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# Physicochemical and morphological properties of complexes made of cationic liposomes and oligonucleotides

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**Abstract**

Cationic liposomes are a potential delivery system for antisense oligonucleotides, but physical chemistry of this system is still poorly understood. We studied physicochemical properties (size distribution, lipid mixing reactions) and the morphology of oligonucleotide–cationic lipid complexes in buffer and in cell culture medium using quasi-elastic light scattering (QELS), resonance energy transfer (RET) and freeze-fracture electron microscopy. In addition, interaction of the complexes with model membranes was studied at extracellular (pH 7.4), intracellular (pH 7.1) and endosomal (pH 5 and 6) conditions. Phosphorothioate oligonucleotides (ODN, 15 mer) were complexed with cationic liposomes composed of DOTAP, or DOTAP with DOPE at 1/1 and 1/2 molar ratios and investigated at different charge ratios ($-/+$, ODN–cationic lipid). The size of the complexes formed in water increased prominently in buffer and DMEM and it was maximal at charge ratio ($-/+$) of 1.2–1.5. In the case of sole DOTAP liposomes the complex sizes were smaller at $-/+$ ratios $<0.5$ and $>1.7$ in all media. In the presence of DMEM the DOPE-containing complexes gave average diameter in μm range irrespect of the charge ratio. Lipid mixing, in general, increased with increasing $-/+$ ratio and with DOPE in the presence of buffer or DMEM. Freeze-fracture electron micrographs showed cationic liposomes undergoing aggregation and fusion during interaction with ODNs. DOPE in cationic liposomes induced hexagonal lipid tubule formation that was most pronounced in cell culture medium. Upon incubation with endosomal model liposomes ODN was partly released from the complexes. The release was more pronounced when the liposomes contained DOPE. It appears that DOPE as a helper lipid affects the behaviour of ODN/lipid complexes at several stages. 

*Keywords:* Cationic liposomes; Oligonucleotides; Particle sizes; Resonance energy transfer; Freeze-fracture electron microscopy; Lipid fusion; Oligonucleotide release

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## 1. Introduction

Oligonucleotides (ODNs) are potential therapeutic agents that are used to block protein translation from mRNA (antisense effect) or transcription of RNA from the gene (antigene effect). Antisense and antigene oligonucleotides are negatively charged and relatively large molecules which do not readily gain access to their sites of action in the cytoplasm and nucleus, respectively (Stein and Cheng, 1993).

Cationic liposomes are an efficient and simple method to deliver oligonucleotides (Bennett et al., 1992) or plasmid DNA or RNA into cells (Felgner and Ringold, 1989; Malone et al., 1989). Oligonucleotides are not encapsulated in cationic liposomes, instead the liposomes bind these anionic compounds electrostatically (Jääskeläinen et al., 1994). Fusogenic and hexagonal phase forming DOPE (dioleoylphosphatidylethanolamine) helps cationic lipid mediated delivery of DNA (Felgner et al., 1994). but in the case of oligonucleotides the effect of DOPE on delivery and physical properties of the complexes have not been systematically studied. Cellular delivery of oligonucleotides and plasmid DNA by cationic liposomes was thought to take place via fusion of cationic lipids with negatively charged cell membranes thus enabling enhanced cytoplasmic delivery (Felgner and Ringold, 1989) but recent reports show, that endocytosis and destabilization of endosomal membrane is the major mechanism of delivery (Legendre and Szoka, 1992; Farhood et al., 1995; Wrobel and Collins, 1995; Zabner et al., 1995; Friend et al., 1996). This may be the case also in oligonucleotide delivery (Zelphati and Szoka, 1996a).

Despite the potential usefulness and apparent simplicity of cationic liposomes as a dosage form for oligonucleotides, the behaviour and morphology of liposome–oligonucleotide complexes with and without DOPE in buffer or cell growth medium is poorly understood. The interactions of oligonucleotides and cationic lipids in buffer were studied only recently (Jääskeläinen et al., 1994; Arima et al., 1997). Oligonucleotides induced aggregation and lipid mixing and depending on the charge ratio the complex size varied substantially. This is interesting since it is known that endocytic liposome delivery is sensitive to liposome size (Mönkkönen et al., 1994).

In the present study, some properties of the cationic liposome/phosphorothioate oligonucleotide complexes in buffer and cell growth medium are established to gain more insight to parameters possibly affecting cellular delivery. We studied the size distributions, lipid fusion, the morphology of the cationic lipid (DOTAP)/oligonucleotide complexes in buffer and growth medium, release of ODN after incubation with endosomal model membranes, and established the impact of DOPE on these properties.

## 2. Materials and methods

### 2.1. Lipids and oligonucleotides

DOTAP, DOPE, *N*-Rh-PE [N-(lissamine rhodamine B sulfonyl)-PE] and *N*-NBD-PE [*N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)-PE] were from Avanti Polar Lipids (Pelham, AL, USA). Phosphatidylcholine (PC, egg hydrogenated), phosphatidylinositol (PI, bovine liver), phosphatidylethanolamine (PE, bovine liver), phosphatidylserine (PS, bovine brain), sphingomyelin (SM, egg yolk), gangliosides (Gang, Type III from bovine brain) and cholesterol (Chol) were from Sigma (St. Louis, MO, USA). DMEM with (Cat No. 31885) or without Phenol Red (Cat No. 11880) was from Gibco Brl Life Technologies Inc. (Gaithesburg, MD, USA).

Phosphorothioate oligonucleotides (S-oligos) were 15-mer and they were synthesized with Applied Biosystems 381 A automatic DNA-synthesizer. Sequences for the unlabeled 15-mer oligos were the same as antisense and random sequences previously used in studies against human c-*myc* in HL-60 cells (Heikkila et al., 1987). FITC-labeled oligonucleotide had a sequence 5′-TGGCGT-CTTCCATTT-3′ (antisense to the initiation codon of luciferase gene).

### 2.2. Liposomes

Cationic liposomes composed of DOTAP or DOTAP/DOPE at molar ratios 1:1 and 1:2 were prepared in sterile water by the thin lipid hydration method followed by sonication until the mean diameter of 40–70 nm was achieved. For *N*-Rh-PE alone (1 mol%) or with *N*-NBD-PE (1 mol% each) labeled liposomes, labeled lipids were evaporated to dryness together with DOTAP and DOPE. DOTAP was 800 μM in all liposome stock formulations. Liposomes with lipid composition (PC:SM:PE:PS:PI:Gang:Chol, 5:1:1:1:1:1:3 by weight) mimicking that of endosomal membranes (Di Simone and Buchmeier, 1994) were prepared similarly in 20 mM Hepes, 150 mM NaCl buffer (pH 7.4) at 60°C. Mean diameter of these liposomes was about 100 nm.

### 2.3. Size determinations

The sizes of oligonucleotide/cationic lipid complexes were determined by quasi-elastic light scattering (Nicomp Submicron Particle Sizer, Model 370, Santa Barbara, CA, USA). Oligonucleotide in $H_2O$ solution (50 μl) was added to cationic liposomes in $H_2O$ (50 μl) solution with subsequent mild vortexing. After about 15 min the complex solution was divided in three aliquots, and 400 μl of either sterile $H_2O$, 20 mM Hepes buffer (pH 7.4) or DMEM was added and the size determinations were performed after about 15 min at ambient temperature. DOTAP concentration was 30 μM in each QELS measurement (total lipid 30, 60 or 90 μM). Ratios of S-oligo to cationic lipid were 0–0.2 (mol/mol ratio) or 0–2.8 ($-/+$ charge ratio). Charge ratio was calculated as 15 mer oligonucleotide having 14 negative charges and each cationic lipid one positive charge per molecule. Size distributions were determined as mean diameter on the basis of vesicle number.

### 2.4. Lipid mixing

Lipid mixing was monitored on a Perkin Elmer LS 50B Luminescence Spectrometer (England) as the increase in the donor (*N*-NBD-PE) fluorescence by resonance energy transfer (RET) at 460 nm (ex) and 530 nm (em) in a cuvette with magnetic stirring at 37°C continuously for 3 min. 20 μl of *N*-Rh-PE and *N*-NBD-PE (1 mol % each) labeled and 80 μl of unlabeled liposome-oligonucleotide complexes were added simultaneously to 1.9 ml Hepes buffer or DMEM (without Phenol Red). Cationic lipid concentration was 40 μM and total lipid concentrations 40, 80 and 120 μM for DOTAP, DOTAP/DOPE 1:1 and DOTAP/DOPE 1:2 liposomes, respectively. Triton X-100 at 0.2% by volume was used to assess total liposomal fluorescence after correcting for the quenching effect of Triton X-100.

### 2.5. Freeze-fracture electron microscopy

Cationic liposomes composed of DOTAP or DOTAP/DOPE at molar ratios 1:1 and 1:2 as well as oligonucleotide/liposome complexes were quenched rapidly for freeze-fracture electron microscopy using the sandwich technique and liquid propane (cooling rate $>10^4$ K $s^{-1}$). The cryofixed specimens were fractured and shadowed in a Balzers BAF 400D freeze-fracture device at $-120$°C and $2 \times 10^{-6}$ torr. The cleaned replicas were examined in a transmission electron microscope (Jeol JEM 100B or Zeiss CEM 902 A) (Sternberg, 1992).

### 2.6. Release of ODN from the complexes

FITC-labeled oligonucleotides were complexed in sterile water with DOTAP or DOTAP/DOPE 1:1 liposomes containing 1 mol% of Rho-PE (total lipid 700 μM) to yield $-/+$ charge ratios of 0.1, 0.25, 0.5, 0.75, 1.0, 1.5 and 2.0. The response of FITC was linear within this range.

Release of ODN from the complexes was measured on 96-well plates with Bio-Tek FL 500 fluorescence plate reader (Bio-Tek Instruments, Vermont, USA) with 485 nm (slit 20) excitation and 530 nm (slit 25) emission. The measurements were made in triplicate after 15 min incubation at 37°C in 20 mM Hepes, 150 mM NaCl, pH 7.4; in DMEM and in 130 mM K-acetate, 10 mM $MgCl_2$, 10 mM Hepes, pH 5, 6 or 7.1. Final lipid concentration–well was 9 μM.

Effect of endosomal model membranes on the fluorescence dequenching was evaluated by further incubation of the above samples with 15.6 μg/well of model liposomal lipids (about 1.5 excess of negative charges) for 15 min at 37°C. Fluorescence dequenching was calculated by comparing with free FITC-oligonucleotide incubated in triplicate simultaneously at the same concentration without liposome complexation. As the fluorescence baseline (at pH 7.1 and 7.4) did not significantly change with increasing the $-/+$ charge ratio up to 2 it is subtracted from values obtained and it is the 0%-level in results shown in Figs. 7 and 8.

## 3. Results and discussion

### 3.1. Size distributions

In $H_2O$ the mean size of the complexes increased with increasing $-/+$ ratio so that at charge ratio of about 1.2 the complex sizes were 5–10 fold that of the original liposomes without oligonucleotides (Fig. 1A). At higher $-/+$ ratios the complex sizes decreased gradually.

In Hepes buffer, similar size distributions were found up to $-/+$ charge ratio of about 0.6–0.8. Thereafter there was a sharp increase in sizes to μm range in case of DOTAP/DOPE liposome complexes (Fig. 1B). Only with DOTAP liposomes small sized complexes (about 100 nm) were seen at higher $-/+$ charge ratios (Fig. 1B).

In cell growth medium (DMEM) DOTAP liposomes showed similar size distribution profile as in Hepes, whereas DOTAP/DOPE liposomes at both molar ratios gave an average diameter of about 700–1200 nm (S.D. up to 60%) irrespect of the charge ratio (Fig. 1C). After 2 h (typical period for oligo-lipid complex exposure to cells) none of DOPE containing complexes, irrespect of the charge ratio, were measurable by QELS and visible particles were formed in many cases. QELS measurements with a single angle instrument of large DOPE containing complexes are not accurate due to the variable shape and size of the aggregated or fused complexes and continuous change in particle sizes during the measurement. However, the difference to DOTAP liposomes is clear. In addition, the electron microscopy revealed important morphological changes due to DOPE (see Section 3.3) as discussed later.

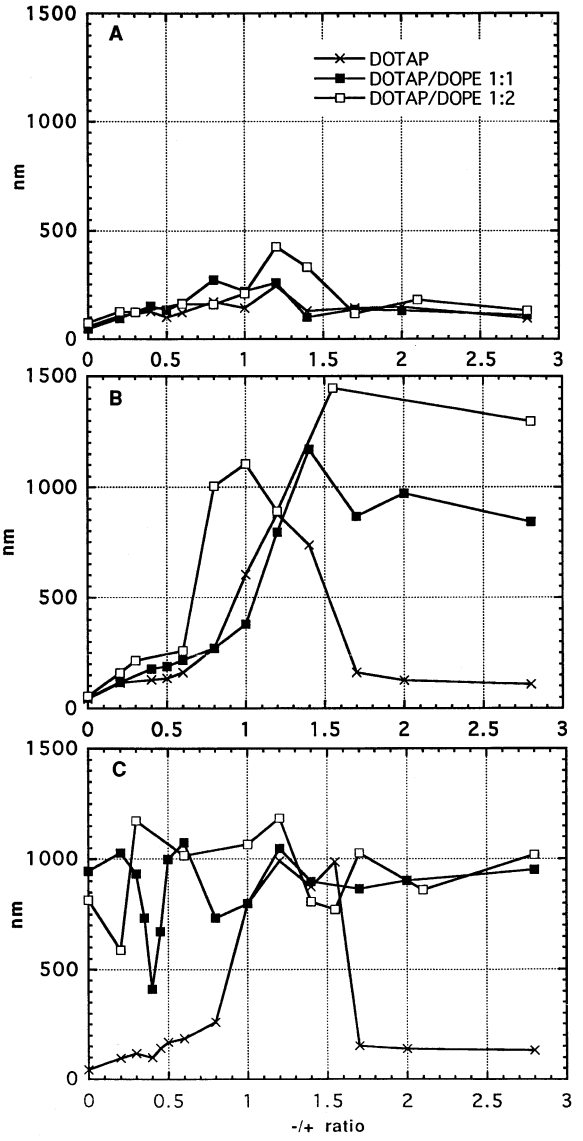


Fig. 1. Vesicle sizes (nm) of a 15-mer *S*-oligonucleotide complexed with cationic liposomes composed of DOTAP (×), DOTAP/DOPE 1:1 by mol (■), or DOTAP/DOPE 1:2 by mol (□) with increasing $-/+$ charge ratio. Vesicles prepared in $H_2O$ and incubated for 15 min (A) and consequently incubated for 15 min in Hepes (B) or DMEM (C).

These results suggest that the bound oligonucleotides add to the effects of ions in buffer on fusion reactions. This may take place via dehydration, as headgroup ionization, which favors $L_\alpha$ phase, is reduced by salts (Seddon et al., 1983) and dehydration is considered to be necessary for $H_{II}$ phase formation (Katsaras et al., 1993). In the case of DMEM, however, oligonucleotide content did not seem to have effect on the size distribution of DOTAP/DOPE complexes. Precise mechanism of DMEM induced size increase in DOPE containing liposomes is hard to specify due to amount of different components in DMEM (inorganic salts, glucose, sodium pyruvate, amino acids and vitamins). Nevertheless, the mean size of DOPE containing complexes exceeded clearly the size limit for liposome endocytosis in most cells (Mönkkönen et al., 1994).

### 3.2. Lipid mixing

In Hepes-buffer the lipid mixing generally increased by increasing the $-/+$ charge ratio up to about 1.2 (Fig. 2). At higher charge ratios ($-/+$) the lipid mixing stayed at about the same level except in the case of DOTAP liposomes, where lipid mixing drastically decreased at higher charge ratios. Maximal lipid mixing was achieved in most cases during 3 min and was about 40% for DOTAP/DOPE and about 15% for DOTAP liposomes (Fig. 2).

In DMEM (Fig. 3) lipid mixing of DOTAP-ODN complexes was seen only at $-/+$ charge ratios $\geq 1.2$. With DOPE substantial lipid mixing was observed at all charge ratios, including zero. Thus, DOPE induces, in addition to aggregation (Fig. 1), also fusion at all $-/+$ ratios in DMEM (Fig. 3).

In this study the cationic lipid concentration was constant and two DOPE concentrations were added. Therefore the total lipid concentration increased with increasing amount of DOPE. In principle, this increases the chance of aggregation. However, it is unlikely that the differences in total lipid concentration would result in different patterns in size distributions, resonance energy transfer and morphology of the complexes.

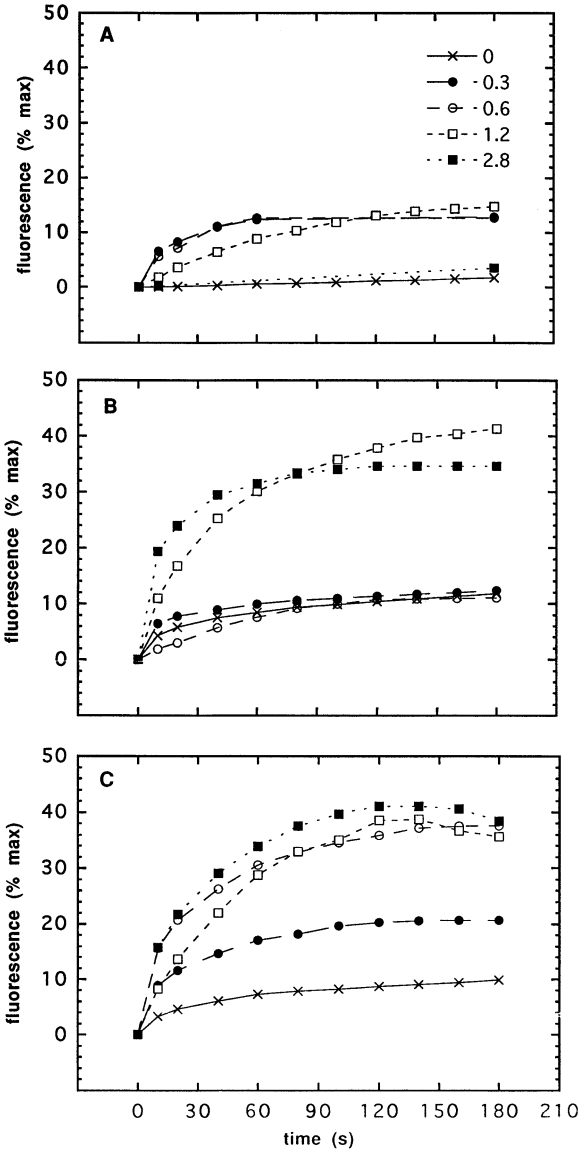


Fig. 2. Time course of lipid mixing after addition of liposome oligonucleotide complexes (80% unlabeled, 20% labeled liposomes) into 20 mM Hepes buffer (pH 7.4) at 37°C. 40 μM DOTAP (A), 80 μM DOTAP/DOPE 1:1 by mol (B), or 120 μM DOTAP–DOPE 1:2 by mol (C) liposomes. Charge ratios in the mixtures were 0 ( × ), 0.3 (●), 0.6 (○), 1.2 (□), 2.8 (■).

As we have shown earlier (Jääskeläinen et al., 1994), oligonucleotides increase lipid mixing in cationic liposomes by enabling their aggregation.

These data show that preformed oligonucleotide-lipid complexes undergo more fusion after exposure to buffer or DMEM. This may alter oligonucleotide delivery properties.

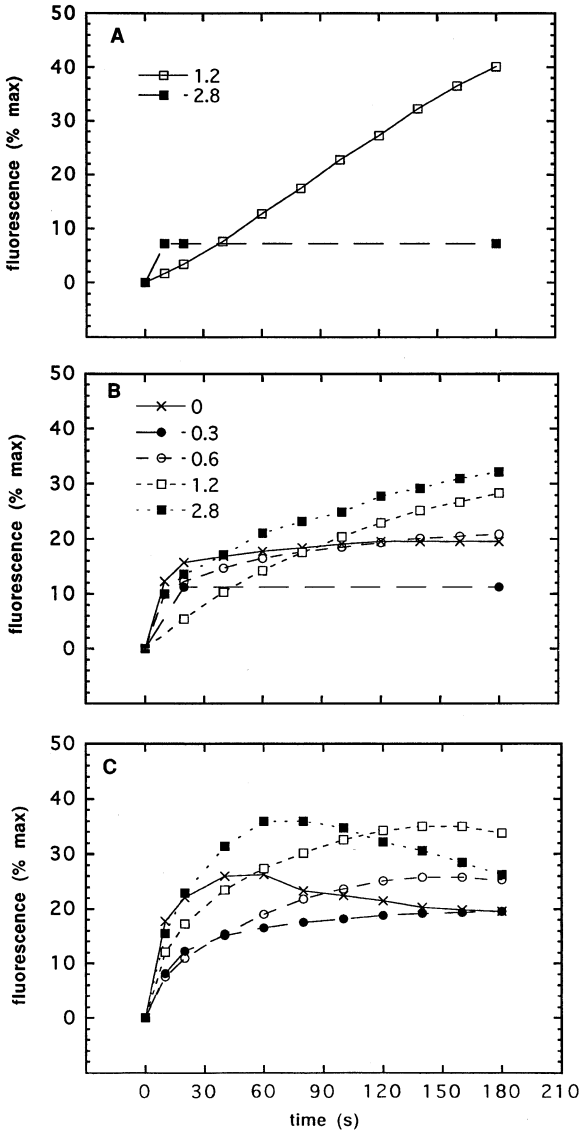


Fig. 3. Time course of lipid mixing after addition of liposome oligonucleotide complexes (80% unlabeled, 20% labeled liposomes) into DMEM at 37°C. 40 μM DOTAP (A), 80 μM DOTAP/DOPE 1:1 by mol (B), or 120 μM DOTAP/DOPE 1:2 by mol (C) liposomes. Charge ratios in the mixtures were 0 (×), 0.3 (●), 0.6 (○), 1.2 (□), 2.8 (■).

### 3.3. Morphology of the formed complexes

*Liposomes without oligonucleotides*: Freeze-fracture electron micrographs of liposomes incubated in Hepes-buffer, made of DOTAP (Fig. 4a), DOTAP/DOPE 1:1 (Fig. 4c) and DOTAP/DOPE 1:2 (Fig. 4E) show mainly small ($<300$ nm) vesicles, well separated from each other in the ice. Due to their positive surface charge, repulsive forces between the liposomes prevent their aggregation (Sternberg et al., 1994).

DOTAP liposomes were similar in cell culture medium (DMEM) as in buffer (Fig. 5A), whereas DOTAP/DOPE liposomes transformed to partial (Fig. 5C) or full (Fig. 5E) hexagonal-tubular ($H_{II}$) structures. Based on its wedge shaped molecular structure, DOPE can adopt highly curved structure, such as $H_{II}$ tubules. They are usually observed at high lipid concentrations, high temperatures (Cullis and Kruijff, 1979), and/or high salt concentrations (Allen et al., 1990). Accordingly, the high concentration of salts in the cell culture medium DMEM induced formation of $H_{II}$-tubules in DOTAP/DOPE liposomes without oligonucleotide (Fig. 5C and E).

*Morphology of oligonucleotide-liposome complexes*: Pictures were taken from lipid/oligonucleotide complexes in Hepes-buffer at $\pm$ charge ratio 0.3 (Fig. 6A–C), 1.2 (Fig. 4B, D and F) and 2.8 (6A′-C′). In addition, we studied the ODN/DOTAP and ODN/DOTAP:DOPE complexes in DMEM at charge ratio of 1.2 (5B, 5D, 5F).

In Hepes-buffer (Fig. 4B) and in DMEM (Fig. 5B), DOTAP/oligonucleotide complexes were aggregated, but without tubular hexagonal structures. In contrast, DOTAP/DOPE liposomes at 1:2 molar ratios were extensively transformed to $H_{II}$ lipid tubules in oligonucleotide complexes, especially at $-/+$ ratio of 1.2 in Hepes (Fig. 4F) and in DMEM (Fig. 5F). Here, massive fusion of liposomes is possibly induced by cations (Allen et al., 1990). Morphological features of liposomes made of DOTAP/DOPE at 1:1 molar ratio were found to be intermediate between DOTAP and DOTAP/DOPE (1:2). From these morphological results we conclude that increasing concentration of DOPE and ions (DMEM > Hepes) favors lipid fusion and $H_{II}$ tubule formation. Same kind of

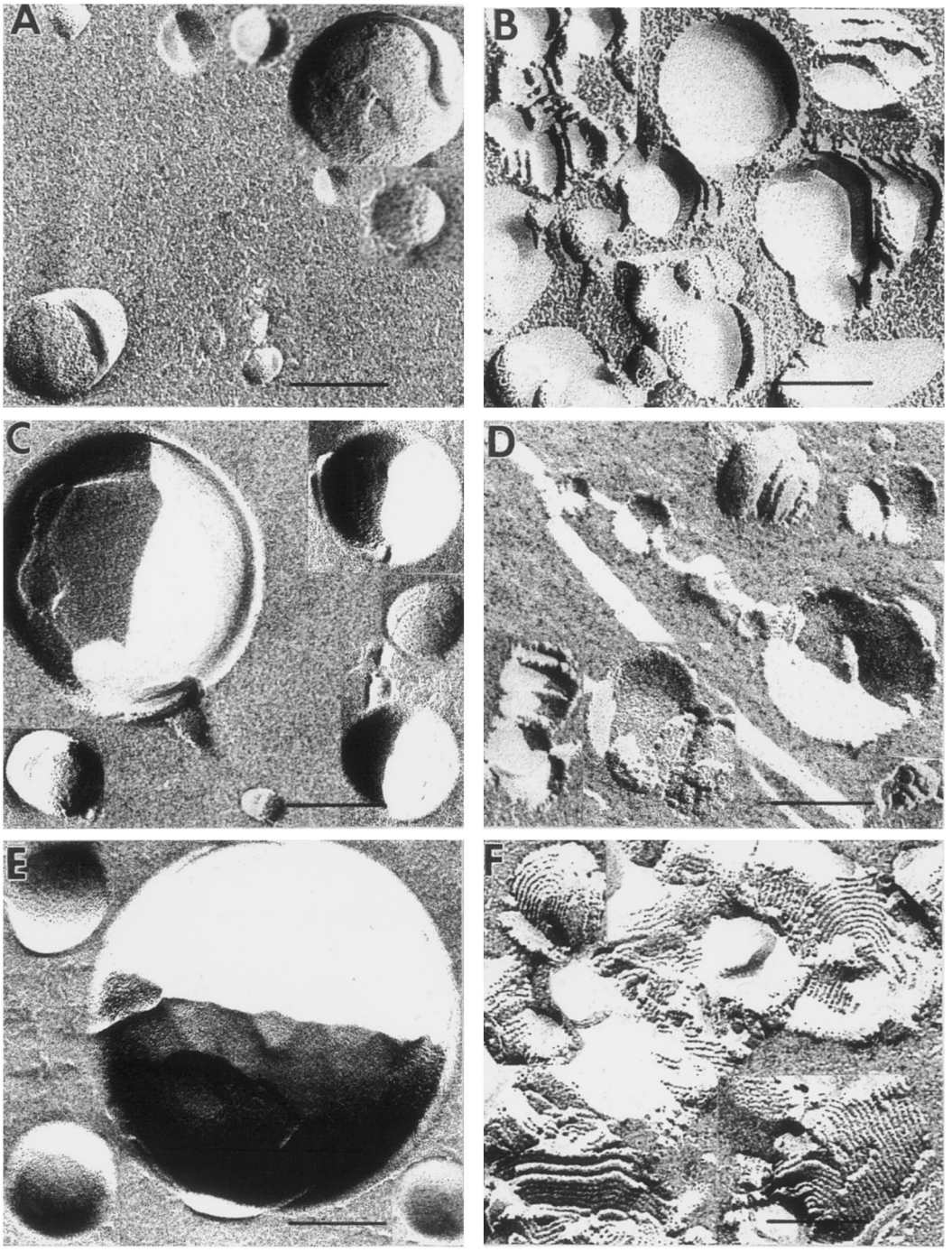


Fig. 4. Freeze-fracture electron micrographs of cationic control liposomes made of DOTAP (A), DOTAP/DOPE (1:1 molar ratios, C), and DOTAP/DOPE (1:2 molar ratios, E) in 20 mM HEPES-buffer, pH 7.4. Freeze-fracture electron micrographs of the related complexes made of a 15-mer *S*-oligonucleotide interacting with DOTAP (B), DOTAP/DOPE (1:1 molar ratios, D) and DOTAP/DOPE (1:2 molar ratios, F) at $-/+$ of 1.2 in HEPES-buffer (pH 7.4). Bar represents always 100 nm and the shadow direction is running from bottom to top of the electron micrographs.

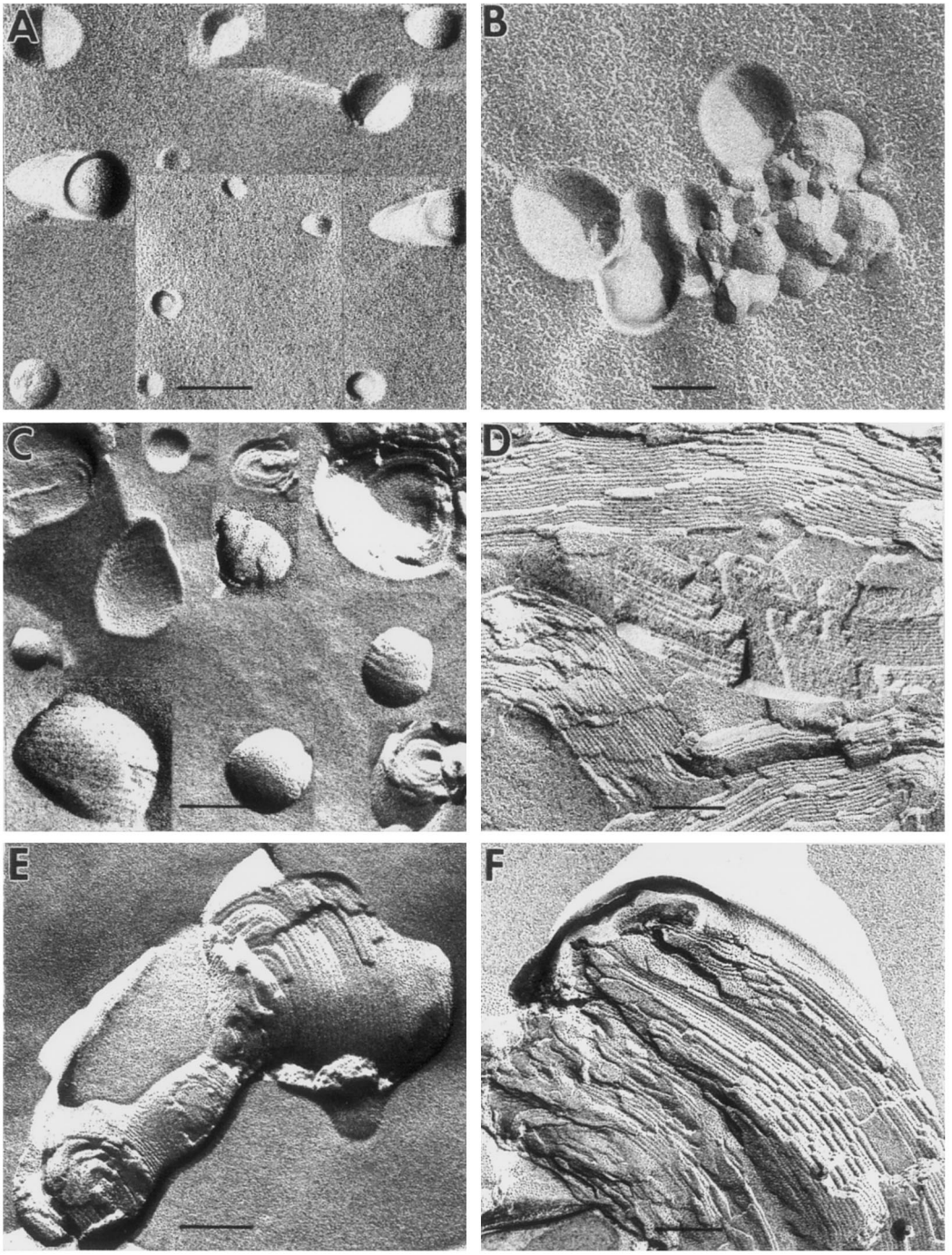


Fig. 5. Freeze-fracture electron micrographs of liposomes and complexes of the same composition as in Fig. 4 but prepared in DMEM instead of Hepes-buffer. Bar represents always 100 nm and the shadow direction is running from bottom to top of the electron micrographs.

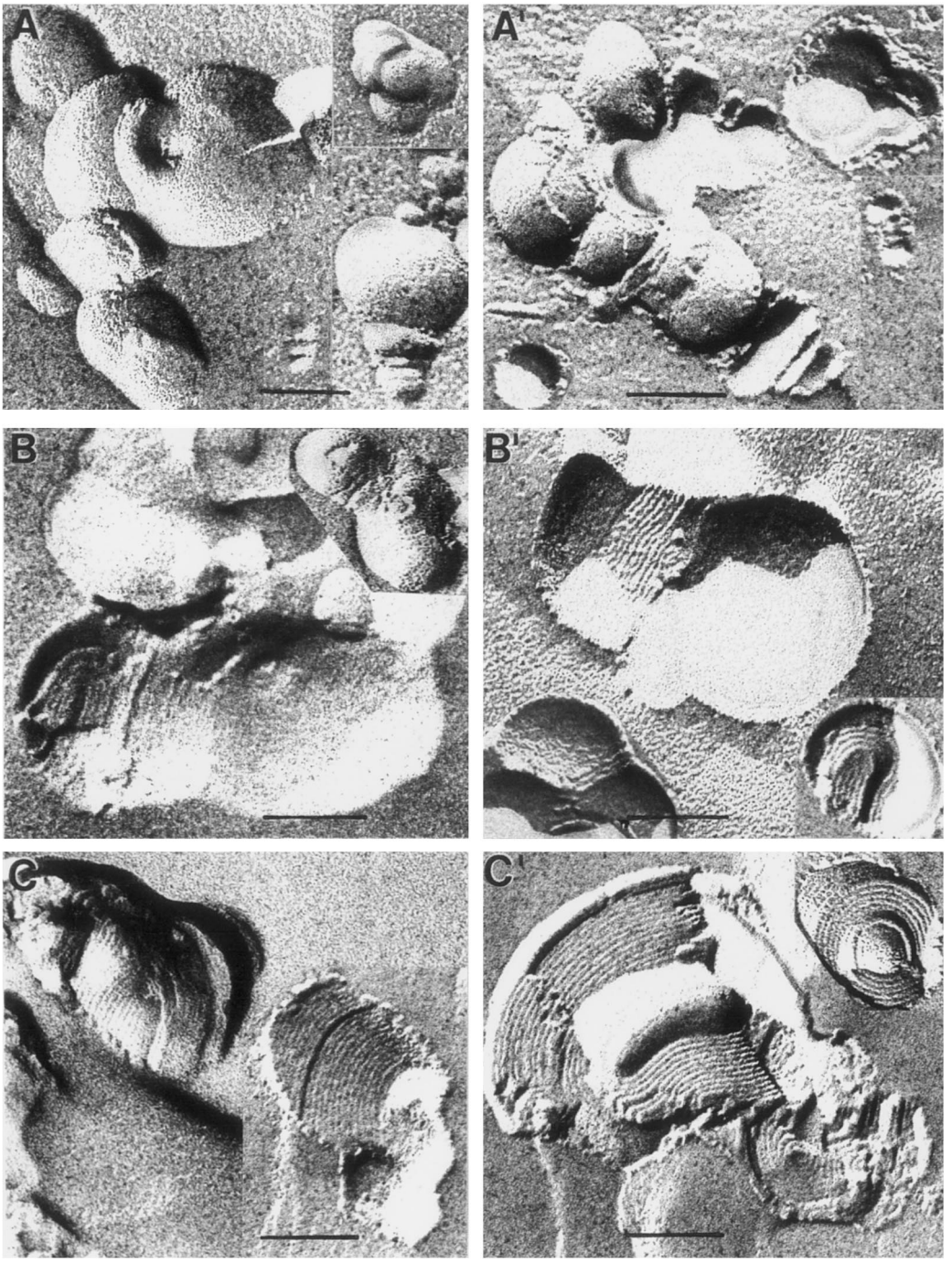


Fig. 6. Freeze-fracture electron micrographs of complexes made of a 15-mer *S*-oligonucleotide interacting with DOTAP (A), DOTAP/DOPE (1:1 molar ratios, B) and DOTAP/DOPE (1:2 molar ratios, C) at charge ratio $-/+0.3$ and respectively A′, B′ and C′ at a charge ratio of $-/+2.8$. All samples were prepared in HEPES-buffer (pH 7.4). Bar represents always 100 nm and the shadow direction is running from bottom to top of the electron micrographs.

conclusion can be drawn also at −/+ ratios of 0.3 and 2.8: without DOPE no $H_{II}$ tubular structures were seen (Fig. 6A, A′), and with increasing amount of DOPE hexagonal structures became evident (Fig. 6B, C′).

Different from plasmid DNA (Sternberg et al., 1994), no spaghetti-like structures are visible during interaction of cationic liposomes with oligonucleotides. It is possible, that the size of a single-strand oligonucleotide of about 2 nm is too small to band around a lipid bilayer tubule or the length of oligonucleotide is too short to be seen as 'spaghetti' in EM.

### 3.4. ODN release from lipid complexes

The lipid-ODN complexes were incubated with liposomes mimicking the endosomal membrane composition (Di Simone and Buchmeier, 1994), based on the plasma membrane composition of Chinese hamster ovary (CHO) cells (Warnock et al., 1993), to gain more insight to ODN release on endosomal level. There was a significant fluorescence dequenching, and probably release of ODN when DOTAP/DOPE complexes of ODNs were incubated with endosomal model membranes at pH 7.1 and 7.4. The release was highest at excess positive charge of the complexes. On the contrary, with DOTAP-ODN complexes there was no significant fluorescence dequenching at −/+ 0.1–2.0 charge ratios (Fig. 7). This suggests that DOPE facilitates ODN release from the complexes in contact with the bilayer.

Next, we studied the effect of the acidic endosomal pH on the complexes. Experiments without endosomal model membranes showed considerable dequenching when ODN-lipid complexes were placed at pH 5 and 6 (Fig. 8). This was most pronounced at −/+ charge ratio of 0.1 and it decreased gradually with higher −/+ ratios. Fluorescence dequenching was higher for DOTAP than DOTAP/DOPE liposomes.

Interestingly, there was additional fluorescence dequenching when ODN complexes with DOTAP/DOPE were incubated with endosomal model membranes at pH 5 or 6 (Fig. 9A), suggesting ODN release upon contact with endosomal

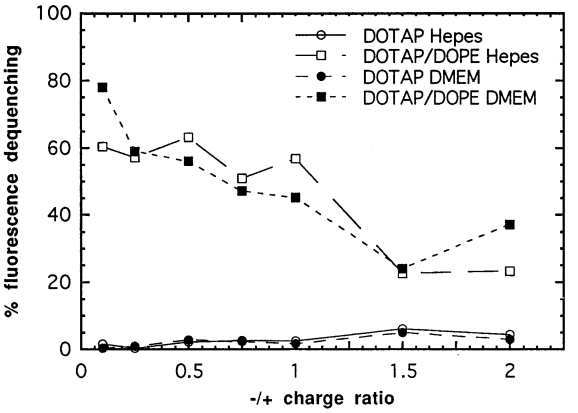


Fig. 7. Fluorescence dequenching (%) after incubation of ODN–cationic liposome complexes for 15 min at 37°C with endosomal model membranes (PC:SM:PE:PS:PI:Gang:Chol, 5:1:1:1:1:1:3 by weight) in 20 mM Hepes, 150 mM NaCl buffer, pH 7.4 or in DMEM.

model membranes. Surprisingly, fluorescence quenching was seen with DOTAP-ODN complexes at pH 5 and 6 when incubated with model liposomes at −/+ charge ratios of less than 1.5 (Fig. 9B). This indicates that the fluorescent labels come in closer proximity upon binding to the endosomal membranes, after partial loosening of the complex at acidic pH.

Fluorescence dequenching data is consistent with previous findings, where DOTAP/DOPE liposomes have been shown to fuse with nega-

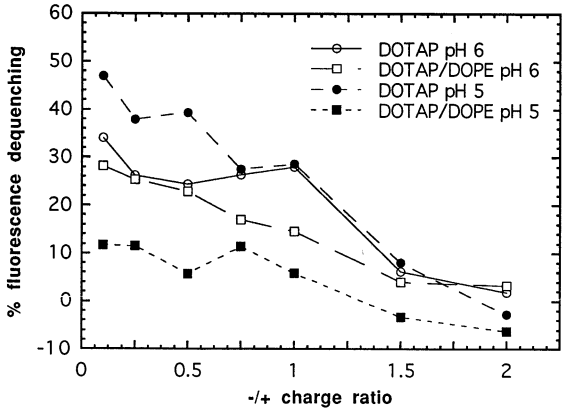


Fig. 8. Fluorescence dequenching (%) as compared to pH 7.1 (0%-level) after incubation of ODN/cationic liposome complexes for 15 min at 37°C in 130 mM *K*-acetate buffer, pH 6 or pH 5.

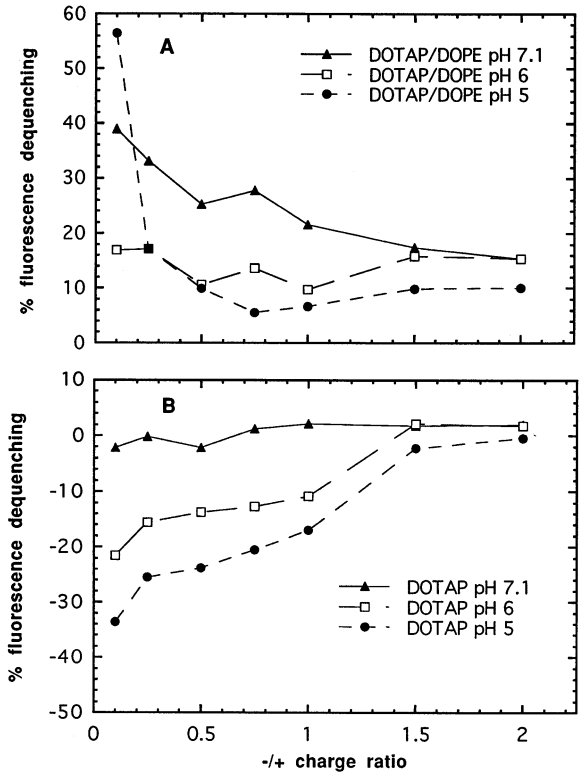


Fig. 9. Fluorescence dequenching (%) after incubation of ODN/cationic liposome complexes for 15 min at 37°C with endosomal model membranes (PC:SM:PE:PS:PI:Gang:Chol, 5:1:1:1:1:1:3 by weight) in 130 mM *K*-acetate buffer, pH 6 or pH 5. (Fig. 9A; DOTAP/DOPE 1:1 by mol, Fig. 9B; DOTAP).

tively charged DOPC/DOPG liposomes at pH range 4.5–7, whereas DOPC/DOTAP liposomes did not fuse (Wrobel and Collins, 1995). Similar results were obtained in lipid mixing experiments with cells, where both DOTAP liposomes showed similar binding and uptake but significant lipid mixing was seen only with DOPE containing liposomes (Wrobel and Collins, 1995). ODNs have been shown to be displaced from DOTAP liposomes when incubated with negatively charged fluid state liposomes (DOPE 50 mol%, 25 mol% DOPC and mol 25% of either PS, PG, PI or PA) but not with neutral (DOPE/DOPC 2:1 by mol) or fluid/solid state liposomes (DPPG/DPPC/DMPE 1:1:2 by mol) demonstrating the importance of the physical state of the target bilayer on ODN release (Zelphati and Szoka, 1996b). Our results are in agreement with the results obtained for solid state membranes with respect to DOTAP (Zelphati and Szoka, 1996b), as endosomal model membranes used in this study are partially composed of solid state lipids at 37°C. However, DOPE in liposomes caused significant release of ODN even in the case of our endosomal liposomes that contain relatively high amounts of cholesterol and sphingomyelin (Belcher et al., 1987; Lange et al., 1989; Warnock et al., 1993). High cholesterol/phospholipid ratio (mol/mol) of up to about 0.6 (Lange et al., 1989; Warnock et al., 1993) has been found in endosomal membranes. Both sphingomyelin and cholesterol increase the tightness of the bilayer membranes (New, 1990). TMA-DPH fluorescence anisotropy values for endosomal membranes for some cell lines are more in agreement with solid state than fluid state bilayers (Illinger et al., 1995). The lipid composition of model liposomes in this study is in agreement with the lipid composition of endosomal membranes and the results show clear difference between endosomal interactions of DOTAP and DOTAP/DOPE complexes. DOPE enhances transfection activity considerably with many, but not all, cell lines (Wheeler et al., 1996). Enhancing effects or lack of enhancement may be due to differences in physicochemical properties of complexes and to complex interactions at endosomal walls. Due to the complexity of the system there has been surprisingly little correlation between lipid mixing, complex size, $\zeta$ potential and transfection activities (Stegmann and Legendre, 1997).

## 4. Conclusions

Medium has a great effect on the size and morphology of the ODN-lipid complexes, as shown by several methods in this study. Especially DOPE containing complexes show great variability in size and morphology of the complexes, which may hamper their use as a drug delivery system. The complexes were sensitive to the medium showing the greatest changes in cell culture medium. The DOTAP/DOPE complexes undergo lipid fusion and $H_{II}$ tubule formation with

the culture medium. Finally, in contact with the endosomal wall model membranes DOTAP/DOPE liposomes released ODN, while DOTAP/ODN complexes did not.

Behaviour of lipid-ODN complexes are affected at several phases and more basic mechanistic research is needed to gain understanding of the cationic lipid mediated oligonucleotide delivery.

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