Molecular Shape of the Cationic Lipid Controls the Structure of Cationic Lipid/Dioleylphosphatidylethanolamine-DNA Complexes and the Efficiency of Gene Delivery*

Jarmila Šmisterová‡‡§§, Anno Wagenaar‡‡, Marc C. A. Stuart‡‡, Evgeny Polushkin**, Gerrit ten Brinke**, Ron Hulst§§, Jan B. F. N. Engberts‡‡, and Dick Hoekstra‡‡

From the ‡Department of Membrane Cell Biology, University of Groningen, Faculty of Medical Sciences, A. Deusinglaan 1, 9713 AV Groningen, the §Biomade Technology Foundation, Nijenborgh 4, 9747 AG Groningen, the ¶Physical Organic Chemistry Unit, Stratingh Institute, University of Groningen, Nijenborgh 4, 9747 AG Groningen, the **Department of Biophysical Chemistry, University of Groningen, Nijenborgh 4, 9747 AG Groningen, and the ‡‡Department of Polymer Chemistry, University of Groningen and Dutch Polymer Institute, Laboratory of Polymer Chemistry, Materials Science Center, Nijenborgh 4, 9747 AG Groningen, The Netherlands

Pyridinium amphiphiles, abbreviated as SAINT, are highly efficient vectors for delivery of DNA into cells. Within a group of structurally related compounds that differ in transfection capacity, we have investigated the role of the shape and structure of the pyridinium molecule on the stability of bilayers formed from a given SAINT and dioleoylphosphatidylethanolamine (DOPE) and on the polymorphism of SAINT/DOPE-DNA complexes. Using electron microscopy and small angle x-ray scattering, a relationship was established between the structure, stability, and morphology of the lipoplexes and their transfection efficiency. The structure with the lowest ratio of the cross-sectional area occupied by polar over hydrophobic domains (SAINT-2) formed the most unstable bilayers when mixed with DOPE and tended to convert into the hexagonal structure. In SAINT-2-containing lipoplexes, a hexagonal topology was apparent, provided that DOPE was present and complex assembly occurred in 150 mM NaCl. If not, a lamellar phase was obtained, as for lipoplexes prepared from geometrically more balanced SAINT structures. The hexagonal topology strongly promotes transfection efficiency, whereas a strongly reduced activity is seen for complexes displaying the lamellar topology. We conclude that in the DOPE-containing complexes the molecular shape and the nonbilayer preferences of the cationic lipid control the topology of the lipoplex and thereby the transfection efficiency.

Because of their negligible immunogenicity, relative ease of large scale production, and amenability for chemical modification, cationic lipids are considered useful alternatives for viral vectors and their low stability in the presence of serum thus far frustrate their use for in vivo application. To overcome these difficulties, new formulations are engineered with the aim of better defining the structure, enhancing stability, reducing polydispersity and size, and improving DNA compaction (7–9). Equally crucial in these developments is the need for a better understanding of the structure-function relationship of cationic lipid-based gene delivery systems. Although several studies, in vitro (10–12), as well as in vivo (4, 13, 14) have been carried out with that aim, a coherent and comprehensive interpretation is often difficult, because of a comparison of different classes of cationic lipids within one study and the use of different cell lines. This is particularly relevant when taking into account that even within a group of structurally related compounds within one class, a small change in the structure may lead to dramatic changes in their biological activity (15). Thus a variety of structural models have been proposed based upon electron microscopy studies, including a DNA encapsulation model (16), spaghetti and meatball structures (17), DNA entrapped into aggregated multilamellar structures (18–20), or DNA internalized within liposomes (21).

By using small angle x-ray scattering (SAXS)† and synchrotron x-ray diffraction, important new insight has been obtained into the structural organization of cationic lipid-DNA complexes. Thus, depending on the absence or presence of the helper lipid dioleoylphosphatidylethanolamine (DOPE), either a lamellar or a two-dimensional hexagonal lattice (H2) organization, respectively, was obtained (22, 23).

The phase preference of the lipoplex for a lamellar or hexagonal phase not only depends on the use of helper lipid as such but also on the chemical nature of the helper lipid (e.g. DOPE versus dioleoylphosphatidylcholine; Refs. 24 and 25) and on the cationic lipid. For example, within a group of cationic lipids that perform well in the absence of co-lipid, like dioctadecylamidoglyclylpermine-4-trifluoroacetic acid and bis-guanidinium-tren-cholesterol and lipopolymamines, hexagonal structures (26), aggregates with lamellar domains (27, 28), or nucleosome-like structures interacting with one other (29) have been observed. On the other hand, the effect of DOPE on the ultimate structure of the complex seems to depend on the class of cationic lipid. For example, bis-guanidinium-tren-cholesterol/DOPE-DNA complexes exhibited lamellar symmetry (27, 28, 30), whereas DOTAP/DOPE-DNA complexes form an inverted hexagonal organization (23). The differences between these two morphologies have been attributed to differences in the charge density.

Received for publication, July 3, 2001, and in revised form, September 17, 2001

This paper is available online at http://www.jbc.org

¶ To whom correspondence should be addressed. Fax: 31-50-3632728; E-mail: d.hoekstra@med.rug.nl.

†† To whom correspondence should be addressed. Fax: 31-50-3632728; E-mail: d.hoekstra@med.rug.nl.

‡‡ To whom correspondence should be addressed. Fax: 31-50-3632728; E-mail: d.hoekstra@med.rug.nl.

‡§ To whom correspondence should be addressed. Fax: 31-50-3632728; E-mail: d.hoekstra@med.rug.nl.

† The abbreviations used are: SAXS, small angle X-ray scattering; DOPE, dioleoylphosphatidylethanolamine; DOTAP, dioleoyl-3-trimethylammonium propane; β-gal, β-galactosidase; cryo-TEM, cryo-transmission electron microscopy.
of the two cationic lipids, differences in hydrophobic moiety, and the concentration of DOPE used in the formulations (27). Within a group of related DOTAP analogs, the length of the hydrophobic tails determined the tendency of the DOTAP/DOPE bilayer to form the hexagonal phase (31).

Although a correlation has been shown between the inverted hexagonal packing of DOTAP/DOPE-DNA lipoplexes and rapid fusion with anionic vesicles followed by DNA dissociation (23), direct insight into the structure-function relationship of lipoplex-mediated transfection is very poor. The purpose of the current study was to obtain such insight by using one particular class of cationic pyridinium-derived lipids, abbreviated as SAINTs (12, 15, 32). The effect of the overall shape of the amphiphiles on the colloidal stability of the bilayers formed with DOPE (1:1) and the impact of this stability/instability on the polymorphism of SAINT/DOPE-DNA complexes within a group of structurally highly related compounds, although differing in their ability to deliver DNA into COS-7 cells, was investigated by electron microscopy, by small angle x-ray scattering, and in transfection studies.

**EXPERIMENTAL PROCEDURES**

**Lipids and Chemicals—**DOPE was purchased from Avanti Polar Lipids, Inc. Pyridinium amphiphiles, abbreviated as SAINT, were synthesized and characterized as previously described (18). All other chemicals were of the highest grade.

**Preparation of Vesicles—**A solution of SAINT, either alone or in 1:1 molar ratio with DOPE in chloroform or methanol, was taken to dryness under a stream of nitrogen. Any residual solvent was removed under vacuum. The lipid film was hydrated at room temperature in water at a final lipid concentration of 1 mM and sonicated to clarity in a bath sonicator (G112SSPIT, 600 V) immediately before use.

**Static Light Scattering—**50 nmol of SAINT/DOPE (1:1, unless indicated otherwise) liposomes were complexed with 3.3 mg of pCMV β-gal plasmid in a final volume of 200 μl of HBS (10 mM HEPES, 150 mM NaCl, pH 7.4). The turbidity was measured in a 96-well microtiter plate at 550 nm for different time intervals by spectrophotometry (Bio-Tek Instruments, Inc., Winooski, VT).

**Cryo-TEM—**A small drop of the lipid or lipoplex suspension was deposited on a glow discharged holey carbon-coated grid. After blotting away the excess of lipid, the grids were plunge-frozen in liquid ethane. Frozen hydrated specimens were mounted in a GaTen (model 626) CRYOSTAGE and examined in a Philips CM 120 cryo electron microscope operating at 120 kV.

**Small Angle X-ray Scattering—**SAXS measurements were performed at 25 °C using a NanoStar device (Bruker AXS and Anton Paar) with a ceramic fine-focus x-ray tube operated in a point focus mode. The tube was powered with a Kristalloflex K760 generator at 35 kV and 40 mA. The primary beam was collimated using cross-coupled Göbel mirrors and a 0.1-mm pinhole providing a CuKα radiation beam (the wavelength λ = 0.154 nm) with a full-width at half-maximum about 0.2 mm in diameter at the sample position. A sample-detector distance of 0.24 m was chosen, because no new reflections were observed at longer distances. The use of a Hi-Star position-sensitive area detector (Siemens AXS) allowed the recording of the scattering intensity in the q range of 0.5–8.5 nm⁻¹. The scattering vector q is defined as

\[ q = \frac{4\pi}{\lambda} \sin \left( \frac{\theta}{2} \right) \quad \text{(Eq. 1)} \]

where \( \lambda \) is the wavelength and \( \theta \) is the scattering angle. The measurements of the samples, prepared by mixing SAINT/DOPE liposomes (1:1, 750 nmol) and pCMV β-gal solution (50 μg, the charge ratio 2.5:1), were performed in flame sealed quartz capillaries with a diameter of 1 mm. After flame sealing, the samples were centrifuged at low speed and left for 2–3 days at room temperature to equilibrate. The measuring time was between 3 and 9 h.

**Transfection Experiments—**A 7.1-kilobase plasmid containing the *Escherichia coli* β-galactosidase gene under the control of the cytomegalovirus immediate-early gene promoter (pCMV β-gal, Clontech, Palo Alto, CA) was used as the reporter gene. DNA was isolated from *E. coli* using a Qiagen plasmid kit. The plasmid concentration was determined by measuring the absorption at 280 nm using the relation 1.0 A = 50 μg/ml. Typically, the A260/A280 value was 1.95.

**RESULTS**

The purpose of this work was to investigate the structure/function relationship of cationic lipid-mediated gene delivery. To this end, one class of such lipids was selected, the so-called SAINT amphiphiles, which are pyridinium-based amphiphiles with two symmetric alkyl chains (Fig. 1). SAINT-2 and SAINT-21 only differ in their head group region, the latter containing an “extended” trimethyl ammonium charge, whereas both amphiphiles contain the same di-C18:1 (oleylmonounsaturated, 85:15 cis/trans) alkyl chains. Instead of two C18:1 alkyl chains, SAINT-27 contains two palmitoyl chains (C16:0) and an additional positive charge in the head group region, provided by the C4 spacer-attached amine group.

**SAINT-mediated Delivery of DNA into COS-7 Cells—**The effect of the different structural features of the various SAINT derivatives on their transfection ability when mixed with an equimolar amount of DOPE is shown in Fig. 2. The transfection efficiency of all three cationic SAINT lipids was found to be optimal at a +/– charge ratio of approx. 2.5 (data not shown and Ref. 15). The replacement of C18:1 chains by the saturated palmitoyl chain in SAINT-27 and the presence of an additional positive charge in the form of a protonated amine group resulted in a lowering of the transfection activity to about 35% of that obtained for SAINT-2/DOPE (1:1). Unexpectedly, the introduction of the (charged) trimethylammonium...
group in the SAINT-2 skeleton, defined as SAINT-21, virtually abolished transfection activity.

DOPE is a common helper lipid that usually improves transfection efficiency. Interestingly, when compared with the results obtained in the absence of DOPE, the activity of SAINT-2 increased ~4–5-fold when lipoplexes were prepared with 50 mol % DOPE. By contrast, the activity of both SAINT-21 and SAINT-27 turned out to be DOPE-independent. To clarify the underlying mechanisms that dictated these remarkable DOPE-dependent differences in SAINT-mediated transfection efficiency and the superior transfection efficiency of SAINT-2 containing lipoplexes, the colloidal and structural properties of the various cationic liposomes and lipoplexes were investigated.

The Colloidal Stability of SAINT Liposomes—In water, all three SAINT derivatives, when mixed with an equimolar amount of DOPE, formed vesicles with diameters in the range of 100–200 nm, as determined by cryo-TEM (Fig. 3A). However, when exposed to physiological salt concentrations, the colloidal stability of the SAINT/DOPE (1:1) vesicles was dependent on the SAINT structure. Liposomes, prepared from SAINT-21/DOPE and SAINT-27/DOPE in water, maintained their vesicular appearance after dilution in HBS buffer (150 mM NaCl, 10 mM HEPES, pH 7.4), and single aqueous compartments bounded by a unilamellar membrane were visualized by cryo-TEM (Fig. 3A). However, the transfer of SAINT-2/DOPE liposomes into HBS buffer resulted in an apparent disintegration of the vesicular structure, leading to the formation of particles with a typical electron-dense fingerprint structure within their lumen, as shown in Fig. 3B, which is thought to originate from the formation of nonbilayer structures. Indeed, structural analysis of these particles by SAXS (Fig. 3C) revealed the ability of SAINT-2/DOPE (1:1) liposomes to undergo a transition from a lamellar (in water) to the hexagonal phase after addition of 150 mM NaCl, with diffraction maxima at \( q = 1.01, 1.76, \) and 2.02, and a periodicity of 7.2 nm. Next we verified how these colloidal and structural features were affected upon lipoplex assembly.

The Colloidal Stability of SAINT Lipoplexes—To determine the stability of SAINT/DOPE bilayers following their interaction with DNA upon assembly of the lipoplex, changes in particle size were monitored by light scattering as a function of the

**Fig. 3.** Effect of physiological salt concentration on the morphology of SAINT-2/DOPE (1:1) liposomes. SAINT-2/DOPE liposomes were prepared as described under “Experimental Procedures,” either in water (A) or in HBS buffer (150 mM NaCl, at pH 7.4) (B and C). The liposomes were visualized by cryo-TEM (A and B) and examined by SAXS (C). Note the vesicular appearance (absence of electron dense material in vesicle lumen) when the liposomes had been prepared in water, in the absence of 150 mM NaCl (A). By contrast, particles with a typical electron-dense fingerprint structure are seen in the presence of salt (B), which reflects a hexagonal organization (HII) (C). The bars represent 100 nm.
SAINT/DNA charge ratio. At 150 mM NaCl, the stability of SAINT/DOPE-DNA complexes reflected a colloidal behavior typical of that of the pure liposomes, as shown in Fig. 4. In these experiments, SAINT/DOPE vesicles were prepared in water and, subsequently, mixed with plasmid in HBS buffer. The turbidity of the complex suspensions at the indicated charge ratios was measured after 10 min. In line with the colloidal stability of their vesicular entities, lipoplexes consisting of SAINT-21/DOPE (1:1) and SAINT-27/DOPE (1:1) were only marginally destabilized in the presence of DNA and did not precipitate over a period of about 3 h. Significant aggregation was only observed at a charge ratio of about 2.5, indicating that around this ratio, the repulsion between the head groups of the cationic lipids was lowest. In contrast to SAINT-21 and SAINT-27, SAINT-2/DOPE (1:1) lipoplexes were highly unstable and precipitated in the presence of 150 mM NaCl. The charge neutralizing effect reached a maximum at a charge ratio of 5.0, representing conditions at which the reduced electrostatic repulsion between the complexes led to their precipitation. Interestingly, the neutralization point for SAINT-2 lipoplexes is reached at a 2-fold higher concentration of cationic lipid than that observed in case of SAINT-27-and SAINT-21 lipoplexes. This difference would imply that the SAINT-2 complexes accommodate twice as much cationic lipid at the same amount of DNA than complexes assembled from either SAINT-21 or SAINT-27. To examine whether this remarkable distinction correlated with differences in structural features as noted above, i.e. involving a lamellar versus nonlamellar transition in case of SAINT-2 but not SAINT-21 or SAINT-27 in the presence of 150 mM NaCl, the SAINT lipoplexes were further characterized by electron microscopy and SAXS.

**The Morphology of SAINT Lipoplexes as Determined by Electron Microscopy**—Both negative staining and cryo-transmission electron microscopy were used to examine the morphology of complexes formed by the three different SAINTs, as prepared in the presence of 150 mM NaCl. As already anticipated from the differences in their colloidal properties, the morphology of SAINT-21 and SAINT-27 lipoplexes on the one hand and that of SAINT-2 lipoplexes on the other hand differed remarkably. At a charge ratio of 2.5, lipoplexes consisting of SAINT-21 and SAINT-27 gave rise to the formation of distinct globules, showing condensed multilayers with a diameter of about 500 nm (Fig. 5). By contrast, SAINT-2-containing complexes (Fig. 6A) showed aggregates that did not display a multilayered structure, and after several hours, these clustered complexes reached diameters up to 1 μm. The electron dense core of these structures revealed again typical striated features (Fig. 6B), very similar to those seen within the lumen of SAINT-2/DOPE vesicles in NaCl/HEPES (Fig. 3B). Note that cryo-TEM images of SAINT-2/DOPE lipoplexes prepared in water display an entirely different morphology, showing a lamellar structure similar to that of SAINT-27 and SAINT-2 lipoplexes (not shown; Fig. 5), as reported previously (12). To further investigate the structural organization underlying the morphological features of these various complexes, SAXS measurements were subsequently carried out.

**The Structure of SAINT Lipoplexes**—The lamellar organization of SAINT-27/DOPE (1:1) lipoplexes at physiological salt concentrations, as suggested by electron microscopy, was confirmed by SAXS measurements (Fig. 7). Diffraction maxima were obtained at \( q_1 = 0.97 \text{ nm}^{-1} \) and \( q_2 = 1.93 \text{ nm}^{-1} \), and the ratio between these values of 1:2 suggests a lamellar morp-
ogy with a periodicity of 6.5 nm. Note that the same structural organization was determined for DNA-devoid SAINT-27/DOPE vesicles (not shown), as described above. Similar analyses of SAINT-2/DOPE (1:1) lipoplexes (Fig. 8C) revealed at least four distinct diffraction peaks at \( q \) values of 1.03, 1.78, 2.06, and 2.69 nm\(^{-1}\), whereas two weaker maxima are apparent at \( q \) values of approx. 1.2 and 1.19, i.e., typical of a lamellar structure. Thus both inclusion of DOPE and complex preparation at physiological salt conditions are needed to form lipoplexes with a hexagonal structure. Interestingly, when the SAINT-2/DOPE lipoplexes were prepared in water and subsequently transferred to HBS buffer, it was still possible for the lamellar phase, obtained at these conditions, to transform into the hexagonal lattice, as shown in Fig. 9. However, the lipoplexes thus obtained showed a lower level of structural organization than that obtained for complexes prepared at physiological salt conditions. This was inferred from the observation that the former complexes showed a periodicity of 6.8 nm, with less distinguished and less sharp diffraction peaks at \( q \) values of 1.09, 1.81, and 2.10 nm\(^{-1}\) (Fig. 9).

**DISCUSSION**

By using chemically and structurally closely related pyridinium-based cationic lipids, we have shown that subtle changes in cationic lipid structure in conjunction with the presence or absence of the helper lipid DOPE and charge neutralizing conditions dramatically affects lipoplex-mediated transfection. In short, the superior transfection properties of SAINT-2 lipoplexes, as described in previous work (12, 15) appears to be related to its ability to undergo a lamellar to a nonlamellar phase transition, which requires the presence of DOPE and which is promoted by charge neutralizing conditions as provided at physiologically relevant conditions. Specifically, the transition from the lamellar to the hexagonal organization of SAINT-2-based lipoplexes enhances the cellular transfection efficiency 2–5-fold, with the net increment depending on the presence of DOPE and physiological salt conditions. The data indicate that DOPE appears to be the major driving force in promoting transfection, because its absence decreased the transfection efficiency more dramatically than the absence of salt. This potency of DOPE in promoting transfection would be consistent with the transformation of the lamellar DOPE-containing complex as prepared in water, to the hexagonal phase, when dispersed in a physiological salt solution (Fig. 9). The suboptimal structural transition accomplished at such condi-
tions, compared with complexes prepared in salt (Fig. 8 versus Fig. 9), explains the lower transfection efficiency obtained at such conditions. Also note that the transfection is not necessarily abolished when a complex maintains the lamellar phase but that the efficiency is drastically reduced, as reflected by the (much) lower efficiencies of the lamellar SAINT-21 and -27 lipoplexes and lamellar i.e. DOPE-devoid SAINT-2 complexes (see also below).

The ability of SAINT-2, compared with the SAINT-21 and -27 derivatives, to readily undergo a lamellar to nonlamellar organization and thereby strongly promote cationic lipid-mediated transfection can be rationalized by taking into account the molecular shape concept (12, 33). Thus SAINT-2 shows the largest imbalance between the cross-sectional areas occupied by the hydrophobic and hydrophilic domains of its structure (Fig. 1). Clearly, the presence of DOPE, which prefers in isolation the hexagonal phase and which promotes in mixed bilayers negative curvature, and to a lesser extent salt-induced head
group charge neutralization, further expand the hydrophobic surface area and promote this delicate imbalance (Fig. 8, B and C). Indeed, the lamellar to nonlamellar phase behavior of a pure lipid is dictated by the packing parameter, the spontaneous curvature, electrostatic interactions, hydration repulsion, and van der Waals’ attraction (33, 10, 13). In addition, the presence of unsaturated bonds in the alkyl chains will also contribute, in particular the cis double bond that represents more than 70% of the chain configuration in SAINT-2. Not only does an unsaturated bond lower the main liquid crystalline phase transition temperature of a bilayer, compared with the saturated or trans-configuration, the area occupied by the cis-hydrophobic domain is also more broadened. Moreover, the more fluid unsaturated oleyl-chains can better accommodate the DNA polymer upon complex assembly than the saturated ones (34).

Even in the absence of DNA, SAINT-2 displays instability to such an extent that already at slightly elevated salt concentrations, the SAINT-2/DOPE packing becomes destabilized. This resulted in the transition to the hexagonal assembly (Fig. 3), which was preserved upon complexation with DNA (Fig. 8C), although lamellar complexes may also acquire (albeit less optimal) hexagonal features when such complexes are suspended in physiological salt solutions, provided they contain DOPE (Fig. 9). As a consequence of the hexagonal lattice, the alkyl chains are oriented toward the aqueous phase, thereby promoting attractive hydrophobic and van der Waals’ forces over the repulsive electrostatic and hydration forces (31). Accordingly, the SAINT-2 containing lipoplexes are colloidal ly highly unstable (Fig. 4).

Evidently, a larger and more hydrated head group (SAINT-21) or a multiply charged head group in conjunction with more orderly packed saturated palmityl chains (SAINT-27) frustrates the lamellar-to-inverted hexagonal reorganization. In fact, the C16:0 chains per se may well suffice to impede this transition because the transfection efficiency of SAINT-1, in which the hydrophobic domain is the same as for SAINT-27 but the head is identical to that of SAINT-2 (Fig. 1), is 5-fold less than the efficiency obtained for SAINT-2 lipoplexes (15).2 Apparently, SAINTs possessing such molecular features are able to accommodate the cone-shaped DOPE in the lamellar phase (Fig. 5). The relative “stability” of this phase, which should display dynamics for perturbation of the target membranes as well as plasmid release (see below), very likely bears important consequences for transfection, because the latter propensity is virtually abolished in case of SAINT-21 lipoplexes. Indeed, it could be argued that the head group area in SAINT-27, relative to that of SAINT-21, is less strongly hydrated, because of the ability of the ammonium group to undergo electrostatic and hydrogen bonding interactions with the phosphate group of DOPE. As noted (10, 13), diminished head group repulsion in case of SAINT-27 favorably affects nonbilayer transitions. This is supported by the observation that substitution of the amine group in SAINT-27 by a trimethylammonium group resulted in a structure that, similarly to SAINT-21, is completely devoid of transfection activity (not shown). Taken together, these data indicate that the bilayer structure and its stability negatively interfere with transfection efficiency, whereas a propensity for transitions to a nonbilayer organization promote plasmid translocation. In addition, DOPE can facilitate this lamellar-to-nonlamellar transition not by its presence per se but in a SAINT structure-dependent manner. Moreover, less completely (re)assembled hexagonal complexes, as obtained when dispersing lamellar (in water preformed) complexes in salt-containing media, lead to a reduced (50%) transfection potential when compared with complexes that adopt the hexagonal phase upon de novo assembly.

The question then arises at which level of the transfection process nonbilayer structures are relevant. Both target membrane perturbation and/or plasmid release will require a distinct degree of membrane dynamics, processes that would be strongly promoted by a departure from a bilayer structure. Koltover et al. (23) showed that hexagonal lipoplexes fused readily with anionic vesicles, a process that was accompanied by a rapid dissociation of DNA, suggesting that nonbilayer structures play a key role in the escape of DNA from the endosome. Mui et al. (35) suggested that the differences in the DNA delivery efficiency of DOPE- and (bilayer-stabilizing) DOPC-containing complexes might originate from the higher tolerance of the target membrane for the exogenous DOPC when compared with DOPE with its nonbilayer preferences rather than by differences in the uptake and extent of lipid mixing. Also the destabilization of the endosomal membrane by the formation of nonbilayer structures has been suggested to play a role in the escape mechanism of DNA from the endosome (36, 37). In this context, we have observed (data not shown) that lamellar and hexagonal complexes are taken up by COS-7 cells to a similar extent. Yet, nonlamellar SAINT-2/DOPE mixtures interact with negatively charged phospholipid vesicles more extensively than lamellar SAINT-21/DOPE and SAINT-27/DOPE complexes, as reflected by larger changes in light scattering and release of an aqueous contents marker from the target vesicles. Clearly, these considerations emphasize that cellular factors, capable of (further) destabilizing the bilayer structure (or promoting nonlamellar structures) of lipoplexes should also be taken into account. Indeed, our data hint that differences in molecular structures of SAINT-21 and SAINT-27, although not reflected in overall differences in the physical and morphological properties of the vesicles or lipoplexes in bulk solution, do become apparent when the complexes interact with cellular membranes. This is indicated by differences in transfection efficiency and may imply differences in DNA release from the complex and/or capacity to destabilize endosomal membranes. The latter events presumably require tight lipoplex-cellular membrane interactions, for example to allow translocation of acidic phospholipids into the complex or that of

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2 J. Šmisterová, R. Hulst, J. B. F. N. Engberts, and D. Hoekstra, unpublished data.

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Fig. 9. SAXS pattern of SAINT-2/DOPE lipoplexes, prepared in water, following a transfer to HBS buffer (150 mM NaCl). SAINT-2/DOPE lipoplexes, displaying a lamellar membrane stacking upon preparation in water, were subsequently suspended in a 150 mM NaCl, 10 mM HEPES buffer at pH 7.4. The complexes were then analyzed by SAXS, showing the hexagonal structure with Bragg peaks at 1.09, 1.81, and 2.10 nm−1 and a periodicity of 6.8 nm.
amphiphiles into the endosomal membrane, facilitating dissociation, as proposed (36), an event that simultaneously may propagate nonlamellar transitions (38) both in the complex and in the endosomal membrane, respectively. In analogy with differences reported for DOPE versus DOPE, as discussed above, the relative state of hydration and the structural balance of the amphiphile likely represent governing parameters in this regard. As noted, the less hydrated and relatively small ammonium head group of (transfecting) SAINT-21 or when replacing the ammonium group on SAINT-27, seem to suffice to dictate such properties in a cellular context.

In conclusion, we have shown that the ability of DOPE to control the structure of the cationic lipid/DOPE-DNA complex depends on the molecular shape of the cationic lipid. The shape and consequently the packing parameter, as determined by the ratio of head group area over hydrophobic area, is particularly sensitive to the presence of multiple charges, which may interact with adjacent charges in the helper lipid, and charge neutralization at physiological conditions. As a result the lipoplex may adopt the hexagonal morphology, which may interact with adjacent charges in the helper lipid, and charge neutralization at physiological conditions. As a

Acknowledgment—The encouragement and interest of Dr. George Robillard in this project is gratefully acknowledged.

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Molecular Shape of the Cationic Lipid Controls the Structure of Cationic Lipid/Dioleylphosphatidylethanolamine-DNA Complexes and the Efficiency of Gene Delivery

Jarmila Šmisterová, Anno Wagenaar, Marc C. A. Stuart, Evgeny Polushkin, Gerrit ten Brinke, Ron Hulst, Jan B. F. N. Engberts and Dick Hoekstra

J. Biol. Chem. 2001, 276:47615-47622. doi: 10.1074/jbc.M106199200 originally published online October 2, 2001

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