ 0.4 nm each) and a monolayer of DNA (the diameter of a DNA molecule is $\sim$2 nm), with ample space for the DNA-associated hydration layers. It is interesting to note that the vesicles have deformed, and not ruptured, despite the fact that they are over 24 h old.

The lipid-DNA interaction is asymmetric

The strong deformation of the DNA-coated unilamellar vesicles in Figs. 3 and 4 indicates that the lipid-DNA interaction is either very strong or very asymmetric, or that the vesicles are very flexible. We believe that the former two possibilities are the more important ones. The asymmetry is caused when DNA adsorbing to only one side of the bilayer induces an asymmetry in packing pressure. This is due to a reduction in the effective headgroup area of the

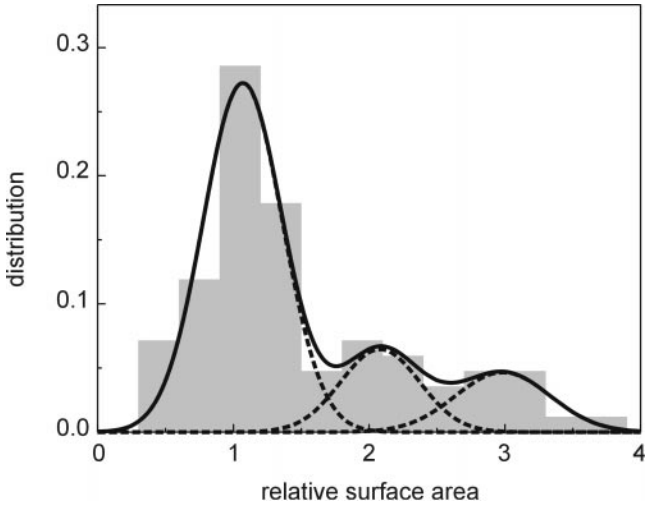

FIGURE 6 A histogram showing the size distribution of DNA-coated unilamellar vesicles. The sizes are given as multiples of the surface area of the vesicles in the suspension before addition of DNA. The data are taken from EM images of 84 near-spherical vesicles. Peaks at the two and threefold surface area of the area of the original vesicles are believed to be indicative of the vesicles being a result of DNA-induced fusion.

cationic lipids, when charges are partially compensated by the oppositely charged DNA on one side of the bilayer, and when the hydrophilicity of polar headgroups is reduced by the presence of adsorbed DNA.

Besides causing deformation, the stress originating from the asymmetry in membrane packing pressure also tends to destabilize the membrane, and thus promotes vesicle fusion. This resembles the observations made with another hydrophilic polymer, poly(ethylene glycol) (PEG), which for some time has been known to cause fusion. It has been argued (Lentz, 1994) that PEG induces an asymmetry in the lipid packing pressure between the inner and the outer leaflets of the membrane bilayer. The stress caused by such asymmetry was postulated to initiate the vesicle fusion.

Multilamellar complexes form when an adsorbed vesicle ruptures and its bilayer rolls over the complex

It is most likely that multilamellar CL-DNA complex growth begins with one DNA-coated "template" vesicle. Two mechanisms for the adsorption of additional bilayers are obvious immediately.

In the first mechanism, the adsorbed layers are stacks of flattened vesicles adsorbed to a template vesicle. Every adsorbed vesicle should contribute two new lamellae to the complex, covering an area identical to one-half its original total area. The flattening could partly be due to the adsorption to the host, partly due to internalized DNA. It is conceivable that vesicles internalize DNA through a temporary leakage that occurs when they are under great deformation stress caused by the adsorption.

In the second mechanism, multilamellar CL-DNA complexes form when adsorbed vesicles rupture and roll over their host, as is sketched in Fig. 7. In this case, every adsorbed vesicle contributes one new lamella to the complex.

If the first mechanism were responsible for the formation of multilamellar complexes, we should always see an even number of lamellae (if the template vesicle is not counted). In contrast to this expectation, even as well as odd numbers of lamellae in approximately equal numbers are observed. Moreover, we never observe lamellae with estimated areas smaller than the surface area of one 65-nm vesicle, namely 14,000 nm^2. Since a flattened vesicle can only adsorb to its host with one-half of its surface area, while the other half forms a second lamella on top of the first one, the simple vesicle adsorption hypothesis is not sustainable. We therefore favor the second explanation for the formation of multilamellar complexes.

DC-Chol may accumulate near open edges

Our electron micrographs indicate that the vesicles rupture and wrap their whole bilayer around a template vesicle or

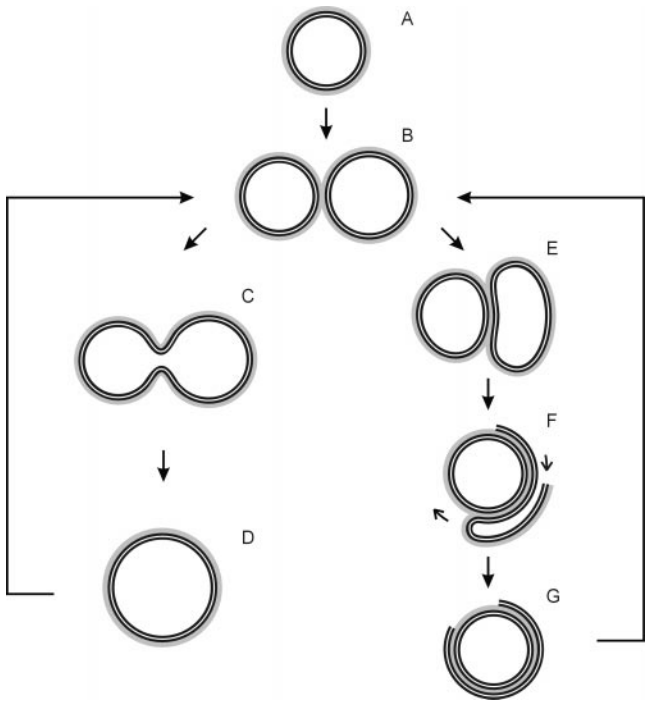

FIGURE 7 Proposed mechanism for the reorganization of lipid bilayers in the presence of DNA. DNA (*gray*) spins around a unilamellar vesicle (*black*), covering it with DNA (*A*). Two vesicles, of which at least one is partly coated with DNA, adsorb to each other (*B*). Fusion occurs if destabilization, due to adsorbed DNA, leads to the formation of a pore (*C*). The vesicle subsequently minimizes its membrane energy by adopting a near-spherical shape (*D*). It then can participate in further fusion or, alternatively, in the formation of multilamellar structures: starting from stage *B*, multilamellar structures form if one vesicle ruptures after deformation (*E*). Such a vesicle then rolls its bilayer over the host vesicle (*F*), forming one adsorbed bilayer with an open edge (*G*). By the same mechanism, further layers adsorb.

complex, as is sketched in Fig. 7. The proposed mechanism entails the formation of an edge where the bilayer does not cover the entire template. This happens if the cost of free energy associated with the edge is smaller than the free energy liberated in the process of bilayer adsorption. For this, we suggest an explanation originally proposed in the literature to explain open bilayers found near the surfactant-induced vesicle-micelle transition (Lasic, 1982; Walter et al., 1991; Edwards et al., 1993). Surfactants prefer geometries with high curvature. It has been suggested in the literature that this prompts the surfactants to gather at the edge of an open bilayer (Lasic, 1982; Cevc et al., 1996). Here, the surfactants form a highly curved lipid mixture, thereby protecting the hydrophobic regions of the lipids from exposure to the solvent (Lasic, 1982; Fromherz, 1983). DC-Chol is a molecule with surfactant-like properties. Its preference for highly curved surfaces can be seen from the fact that in the absence of a helper lipid, such as phosphatidylethanolamine (PE) or phosphatidylcholine, DC-Chol does not form bilayers at all. Therefore, it is likely that DC-Chol shields the open edges in the described fashion. This reduces the free energy cost of edge formation and catalyzes the complex-formation process outlined in previous paragraphs.

Furthermore, the high charge density at the edges, due to the accumulation of cationic DC-Chol, must result in an accumulation of oppositely charged DNA near the edge. Indeed, dark dots are seen at the open bilayer edges relatively often, indicating an elevated electron density in this region (e.g., Fig. 3). We suggest that these dark areas are DNA densely adsorbed to or coiled near the bilayer edges. At this point, we are not convinced that the observed open bilayers are equilibrium structures as they might be long-lived metastable structures as well.

Complex formation may be a cooperative process

It is striking that in the suspensions studied, large multilamellar complexes as well as unilamellar vesicles are found in large numbers, while few oligolamellar complexes are seen. The complexes seem to have grown around one or more closed spherical vesicles.

We have argued previously (Battersby et al., 1998) that the presence of a fingerprint-like pattern across the large multilamellar complexes is indicative of an alignment of DNA helices in different DNA monolayers across the bilayer stack. We believe that this correlation is mediated by a structuring of the adjacent lipid bilayers. The structuring must involve electrostatic attractions between the DNA, the cationic lipids and, perhaps, ions; this leads to a redistribution of the lipids in the bilayer (May and Ben-Shaul, 1997) and of ions in the interfacial region.

After such structuring, the outermost bilayer in a multilamellar complex should be organized as well, while the original, virgin unilamellar vesicles should lack similar structure. It can be argued that the DNA molecules have a higher affinity for the prestructured bilayers and that multilamellar liposomes therefore tend to grow faster by attracting more DNA than unilamellar vesicles. Correlation mediated attraction as well as diminished membrane flexibility, which lowers the repulsion near a membrane, could be responsible for this. We believe that such a mechanism could help to explain the remarkable lack of small multilamellar complexes in the investigated suspensions.

CL-DNA complex formation can be seen as a process that involves several linked paths

In Fig. 7, the possible complex evolution pathways suggested by the results of our study are sketched. Starting with a DNA-coated vesicle (*A*), fused DNA-coated vesicles (*D*), vesicle clusters (*E*) and, eventually, multilamellar complexes (*G*) emerge (cf. Fig. 7). The relative DNA/cationic lipid ratio and the absolute amount of these components in the sample affect which pathway is preferred. It stands to reason, however, that the crucial factors are the *local* concentrations of lipids and DNA. One would expect that a high local lipid concentration provides the vesicles with more opportunity to come into close contact and to form large multilamellar complexes. A high local DNA concentration, however, will saturate or neutralize the cationic charges on the outer monolayer of the vesicles, and thus preclude complex growth.

CL-DNA complex formation results in a broad distribution of complex morphologies. The results vary widely with the CL system and the preparation procedure. In this work, we discussed only a fraction of the multitude of structures found under our particular experimental conditions in our particular system. The structures and mechanisms of DNA-membrane interaction discussed are therefore not exhaustive. They do represent, however, a fairly complete scenario for the development of DNA-lipid structures with multilamellar geometry (see Fig. 7), which was not known, or reported, in such complexity to date.

CONCLUSIONS

Three distinct types of complex that can form in the mixture of DNA and cationic vesicles comprising the charged lipid DC-Chol and DMPC as a zwitterionic helper lipid were studied. They are 1) DNA-coated, and fused DNA-coated, unilamellar vesicles; 2) clusters of DNA-coated vesicles in which the vesicles are deformed and flattened in the contact regions; and 3) highly compact multilamellar complexes that consist of a stack of tightly associated, alternating sheets of DNA and lipid bilayers, often built around a single central vesicle. Frequently, the outer layers of such complexes are not completely closed, but rather have open edges with indications of increased DNA concentration in such regions.

Paths that lead to the various DNA/CL structures are proposed. Steps along these paths include vesicle adsorption, fusion, and a process in which the adsorbing vesicles rupture and roll their bilayer over a template vesicle or the original DNA/CL complex. The final outcome and the prevailing complex formation in the suspension depend on the path chosen and on the amount of DNA and lipid present. Specification of DNA/CL, or of the corresponding charge ratio alone, is insufficient for complete sample description and also cannot be used on its own to predict the final complex formation.

Moreover, we argue that the prevalence of large multilamellar complexes over the relatively small number of complexes with only a few lamellae hints at a cooperative DNA-membrane association process. It is suggested that such cooperativity might at least partly be due to the structuring of the lipid bilayers caused by the layers of DNA in the complex.

The system discussed in this work also exemplifies how the structure of the lipid-DNA complexes is affected by the preference of the lipids used to form lamellae. Analogous studies with lipids such as PE (Koltover et al., 1998), which lead to different complex structures, should be revealing.

We believe that our findings give valuable insight into fundamental mechanisms that play a role in lipid-DNA complex formation. Dense multilamellar complexes such as those visualized in this study might prove to be an effective gene delivery system, as has been suggested for related lipid-DNA complexes in the past. In any case, understanding their formation should be useful for future development and specific biological applications.

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