On the Transport of Lipoplexes through Cystic Fibrosis Sputum

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Purpose. The aim of this study was to examine the extent to which plasmid DNA (pDNA) complexed to cationic liposomes diffuse through cystic fibrosis (CF) sputum. The influence of the physical and chemical properties of the sputa was evaluated. We further investigated whether degradation of the sputa by recombinant human DNase I (rhDNase I) enhances the transport.

Methods. The transport of lipoplexes was studied through layers of CF sputa placed between the donor and acceptor compartment of vertical diffusion chambers. The content of the acceptor compartment was analyzed by confocal fluorescence microscopy, gel electrophoresis and Southern blotting. The influence of linear DNA present in the CF sputa on the size, surface charge and gene expression of the lipoplexes was evaluated by dynamic light scattering, particle electrophoresis and transfection experiments.

Results. Lipoplexes were observed in the acceptor compartments. However, the percent of diffused lipoplexes was low: 0.05% ± 0.01%. It was found that both steric obstruction by the sputa as well as the "long" distance the lipoplexes have to travel were responsible for this low transport. Surprisingly, the transport occurred better through more viscoelastic sputa. The DNA in the CF sputa also retarded the transport, which was attributed to aggregation of the lipoplexes by the DNA. Finally, rhDNase I moderately enhanced the diffusion of lipoplexes.

Conclusions. CF sputum drastically retards the diffusion of lipoplexes. DNA in the sputa aggregates the lipoplexes. This may lower the transport of lipoplexes through the sputa and gene expression. Pretreatment of CF patients with rhDNase I may enhance the efficiency of CF gene therapy, as it allows a better transport of the lipoplexes through the sputum and as it partly removes the sputum which will result in a thinner sputum layer on top of the epithelial cells.

KEY WORDS: CF gene therapy; diffusion; gene complexes; mucus; rhDNase I; gene expression.

INTRODUCTION

After the discovery of the genetic basis of cystic fibrosis (CF) (1) much attention has been given to tackle CF via gene therapy (2–5). This led to promising results *in vitro* and in CF mouse lungs using either viruses or cationic liposomes as carrier for the cystic fibrosis conductance regulator (CFTR) gene (2,3). However, clinical trials in which these gene carriers were used to deliver the CFTR gene to the nose or lower respiratory tract of CF patients showed insufficient correction of the ion transport (6,7). This discrepancy is probably attributed to differences between the CF lung epithelium at one hand, and cell culture or mice models at the other hand. In CF patients, the target cells in the lung are covered by a huge amount of thick mucus. Especially, compared with airway secretions of healthy people, CF mucus contains high amounts of DNA (mainly from died neutrophils), mucin, albumin, phospholipids and inflammatory products (8–11). Therefore it seems very plausible that, to some extent, CF mucus may be responsible for the inefficient gene transfer in CF patients. Indeed, Stern *et al.* and Kitson *et al.* showed that when diluted or native CF sputum was applied on top of a cell layer or on sheep airway epithelia *in vitro* gene transfer using viruses or cationic liposomes was significantly decreased (7,12). The reasons why CF sputum decreases gene transfer were not elucidated in these studies, although this could be crucial information to further optimize the CFTR gene carriers. In a previous report we have shown that CF sputum dramatically retards, in a size depended way, the movement of negatively charged polystyrene nanospheres having sizes comparable to lipoplexes. For the largest particles (560 nm) an almost complete blockage was even observed (13). We also recently found that CF sputum components destabilize cationic lipoplexes: albumin and mucin changes the surface charge of the cationic lipoplexes to negative. However, the most destructive interaction occurred by linear DNA, which is abundantly present in CF mucus: linear DNA aggregates the lipoplexes while it also partially releases pDNA from the cationic lipoplexes (14). Also, other groups have shown that viral carriers become coated by preexisting antibodies in the CF sputum which also results in a decreased gene transfer (15).

The aim of the study reported here was 1) to evaluate to which extent cationic lipoplexes can move through a CF sputum layer, 2) to elucidate the influence of the physicochemical characteristics of the sputa on the transport of lipoplexes, and 3) to find out whether recombinant human deoxyribonuclease I (rhDNase I) can enhance the diffusion of lipoplexes through CF sputum.

MATERIALS AND METHODS

Materials

Dioleoylglycerophosphoethanolamine (DOPE); 1,2-dioleoyl-3-trimethylammoniumpropane (DOTAP) and nitrobenzoxadiazol labeled DOPE (NBD-DOPE) were purchased from Avanti Polar Lipids (Alabaster, AL, USA). The Maxi Prep kit was obtained from Qiagen (Leusden, The Netherlands). The Label IT® fluorescein nucleic acid labeling kit was obtained from Panvera (Madison, WI, USA). Sybr® Green I nucleic acid gel stain was from Molecular Probes (Leiden, The Netherlands). Electrophoresis grade agarose, Dulbecco's phosphate buffered saline (PBS), Dulbecco's modified eagle medium (DMEM), penicillin-streptomycin mix, fetal calf serum (FCS) and the Blugene® non-radioactive nucleic acid detection kit were obtained from GibcoBRL®, Life Technologies (Merelbeke, Belgium). We used self-made sterile Hanks' buffered salt solution (HBSS). Pulmozyme® containing 1 mg (1000 genentech units) of recombinant human deoxyribonuclease I (E.C.3.1.21.1) per mL was a gift from N.V. Roche S.A. (Brussels, Belgium).

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Physicochemical Characterization of the CF Sputa

Approval for the collection of CF sputum was obtained from the ethics committee of the University Hospital of Ghent. The sputum was expectorated spontaneously by CF patients during chest physiotherapy sessions. The elastic modulus (G'), the viscous modulus (G"), the mucin, DNA and protein concentrations were determined on "sputum fractions" taken out of the "sputum samples" and analyzed as previously described (13). In this study, "a sputum sample" is defined as the collected amount of sputum from one patient during a physiotherapy session. "Sputum fractions" are small fractions of sputum obtained from the "sputum samples."

Preparation of Cationic Liposomes

Cationic liposomes containing DOTAP and DOPE in a 1:1 molar ratio with or without 2 mol % NBD-DOPE were prepared as follows. The lipids were dissolved in a 1:1 (volume) mixture of chloroform:methanol. Consequently, the solution of lipids was placed in a round-bottomed flask and the solvents were evaporated under vacuum at 30°C for about 30 min. The resulting lipid film was further dried under a flow of nitrogen for 1 h The lipids were resuspended in HEPES buffer (20 mM HEPES pH 7.4) at a final concentration of 5 mM DOTAP and rehydrated overnight at 4°C to form cationic liposomes. The following day the liposomes were extruded at room temperature through a polycarbonate membrane (pore size of 0.1 μm) using the Avanti Polar Lipids Mini-extruder. The hydrodynamic size and the zeta potential (ζ) of the resulting cationic liposomes were measured by dynamic light scattering (DLS) (Autosizer 4700, Malvern Instruments, Worcestershire, UK) and particle electrophoresis (Zetasizer 2000, Malvern Instruments, Worcestershire, UK), respectively. The hydrodynamic size and ζ of the cationic liposomes, dispersed in HEPES buffer were 125 nm ± 10 nm and +50 mV ± 2 mV, respectively.

Preparation, Purification, and Fluorescent Labeling of Plasmid DNA

The pDNA consisted of 5803 base pairs and contained as reporter gene secretory alkaline phosphatase (SEAP) under the control of the simain virus 40 promoter. The pDNA was amplified in *Escherichia coli*. For the isolation and purification of the pDNA from the bacteria by alkaline lysis, the Maxi Prep kit from Qiagen was used. After precipitation of the pDNA by isopropanol, it was dissolved in HEPES buffer. The pDNA concentration was set at 1.0 mg/mL assuming that the absorption of 50 μg DNA/mL at 260 nm equals one. The pDNA showed a high purity as the 260 nm/280 nm absorption ratio was between 1.8 and 2.0. Fluorescein labeled pDNA (fluorescein-pDNA) was prepared using the Label IT® fluorescein nucleic acid labeling kit. Via this technique fluorescein was covalently attached to the pDNA. To remove unbound fluorescein the pDNA was precipitated by adding 0.1 volume of 5 M NaCl and 2 volumes of ice cold 100% ethanol to the pDNA. After centrifugation, the pellet was washed with 70% ethanol until the supernatant contained no fluorescein. Consequently the pellet was dissolved in HEPES buffer and the concentration was determined by absorption at 260 nm.

Preparation of Lipoplexes

Plasmid DNA was first diluted in HEPES buffer to a concentration of 0.41 mg/mL. Subsequently, the diluted pDNA was added to an equal volume of cationic NBD-DOPE containing liposomes (5 mM DOTAP) resulting in a ± charge ratio of 4. Immediately after the addition of pDNA to the cationic liposomes, HEPES buffer was added till the final concentration of pDNA in the system was 0.126 mg/mL. This mixture was then vortexed and incubated at room temperature for 30 min. This resulted in the formation of lipoplexes. Fluorescein-pDNA containing lipoplexes were prepared in a similar way, except that the cationic liposomes did not contain NBD-DOPE and that the pDNA contained 20% (by pDNA mass) fluorescein-pDNA. The Z-average size and ζ of the lipoplexes, dispersed in HEPES buffer, were routinely determined and equaled 280 nm ± 40 nm and +50 mV ± 2 mV, respectively. The positive ζ (+50 mV) of the lipoplexes indicates that the pDNA becomes completely entrapped and surrounded by a lipid (bi)layer as previously described (16).

Influence of Linear DNA on Lipoplex Size, Surface Charge, and Gene Expression

Lipoplexes were incubated with linear DNA which was dissolved in "sputum buffer" (85 mM Na^+, 75 mM Cl^-, 3 mM Ca^{2+} and 20 mM HEPES pH 7.4). This sputum buffer represents the average content of these ions in sputum. The linear DNA:complexed pDNA concentration ratios which were considered agreed with the concentration ratios as expected to occur *in vivo* (6,14). The effect of the linear DNA on lipoplex size, surface charge and transfection efficiency was studied using respectively dynamic light scattering, particle electrophoresis and COS 1 cells. COS 1 cells (1.6×10^4 cells per well) were grown (at 37°C under 5% CO_2) for 48 hrs in 24 well plates using DMEM supplemented with penicillin and streptomycin sulfate (respectively 50 IU/mL and 50 μg/mL) and 10% fetal calf serum as grow medium. Just before transfection, cells were washed twice with sterile HBSS. Subsequently, 5 μL of the linear DNA/lipoplex mixtures were mixed with 495 μL of sputum buffer supplemented with 1.7% glucose and applied on the cells. The amount of pDNA per well equaled 0.315 μg. After 4 hrs the linear DNA/lipoplex mixtures were removed, cells were rinsed two times with PBS and 500 μL grow medium was added. 48 hrs later, SEAP activity was determined in the medium using 4-methylumbelliferyl phosphate (17).

Diffusion of Lipoplexes through CF Sputum

As the sputum may undergo enzymatic degradation during the diffusion experiments, we first added 0.21 mg/mL phenylsulfonylfluoride, a protease inhibitor, and 0.40 mg/mL NaN_3, an antibacterial agent, to the sputa (13). To determine the diffusion of lipoplexes through CF sputa, we made use of modified vertical diffusion chambers. These modified diffusion chambers consisted out of a donor and an acceptor compartment, separated from each other by a 220 μm thick center compartment which contained CF sputum (13). The donor and the acceptor compartment were simultaneously filled with respectively 4 mL of the lipoplex dispersion and buffer. The lipoplex dispersion in the donor compartment was obtained by diluting (3.8 fold) the prepared lipoplex dispersion

with sputum buffer. The buffer in the acceptor compartment was a 3:1 (vol) mixture of HEPES and sputum buffer.

Mixing in the compartments occurred by a flow of nitrogen. We conducted the transport experiments at 20°C for 150 min as longer times and higher temperatures degraded the CF sputa, even when phenylsulfonylfluoride and NaN_3 were added to the sputa (13). After this time frame the concentration of the lipoplexes in the acceptor compartment was determined fluorometrically. An excitation wavelength (λ_{ex}) of 460 nm and an emission wavelength (λ_{em}) of 545 nm was used for the NBD containing lipoplexes, while for the fluorescein-pDNA containing lipoplexes the λ_{ex} and λ_{em} were 490 nm and 530 nm, respectively.

To determine the diffusion of lipoplexes through a 220 μm thick layer of buffer we applied a viscous solution (80% sucrose dissolved in sputum buffer) in the center compartment. A sucrose solution was used as it was difficult to apply a (low viscous) buffer layer in the center compartments. Subsequently, the small sucrose molecules were removed from the center compartment by filling the donor and acceptor compartment with sputum buffer. During 5 days, the sputum buffer in the donor and acceptor compartments was daily removed and replaced by fresh buffer. Consequently, we measured the transport of lipoplexes through this buffer layer as described above.

The binding of lipoplexes to the walls of the diffusion chamber was evaluated. At low concentration, the binding of lipoplexes was significant. Therefore the concentration of the lipoplexes in the acceptor compartment was corrected as described previously (13).

Influence of rhDNase I on the Diffusion of Lipoplexes through CF Sputum

To evaluate the effect of rhDNase I on the diffusion of lipoplexes, we first degraded the CF sputum by adding 6 μg rhDNase I per mL to the sputum before it was placed between the donor and acceptor compartments.

Confocal Microscopy

Confocal images of the dispersion in the acceptor and donor compartments were taken using a 60× lens. Therefore, a confocal laser scanning microscope (MRC1024, Bio-Rad, Hemel Hempstead, UK) was used.

Agarose Gel Electrophoresis and Southern Blotting

After the diffusion of the lipoplexes through the CF sputa the dispersion in the acceptor compartment was vacuum dried. Consequently, the dry material was dissolved in 100 μL of distillated water and 30 μL of this solution was mixed with 7.3 μL of a 2.4% sodium dodecyl sulfate (SDS) solution (to release pDNA from the lipoplexes). After 30 min 3.6 μL of a 50% sucrose solution was added to this mix and the resulting mixture was loaded on a 1% agarose gel prepared in TBE buffer (10.8 g/L Tris base, 5.5 g/L boric acid and 0.85 g/L ethylenediaminetetraacetic acid). Electrophoresis was done at 6.5 V/cm in TBE buffer during 1 h The gel was stained with Sybr® Green I and visualized under UV light. Consequently, the DNA on the gel was transferred onto a supported nitrocellulose membrane and hybridized with a biotinylated probe. To visualize the hybridized probes the Blugene® non-radioactive Nucleic Acid Detection kit was used.

Statistical Analysis of the Results

The experimental results in this paper are expressed as means ± standard deviations. In studying the effect of sputum viscoelasticity and DNA concentration on the diffusion of the lipoplexes, we tested the equality of the means through analysis of variance. Student's t test was used in evaluating the effect of rhDNase I on the diffusion of the lipoplexes. Significance was set at $P = 0.05$.

RESULTS AND DISCUSSION

The rheological moduli, the DNA, mucin and protein concentration of the CF sputa used in this study are given in Table I. Also the percent of lipoplexes transported through these sputa are shown.

As the concentration of the diffused lipoplexes was determined by measuring the fluorescence of the NBD-DOPE lipids in the acceptor compartments and as lipoplexes may disintegrate in sputum, we preferred to confirm that the fluorescence in the acceptor compartment was really attributed to lipids still associated with the pDNA. First, by confocal fluorescence microscopy we visualized the content of both the donor and acceptor compartments and wondered whether fluorescent "particles" could be identified. For this purpose we diluted the content of the donor compartment and con-

Table I. Rheological Moduli, Mucin, DNA, and Protein Concentration in the CF Sputum Samples[a]

Sputum sample	G′ (Pa)	G″ (Pa)	DNA concentration (mg/mL)	Mucin concentration (mg/mL)	Protein concentration (mg/mL)	% Lipoplexes transported
1	1.5 ± 0.2	0.69 ± 0.03	1.1 ± 0.1	10 ± 1	8*	0.045 ± 0.002
2	2.0 ± 0.3	0.9 ± 0.1	3.3 ± 0.3	10 ± 1	13 ± 3	0.029 ± 0.003
3	2.9 ± 0.4	1.2 ± 0.1	1.8 ± 0.3	11 ± 1	21 ± 3	0.045 ± 0.004
4	3.6 ± 0.2	1.16 ± 0.03	1.8 ± 0.2	13 ± 1	16 ± 2	0.044 ± 0.006
5	25 ± 7	5 ± 1	1.8 ± 0.7	15 ± 3	13 ± 5	0.066 ± 0.007
6	32 ± 8	7 ± 1	3.5 ± 0.2	17 ± 3	25 ± 5	0.043 ± 0.004
7	600 ± 12	129 ± 6	10 ± 1	15 ± 3	87*	0.057 ± 0.004
mean	95 ± 223	21 ± 48	3 ± 3	13 ± 3	26 ± 27	0.05 ± 0.01

[a] The out most right column shows the percentage of the lipoplexes that arrived in the acceptor compartment 150 min after the start of the experiment. Besides the values indicated by *, all other values are the outcome of experiments on four sputum fractions taken out of each sputum sample.

centrated the content of the acceptor compartment. Figure 1 (A and B) shows that in both compartments fluorescent particles were present. To further determine whether these fluorescent particles also contained the pDNA we analyzed the content of the acceptor compartments by gel electrophoresis and Southern blotting (Fig. 2A and B). Southern blotting was necessary as after gel electrophoresis a DNA smear was observed (due to the leakage of small amounts of DNA from the sputum into the compartments (13)), which could mask the presence of free pDNA. Figure 2 B shows the presence of non degraded pDNA in the compartments. Moreover, as expected, the higher the measured fluorescence in the acceptor compartment, the higher the intensity of the pDNA bands. As it was still possible that the pDNA in the acceptor compartment was not derived from diffused lipoplexes but from free pDNA diffused from the sputa into the acceptor compartment, we measured the transport of an equal amount of free pDNA placed in the donor compartment. Allowing 150 min for transport, no pDNA was detected in the acceptor compartments. Finally, we also evaluated the transport of lipoplexes containing fluorescein labeled pDNA. The concentration of lipoplexes in the acceptor compartments was now determined from the fluorescence of the fluorescein-pDNA. The percent of lipoplexes transported through the sputum was in the same range as obtained using lipoplexes containing NDB-DOPE. This indicates again that pDNA and NBD-DOPE were most likely transported in an associated form through the sputa.

Table I shows that the amount of lipoplexes transported through the mucus after 150 min was extremely low. We conducted the transport experiments at 20°C for 150 min as longer times and higher temperatures degraded the CF sputum (13). On the average, only 0.05% ± 0.01% moved through the sputum layer. This agrees with previous work in which we studied, using the same experimental conditions as reported here, the transport of negatively charged polystyrene through CF sputum (13). We found that the percents of nanospheres transported after 150 min through different CF sputa were 0.24% ± 0.08%, 0.022% ± 0.008% and 0.0017% ± 0.0009%, for respectively 124 nm, 270 nm and 560 nm large nanospheres. Due to the presence of massive amounts of negatively charged biopolymers in CF sputa, one would expect that the positively charged lipoplexes would diffuse through the sputum even to a lower extent than the negatively charged 270 nm nanospheres. However, the number of cationic lipoplexes that arrived in the acceptor compartment was about 2.5 fold higher than the number of transported polystyrene nanospheres, having a similar hydrodynamic size (as obtained by DLS) (13). This is probably attributed to the broader size distribution of the lipoplexes compared to the more monodisperse properties of the polystyrene nanospheres: the better transport of the lipoplexes may be due to the presence of a significant amount of lipoplexes which are smaller than the measured average hydrodynamic size.

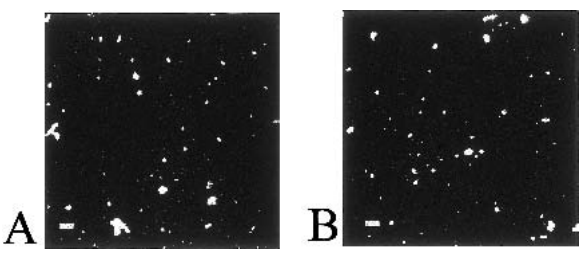

Fig. 1. Confocal images of the content of the donor compartment (A) and acceptor compartment (B) after diffusion of the lipoplexes. The lipoplexes are smaller than the resolution of the microscope, but due the emission of fluorescent light they appear as white spots which are larger than the actual size of the lipoplexes. Bars = 10 μm.

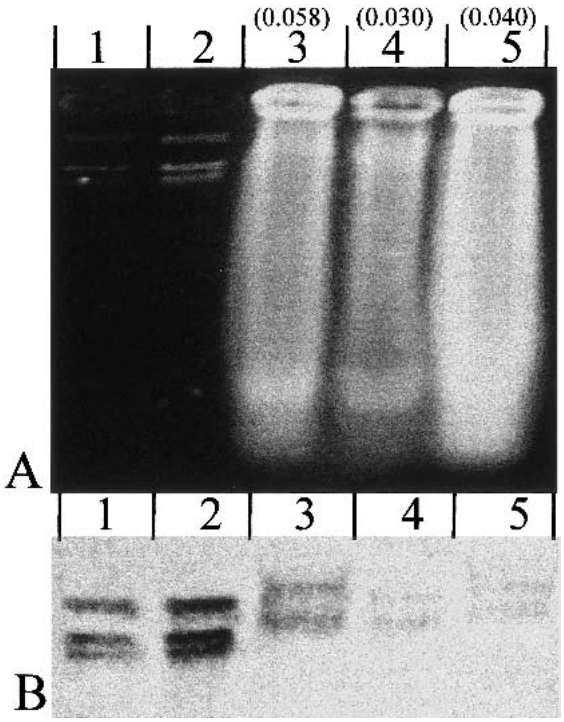

Fig. 2. (A) Gel electrophoresis on the content of the acceptor compartments. Lane 1 and 2 contain 0.005 μg and 0.01 μg free pDNA, respectively. Lane 3 to 5 concern three acceptor compartments in which respectively 0.058%, 0.030% and 0.040% of the lipoplexes were transported (as measured by fluorescence). On the corresponding Southern blot of the gel (B) pDNA is detected in all the lanes. The intensities of the pDNA in lane 3 to 5 correlate with the fluorescence in the acceptor compartments (these values are indicated on top of lane 3 to 5).

The mean percent of lipoplexes diffused after 150 min at 20°C through a layer of buffer equaled 0.17% ± 0.02%. This is only a factor 3.4 higher than the percent of lipoplexes diffused through CF sputum. This indicates that the low amount of lipoplexes in the acceptor compartments after 150 min is due to both a steric hindrance of the lipoplexes by the sputum as well as due to the "long" distance the lipoplexes have to travel to reach the acceptor compartment.

Figure 3 shows the percentage of lipoplexes transported through the CF sputa as a function of the elastic modulus of the sputa. A similar trend was obtained when the viscous modulus of the sputa was plotted in the x-axis. At a first sight, no correlation exists between the viscoelasticity of the sputa and the amount of transported lipoplexes. Clearly, while one could expect a better transport through less viscoelastic sputa, this was not observed. A closer look shows that, if we compare the transport through sputa with comparable DNA concentrations (compare sputum samples 3, 4 with 5 and 2 with 6) even a significant ($P < 0.05$) higher transport occurred through the more viscoelastic sputa. This agrees with our pre-

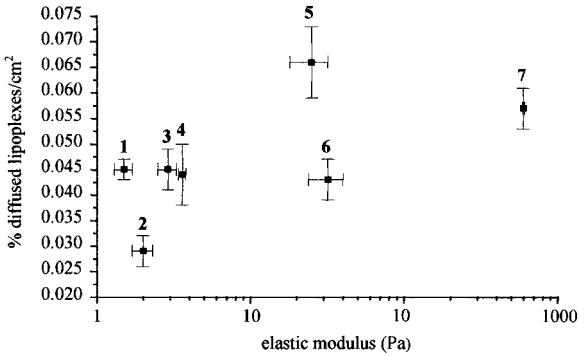

Fig. 3. Percent of lipoplexes that were transported (after 150 min) through a 220 μm thick layer of CF sputum as a function of the elastic moduli of the sputa (n = 4). The numbers in the graph refer to the CF sputa shown in Table I.

vious observations on polystyrene nanospheres which also moved more easily through sputa with a stronger viscoelasticity (13). To explain this we hypothesized that the biopolymer network in CF sputum changes from a homogeneous microporous structure toward a more heterogeneous macroporous structure when the sputa become more viscoelastic (13).

We wondered whether the DNA in the sputa influences the lipoplex transport. The transport of lipoplexes through sputa with a similar viscoelasticity (i.e., no significant difference) was significantly lowered in sputa with a higher DNA concentration (compare sputum sample 2 with 3 and 5 with 6). Figure 4A shows that adding linear DNA to the lipoplex dispersions may aggregate the lipoplexes. Moreover, a dramatic change of the surface charge also occurs. The aggregation of the lipoplexes induced by DNA, which is abundantly present in the sputum, will further decrease the transport of lipoplexes through CF sputum. The concentration ratios at which linear DNA destabilizes lipoplexes corresponds with the concentration ratios one can expect to occur *in vivo* (6,14). Based on these observations we wondered whether these alterations are also reflected in the extent of gene expression in cell cultures. Figure 4B shows the influence of linear DNA on gene expression in COS 1 cells. The lowest gene expression was indeed observed at linear DNA:pDNA concentration ratios in which the linear DNA caused lipoplex aggregation. This is most likely due to a decreased endocytosis of large aggregates. Interestingly, lipoplexes with a pronounced negative surface charge, as a result of the interaction with the linear DNA, showed expression as efficient as the control (i.e. lipoplexes in the absence of linear DNA). As it is highly likely that the linear DNA in CF sputa aggregates the lipoplexes into micrometer sized particles, and as we showed previously that polystyrene nanospheres larger than 560 nm become completely blocked in the sputa, linear DNA may further decrease *in vivo* gene expression in epithelial cells covered with CF sputum.

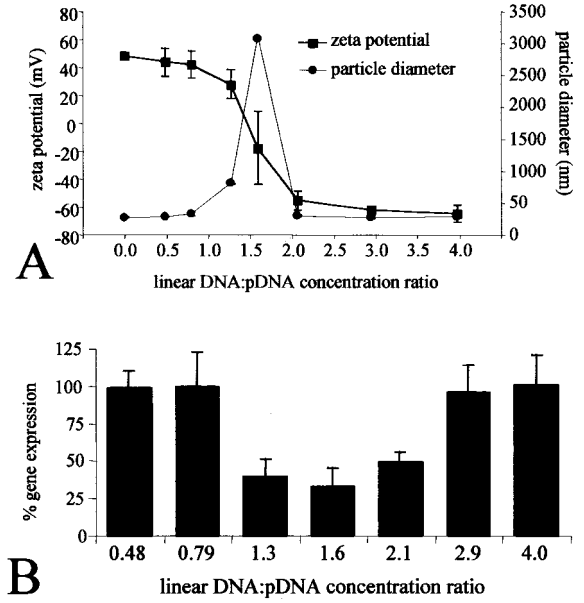

Fig. 4. (A) The zeta potential and size of the lipoplexes as a function of the linear DNA:pDNA concentration ratio (n = 3). The measurement indicated in gray (•) was considered to be inaccurate as dynamic light scattering does not allow to measure accurately micrometer sized particles. In (B) the influence of linear DNA on the gene expression efficiency of lipoplexes is shown (n = 3). The gene expression in the absence of linear DNA was considered as the "control" value.

RhDNase I is often used in CF therapy to reduce sputum viscoelasticity. Because it partly disrupts the biopolymer network in CF sputum by cleaving DNA chains, we wondered whether rhDNase I would also enhance the transport of lipoplexes through CF sputum. For this purpose, the diffusion of lipoplexes (containing NBD-DOPE) through 4 untreated and 4 rhDNase I treated sputum fractions (6 μg rhDNase I/mL sputum) was investigated. A sputum sample having a high DNA concentration was used for this purpose. The rhDNase I decreased the viscoelasticity of this sputum sample to 73% of its start value. As shown in Figure 5, the transport of the lipoplexes increased moderately (although significantly with a factor 1.4). On treatment of the sputa with rhDNase I we previously observed a 2.5-fold increase in the transport of polystyrene nanospheres having a size comparable to the lipoplexes (13). This stronger influence is probably due to the fact that in the study with polystyrene nanospheres the viscoelasticity of the sputa was lowered to a larger extent (to 37% of its start value). As we explained above, the low transport of lipoplexes into the acceptor compartment is mainly due to the "large distance" they have to travel through the sputum and also partly due to a steric obstruction by the sputum. Therefore, it was not a surprise that the treatment of the CF sputum with rhDNase I only moderately enhanced the

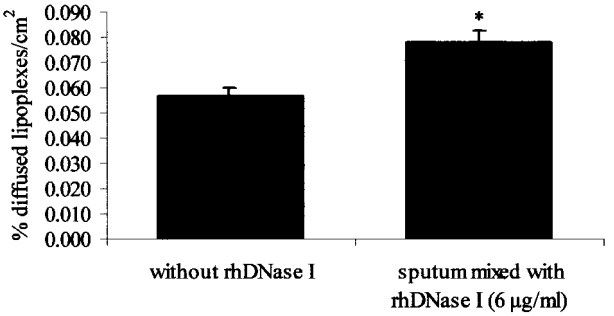

Fig. 5. Percent of lipoplexes that were transported (after 150 min) through a 220 μm thick layer of CF sputum before and after treatment of the sputum with rhDNase I (*$P < 0.05$, n = 4).

transport of the lipoplexes. Nonetheless, as cationic liposomes protect pDNA against degradation by DNase (18), administration of the gene complexes after rhDNase I therapy may clinically be beneficial as it facilitates the removal of CF sputum and, consequently, will decrease the thickness of the CF mucus layer on top of the target cells.

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