

DSC XVI - Nucleic Acid-Lipid Studies

Aljaberi A., Spelios M., Kearns M., Selvi B. and Savva M. (2007) Physicochemical properties affecting lipofection potency of a new series of 1,2-dialkoylamidopropane-based cationic lipids. *Colloids Surf B Biointerfaces* **57**, 108-117.

Abstract: The in vitro transfection activity of a novel series of N,N'-diacyl-1,2-diaminopropyl-3-carbamoyl-(aminoethane) derivatives was evaluated against a mouse melanoma cell line at different +/- charge ratios, in the presence and absence of helper lipids. Only the unsaturated derivative N,N'-dioleoyl-1,2-diaminopropyl-3-carbamoyl-(aminoethane), (1,2Imp[5]) mediated significant increase in the reporter gene level which was significantly boosted in the presence of DOPE peaking at +/- charge ratio of 2. The electrostatic interactions between the cationic liposomes and plasmid DNA were investigated by gel electrophoresis, fluorescence spectroscopy, dynamic light scattering and electrophoretic mobility techniques. In agreement with the transfection results, 1,2Imp[5]/DOPE formulation was most efficient in associating with and retarding DNA migration. The improved association between the dioleoyl derivative and DNA was further confirmed by ethidium bromide displacement assay and particle size distribution analysis of the lipoplexes. Differential scanning calorimetry studies showed that 1,2Imp[5] was the only lipid that exhibited a main phase transition below 37 degrees C. Likewise, 1,2Imp[5] was the only lipid found to form all liquid expanded monolayers at 23 degrees C. In conclusion, the current findings suggest that high in vitro transfection activity is mediated by cationic lipids characterized by increased acyl chain fluidity and high interfacial elasticity.

Antipina M. N., Schulze I., Dobner B., Langner A. and Brezesinski G. (2007) Physicochemical investigation of a lipid with a new core structure for gene transfection: 2-amino-3-hexadecyloxy-2-(hexadecyloxymethyl)propan-1-ol. *Langmuir* **23**, 3919-3926.

Abstract: Cationic liposomes/DNA complexes can be used as nonviral vectors for direct delivery of DNA-based biopharmaceuticals to damaged cells and tissues. In order to obtain more effective and safer liposome-based gene transfection systems, the new cationic lipid 2-amino-3-hexadecyloxy-2-(hexadecyloxymethyl)propan-1-ol (AHHP) was synthesized. In this paper we report on the synthesis of AHHP and investigations of its physical-chemical properties. Langmuir monolayers of AHHP were studied at the air/buffer interface by film balance measurements, grazing incidence X-ray diffraction (GIXD), and infrared reflection absorption spectroscopy (IRRAS). Structure and thermotropic phase behavior of AHHP in aqueous dispersion were examined by small-angle and wide-angle X-ray scattering (SAXS/WAXS) and differential scanning calorimetry (DSC). The results show clear differences in structure and phase behavior of AHHP, both in the monolayer system and in aqueous dispersions, in dependence on the subphase pH due to protonation or deprotonation of the primary amine in the lipid head group. Thermodynamic data derived from pi-A isotherms provide information about the critical temperature (T_c), which is in rough agreement with the temperature of the lipid phase transition from gel to fluid state (T_m) found by X-ray and calorimetry studies of AHHP aqueous dispersions. The packing properties of the molecules in mono- and bilayer systems are very similar. DNA couples to the monolayer of the new lipid at low as well as at high pH but in different amounts. The DNA coupling leads to an alignment of adsorbed DNA strands indicated by the appearance of a Bragg peak. The distance between aligned DNA strands does not change much with increasing monolayer pressure.

Balasubramaniam R. P., Bennett M. J., Aberle A. M., Malone J. G., Nantz M. H., and Malone R. W. (1996) Structural and functional analysis of cationic transfection lipids: the hydrophobic domain. *Gene Ther* **3**, 163-172.

Abstract: Cationic lipids (cytofectins) have gained widespread acceptance as pharmaceutical polynucleotide delivery agents for both cultured cell and in vivo transfection, and the cytofectins DOTAP and DC-Cholesterol are being tested in clinical human gene therapy trials. This study reports the effects of modifications in the hydrophobic domain of a prototypic cytofectin (DORI), including modifications in lipid side-chain length, saturation, and symmetry. A panel of related compounds was prepared and analyzed using DNA transfection, electron microscopy, and differential scanning calorimetry (DSC). Lipid formulations were prepared with dioleoylphosphatidylethanolamine (DOPE) as unsonicated preparations and sonicated preparations. Transfection analyses were performed using cultured fibroblasts, human bronchial epithelial, and Chinese hamster ovarian cells as well as a mouse model for pulmonary gene

delivery. In general, cytofectins containing dissymmetric hydrophobic domains were found to work as well or better than the best symmetric analogs. Optimal side-chain length and symmetry varied with cell type. Compounds with phase transitions (T_c) above and below physiological temperature (37 degrees C) were tested for DNA transfection activity. In contrast to previous reports, cytofectin T_c was not found to be predictive of transfection efficacy. Pulmonary treatment with free DNA was found to be at least as effective as treatment with commonly used cytofectin:DNA complexes. However, cytofectins that incorporate a hydroxyethylammonium moiety in the polar domain were found to enhance in vivo gene delivery relative to free DNA.

Bennett C. F., Mirejovsky D., Crooke R. M., Tsai Y. J., Felgner J., Sridhar C. N., Wheeler C. J., and Felgner P. L. (1998) Structural requirements for cationic lipid mediated phosphorothioate oligonucleotides delivery to cells in culture. *J Drug Target* **5**, 149-162.

Abstract: A series of 2,3-dialkyloxypropyl quaternary ammonium lipids containing hydroxyalkyl chains on the quaternary amine were synthesized, formulated with dioleoylphosphatidylethanolamine (DOPE) and assayed for their ability to enhance the activity of an intercellular adhesion molecule 1 (ICAM-1) antisense oligonucleotide, ISIS 1570. Cationic liposomes prepared with hydroxyethyl, hydroxypropyl and hydroxybutyl substituted cationic lipid all enhanced the activity of the ICAM-1 antisense oligonucleotide. Cationic lipids containing hydroxypentyl quaternary amines only marginally enhanced the activity of ISIS 1570. Hydroxyethyl cationic lipids synthesized with dimyristyl (C14:0) and dioleyl (C18:1) alkyl chains were equally effective. Activity of cationic lipids containing saturated alkyl groups decreased as the chain length increased, i.e. the dimyristyl (C14:0) was more effective than dipalmityl (C16:0) lipid, which was more effective than distearyl (C18:0). The phase transition temperature of cationic lipids containing saturated aliphatic chains was 56 degrees C for the distearyl lipid, 42 degrees C for the dipalmityl lipid and 24 degrees C for the dimyristyl lipid. Cationic lipids with dioleyl alkyl chains required DOPE for activity, with optimal activity occurring at 50 mole%. In contrast, a dimyristyl containing cationic lipid did not require DOPE to enhance the activity of ISIS 1570. Formulation with different phosphatidylethanolamine derivatives, revealed that optimal activity was obtained with DOPE. These studies demonstrate that several cationic lipid species enhance the activity of phosphorothioate antisense oligonucleotides and provide further information on the mechanism by which cationic lipids enhance the activity of phosphorothioate oligodeoxynucleotides.

Bunge A., Kurz A., Windeck A. K., Korte T., Flasche W., Liebscher J., Herrmann A. and Huster D. (2007) Lipophilic oligonucleotides spontaneously insert into lipid membranes, bind complementary DNA strands, and sequester into lipid-disordered domains. *Langmuir* **23**, 4455-4464.

Abstract: For the development of surface functionalized bilayers, we have synthesized lipophilic oligonucleotides to combine the molecular recognition mechanism of nucleic acids and the self-assembly characteristics of lipids in planar membranes. A lipophilic oligonucleotide consisting of 21 thymidine units and two lipophilic nucleotides with an alpha-tocopherol moiety as a lipophilic anchor was synthesized using solid-phase methods with a phosphoramidite strategy. The interaction of the water soluble lipophilic oligonucleotide with vesicular lipid membranes and its capability to bind complementary DNA strands was studied using complementary methods such as NMR, EPR, DSC, fluorescence spectroscopy, and fluorescence microscopy. This oligonucleotide inserted stably into preformed membranes from the aqueous phase. Thereby, no significant perturbation of the lipid bilayer and its stability was observed. However, the non-lipidated end of the oligonucleotide is exposed to the aqueous environment, is relatively mobile, and is free to interact with complementary DNA strands. Binding of the complementary single-stranded DNA molecules is fast and accomplished by the formation of Watson-Crick base pairs, which was confirmed by ¹H NMR chemical shift analysis and fluorescence resonance energy transfer. The molecular structure of the membrane bound DNA double helix is very similar to the free double-stranded DNA. Further, the membrane bound DNA double strands also undergo regular melting. Finally, in raft-like membrane mixtures, the lipophilic oligonucleotide was shown to preferentially sequester into liquid-disordered membrane domains.

de Oliveira M. C., Fattal E., Couvreur P., Lesieur P., Bourgaux C., Ollivon M., and Dubernet C. (1998) pH-sensitive liposomes as a carrier for oligonucleotides: a physico-chemical study of the interaction between DOPE and a 15-mer oligonucleotide in quasi-anhydrous samples. *Biochim Biophys Acta* **1372**, 301-310.

Abstract: pH-sensitive liposomes made of dioleoylphosphatidylethanolamine (DOPE)/oleic acid

(OA)/cholesterol (CHOL) mixtures were shown to be very promising carriers for oligonucleotides (ON). However, it appeared necessary to clarify the structural consequence of the interactions of ON with the liposome, and especially on DOPE, the lipid responsible for the pH sensitivity. The present study was carried out by differential scanning calorimetry and X-ray diffraction, at low hydration. In such a case, DOPE generally adopt a hexagonal phase. It could be shown that ON increased DOPE transition temperature and increased v/v_0 , as a result of electrostatic interactions between ON and DOPE headgroups. OA was found to have exactly opposite effects, its presence between DOPE molecules inhibiting the formation of hydrogen bonds. The presence of both ON and OA allowed the system to organize in a lamellar phase below the solid/liquid transition, whereas above this temperature ON preferably interacted with DOPE in a hexagonal phase and led OA to separate.

de Oliveira M. C., Rosilio V., Lesieur P., Bourgaux C., Couvreur P., Ollivon M., and Dubernet C. (2000) pH-sensitive liposomes as a carrier for oligonucleotides: a physico-chemical study of the interaction between DOPE and a 15-mer oligonucleotide in excess water. *Biophys Chem* **87**, 127-137.

Abstract: The cytoplasmic delivery of drugs encapsulated into pH-sensitive liposomes is under the control of a lamellar-to-hexagonal transition. In a previous study, under anhydrous conditions, oligonucleotides (ODN) encapsulated in pH-sensitive liposomes composed of dioleoylphosphatidylethanolamine (DOPE)/oleic acid (OA)/cholesterol (CHOL) were shown to modify the phase behaviour of DOPE. In the present study, the lipid/ODN interactions were evaluated in fully hydrated samples by surface tension measurements, differential scanning calorimetry, X-ray diffraction and turbidimetry. Concerning the lipids, it was shown that OA provoked a disorganisation of DOPE lamellar phases and led to the complete disappearance of hexagonal transition along with heating. The addition of CHOL further decreased the lipid packing in the bilayers. Concerning ODN, these molecules provoked an increase in the surface pressure of a DOPE/OA/CHOL monolayer, indicating the existence of molecular interactions with the lipids. At a supramolecular level, ODN induced a more ordered organisation of DOPE molecules in the lamellar and hexagonal phases, and completely abolished the disorganisational effect of OA and CHOL.

Fielden M. L., Perrin C., Kremer A., Bergsma M., Stuart M. C., Camilleri P., and Engberts J. B. (2001) Sugar-based tertiary amino gemini surfactants with a vesicle-to-micelle transition in the endosomal pH range mediate efficient transfection in vitro. *Eur J Biochem* **268**, 1269-1279.

Abstract: Novel reduced sugar gemini amphiphiles linked through their tertiary amino head groups via alkyl spacers of 4 or 6 carbons, and with varying (unsaturated) alkyl tail lengths of 12--18, have been synthesized and tested for transfection in vitro in an adherent Chinese hamster ovary cell line (CHO-K1). Transfection efficiencies peaked at 2.7 times that of the commercial standard Lipofectamine Plus/2000 for pure solutions of the compound bearing unsaturated (oleyl) alkyl tails. For those compounds bearing saturated alkyl tails, transfection efficiency peaked at a tail length of 16, at a level similar to Lipofectamine Plus/2000. All of the amphiphiles formed bilayer vesicles at physiological pH. Some of the amino groups at the surface were protonated, and vesicles therefore bore a positive charge. Increased protonation with reduced pH resulted in greatly increased monomer solubility and a morphology change from vesicle to micelle at characteristic pH values, dependent on the tail length. For the compounds promoting high transfection efficiency, this characteristic pH was within the range found in the endosomal compartment (7.4--4.0). Formation of mixed micelles between gemini surfactant and membrane phospholipids at reduced pH may therefore provide a method of endosome rupture and subsequent escape of entrapped DNA, thus discarding the need for extra fusogenic or endosomolytic agents. The positive charge on the vesicles at physiological pH drives the colloidal association with DNA. Small angle X-ray scattering measurements indicate that lamellar aggregates are formed, which have a d spacing of 48--54 Å. Preliminary differential scanning calorimetric measurements suggest that reduction of pH causes a disordering of the hydrocarbon region of the DNA-surfactant complex.

Gaucheron J., Boulanger C., Santaella C., Sbirrazzuoli N., Boussif O., and Vierling P. (2001) In vitro cationic lipid-mediated gene delivery with fluorinated glycerophosphoethanolamine helper lipids. *Bioconjug Chem* **12**, 949-963.

Abstract: There is a need for the development of nonviral gene transfer systems with improved and original properties. "Fluorinated" lipoplexes are such candidates, as supported by the remarkably higher in vitro and in vivo transfection potency found for such fluorinated lipoplexes as compared with conventional ones or even with PEI-based polyplexes (Boussif, O., Gaucheron, J., Boulanger, C., Santaella, C., Kolbe,

H. V. J., Vierling, P. (2001) Enhanced in vitro and in vivo cationic lipid-mediated gene delivery with a fluorinated glycerophosphoethanolamine helper lipid. *J. Gene Med.* 3, 109-114). Here, we describe the synthesis of fluorinated glycerophosphoethanolamines (F-PEs), close analogues of dioleoylphosphatidylethanolamine (DOPE), and report on their lipid helper properties vs that of DOPE, as in vitro gene transfer components of fluorinated lipoplexes based on pcTG90, DOGS (Transfectam), or DOTAP. To evaluate the contribution of the F-PEs to in vitro lipoplex-mediated gene transfer, we examined the effect of including the F-PEs in lipoplexes formulated with these cationic lipids (CL) for various CL:DOPE:F-PE molar ratios [$1:(1-x):x$ with $x = 0, 0.5$ and 1 ; $1:(2-y):y$ with $y = 0, 1, 1.5$, and 2], and various N/P ratios (from 10 to 0.8, N = number of CL amines, P = number of DNA phosphates). Irrespective of the F-PE chemical structure, of the colipid F-PE:DOPE composition, and of the N/P ratio, comparable transfection levels to those of their respective control DOPE lipoplexes were most frequently obtained when using one of the F-PEs as colipid of DOGS, pcTG90, or DOTAP in place of part of or of all DOPE. However, a large proportion of DOGS-based lipoplexes were found to display a higher transfection efficiency when formulated with the F-PEs rather than with DOPE alone while the opposite tendency was evidenced for the DOTAP-based lipoplexes. The present work indicates that "fluorinated" lipoplexes formulated with fluorinated helper lipids and conventional cationic lipids are very attractive candidates for gene delivery. It confirms further that lipophobicity and restricted miscibility of the lipoplex lipids with the endogenous lipids does not preclude efficient gene transfer and expression. Their transfection potency is rather attributable to their unique lipophobic and hydrophobic character (resulting from the formulation of DNA with fluorinated lipids), thus preventing to some extent DNA from interactions with lipophilic and hydrophilic biocompounds, and from degradation.

Hirsch-Lerner D. and Barenholz Y. (1998) Probing DNA-cationic lipid interactions with the fluorophore trimethylammonium diphenyl-hexatriene (TMADPH). *Biochim Biophys Acta* **1370**, 17-30.

Abstract: The aim of this study is to get a better understanding of DNA-cationic lipid complex formation and its characterization through the properties of the lipid assembly, using fluorescent probes known to have different locations in the vesicle bilayer, 1,6-diphenylhexa-1,3,5-triene (DPH) and 1-(4-trimethylammoniumphenyl)-6-phenyl-1,3,5-hexatriene (TMADPH). The location of these two fluorescent probes in the membrane differs; the positive charge of TMADPH is localized close to the water/lipid interface and its fluorophore is present in the upper part of the acyl chain region while DPH (lacking polar group) is embedded deeper in the hydrophobic part of the bilayer. Unilamellar vesicles (approximately 100 nm size) composed of N-(1-(2, 3-dioleoyloxy)-propyl)-N,N,N-trimethylammonium chloride (DOTAP) and 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) as a helper lipid (at 1 : 1 mole ratio) were used as a model of cationic liposomes. Both linear and circular DNA gave almost identical results. DNA-/L+ (mole charge ratio of DNA negatively-charged phosphate to positively-charged lipid) ratios have large effects on the measured parameters. The effects monitored through TMADPH are much more striking than those obtained through the use of DPH, suggesting that the major DNA-lipid interaction occurs at the lipid/water interface. The fact that DNA induced much larger changes in TMADPH fluorescence intensity in H₂O than in D₂O suggests that the changes in the exposure of TMADPH to water and solvent relaxation effects are involved in the interaction. At DNA-/L+>=1, fluorescence intensity increased concomitantly with a small increase in TMADPH fluorescence anisotropy without much affect in the size of the complex. At DNA-/L+<0.6, fluorescence quenching proportional to DNA-/L+ occurred, as well as a large increase in TMADPH fluorescence anisotropy and in complex size. These results suggest that at low DNA-/L+, negatively-charged DNA condenses positively-charged lipid headgroups, thereby inducing formation of lipid-ordered domains. This phase separation results in membrane defects at the lipid/water interface and increased exposure of the hydrophobic upper parts of the acyl chains to water, as indicated by the quenching of TMADPH. This leads to instability and aggregation/fusion of the DNA-lipid complexes. On the other hand, at DNA-/L+>=1, the condensing effect is smaller, involving homogeneous lateral condensation of all the lipids, leading to a reduction in water content near the probe, and the DNA-lipid complexes are relatively small and stable.

Kinnunen P. K., Rytomaa M., Koiv A., Lehtonen J., Mustonen P., and Aro A. (1993) Sphingosine-mediated membrane association of DNA and its reversal by phosphatidic acid. *Chem Phys Lipids* **66**, 75-85.

Abstract: Resonance energy transfer was measured between egg phosphatidylcholine liposomes containing the intramolecular excimer forming pyrene-labelled phospholipid analogue 1,2-bis[pyren-1(-

yl)]decanoyl-sn-glycero-3-phosphocholine (bisPDPC) as a donor and DNA-bound adriamycin as an acceptor. Membrane association of DNA turned out to be critically dependent on the presence of sphingosine in the liposomes. Identical result was obtained by measuring the extent of quenching of the fluorescent DNA-bound dye Hoechst 33258 due to energy transfer to the lipophilic stain Nile Red incorporated in egg phosphatidylcholine liposomes containing varying amounts of sphingosine. The attachment of DNA to sphingosine-containing membranes could be reversed by the further inclusion of the negatively charged phosphatidic acid up to approximately 1:2 PA/sphingosine molar ratio in the liposomes, thus suggesting the involvement of electrostatic interactions. Differential scanning calorimetry measurements confirmed a lack of association between DNA and dimyristoylphosphatidylcholine liposomes. Instead drastic changes were produced by DNA in the heat capacity scans measured for liposomes also incorporating sphingosine. Fluorescence microscopy revealed an extensive aggregation of sphingosine containing pyrene-phosphatidylcholine-labelled egg phosphatidylcholine liposomes in the presence of DNA. Together with other available data on the effects of sphingosine, the present findings suggest that sphingosine could directly alter the chromatin structure. Accordingly, such alterations may contribute to the control of replication and gene expression.

Koiv A., Mustonen P., and Kinnunen P. K. (1994) Differential scanning calorimetry study on the binding of nucleic acids to dimyristoylphosphatidylcholine-sphingosine liposomes. *Chem Phys Lipids* **70**, 1-10. **Abstract:** Binding of DNA and RNA to sphingosine-containing dimyristoylphosphatidylcholine (DMPC) liposomes was characterized by differential scanning calorimetry. The thermal phase behaviour of neat DMPC liposomes was unaffected by the presence of the nucleic acids. However, significant alterations in the melting profiles of the DMPC/sphingosine composite membranes were produced by DNA and RNA, thus revealing their binding to the liposomes. For example, for 79:21 (molar ratio) DMPC/sphingosine liposomes a single endotherm at 29.1 degrees C with an enthalpy of 6.3 kcal/mol lipid was observed. In the presence of DNA at the nucleotide/sphingosine ratio of 0.6 this endotherm separated into three distinct peaks at 28.0, 31.4 and 35.1 degrees C, together with an approximately 22% reduction in the total enthalpy. Further increase in DNA concentration up to 1.5 nucleotides per sphingosine led to complete loss of the original heat absorption peak of the DMPC/sphingosine liposomes, while an endotherm at 34.3 degrees C with ΔH of 2.7 kcal/mol developed. By visual inspection, rapid and extensive aggregation of the liposomes due to DNA was evident. Evidence for DNA-induced phase separation was also provided by compression isotherms of sphingosine containing DMPC monolayers recorded over an aqueous buffer both in the presence and absence of DNA. The effects of RNA on the thermal phase behaviour of the composite liposomes were qualitatively similar to those described above for DNA. Notably, the presence of eggPA abolished the nucleic acid induced heat capacity changes for DMPC/sphingosine liposomes probably because of neutralization of the positive charge of sphingosine. The binding of DNA to DMPC/sphingosine liposomes occurred both below and above the lipid phase transition temperature, as shown by fluorescence resonance energy transfer utilizing adriamycin-labelled DNA as a quencher and membrane incorporated pyrene-labelled phospholipid as a donor. However, the apparent binding to liquid crystalline liposomes was slightly more effective.

Koiv A. and Kinnunen P. K. (1994) Binding of DNA to liposomes containing different derivatives of sphingosine. *Chem Phys Lipids* **72**, 77-86. **Abstract:** Binding of DNA to dimyristoylphosphatidylcholine (DMPC) liposomes containing different sphingosine derivatives was investigated. DNA labelled with adriamycin was used as a fluorescence quencher and its membrane association was observed by resonance energy transfer from liposomes incorporating a pyrene-derivatized lipid bisPDPC as a donor and containing 19 mol% of sphingosine, dihydro-, phyto- or dimethylsphingosine. As revealed by differential scanning calorimetry, the thermal phase behaviour of multilamellar liposomes containing these sphingolipids was also significantly altered by DNA. Attachment of DNA to liposomes containing sphingosylphosphorylcholine was much weaker, and no binding of DNA to membranes containing N-acetylsphingosine, N-stearoylsphingosine or sphingomyelin was observed. The membrane binding of DNA was dependent on pH and could be reversed by the inclusion of phosphatidic acid (eggPA) into the liposomes. Analogously, the association of cytochrome c with eggPA could be reversed by the DNA-binding sphingosines. These findings lend support to our previous proposal that the DNA-sphingosine interaction is electrostatic and requires the presence of a positive charge in the latter. Accordingly, sphingosines carrying a protonated amino group attach DNA to membranes, while blocking of the amino group by N-acylation abolishes this interaction.

Koynova R. and MacDonald R. C. (2005) Lipid transfer between cationic vesicles and lipid-DNA lipoplexes: effect of serum. *Biochim Biophys Acta* **1714**, 63-70.

Abstract: Differential scanning calorimetry was used to examine the lipid exchange between model lipid systems, including vesicles of the cationic lipids ethyldimyristoylphosphatidylcholine (EDMPC), ethyldipalmitoylphosphatidylcholine (EDPPC) or their complexes with DNA (lipoplexes), and the zwitterionic lipids (DMPC, DPPC). The changes of the lipid phase transition parameters (temperature, enthalpy, and cooperativity) upon consecutive temperature scans was used as an indication of lipid mixing between aggregates. A selective lipid transfer of the shorter-chain cationic lipid EDMPC into the longer-chain aggregates was inferred. In contrast, transfer was hindered when EDMPC (but not EDPPC) was bound to DNA in the lipoplexes. These data support a simple molecular lipid exchange mechanism, but not lipid bilayer fusion. Exchange via lipid monomers is considerably more facile for the cationic ethylphosphatidylcholines than for zwitterionic phosphatidylcholines, presumably due to the higher monomer solubility of the charged lipids. With the cationic liposomes, lipid transfer was strongly promoted by the presence of serum in the dispersing medium. Serum proteins are presumed to be responsible for the accelerated transfer, since the effect was strongly reduced upon heating the serum to 80 degrees C. The effect of serum indicates that even though much lipoplex lipid is inaccessible due to the multilayered structure, the barrier due to buried lipid can be easily overcome. Serum did not noticeably promote the lipid exchange of zwitterionic liposomes. The phenomenon is of potential importance for the application of cationic liposomes to nonviral gene delivery, which often involves the presence of serum in vitro, and necessarily involves serum contact in vivo.

Koynova R. and MacDonald R. C. (2003) Mixtures of cationic lipid O-ethylphosphatidylcholine with membrane lipids and DNA: phase diagrams. *Biophys J* **85**, 2449-2465.

Abstract: Ethylphosphatidylcholines are positively charged membrane lipid derivatives, which effectively transfect DNA into cells and are metabolized by the cells. For this reason, they are promising nonviral transfection agents. With the aim of revealing the kinds of lipid phases that may arise when lipoplexes interact with cellular lipids during DNA transfection, temperature-composition phase diagrams of mixtures of the O-ethyldipalmitoylphosphatidylcholine with representatives of the major lipid classes (phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, cholesterol) were constructed. Phase boundaries were determined using differential scanning calorimetry and synchrotron x-ray diffraction. The effects of ionic strength and of DNA presence were examined. A large variety of polymorphic and mesomorphic structures were observed. Surprisingly, marked enhancement of the affinity for nonlamellar phases was observed in mixtures with phosphatidylethanolamine and cholesterol as well as with phosphatidylglycerol (previously reported). Because of the potential relevance to transfection, it is noteworthy that such phases form at close to physiological conditions, and in the presence of DNA. All four mixtures exhibit a tendency to molecular clustering in the gel phase, presumably due to the specific interdigitated molecular arrangement of the O-ethyldipalmitoylphosphatidylcholine gel bilayers. It is evident that a remarkably broad array of lipid phases could arise in transfected cells and that these could have significant effects on transfection efficiency. The data may be particularly useful for selecting possible "helper" lipids in the lipoplex formulations, and in searches for correlations between lipoplex structure and transfection activity.

Liu W. G., Zhang X., Sun S. J., Sun G. J., Yao K. D., Liang D. C., Guo G., and Zhang J. Y. (2003) N-alkylated chitosan as a potential nonviral vector for gene transfection. *Bioconjug Chem* **14**, 782-789.

Abstract: Alkylated chitosans (ACSs) were prepared by modifying chitosan (CS) with alkyl bromide. The self-aggregation of ACSs in acetic acid solution was characterized by fluorescence spectroscopy and dynamic light scattering method. The results indicate that introducing alkyl side chains leads to the self-aggregation of ACSs, and CS with a 99% deacetylation degree shows no aggregation due to the electrostatic repulsion. The electrophoresis experiment demonstrates that the complex between CS and DNA was formed at a charge ratio (+/-) of 1/1; ACS/DNA complexes were formed at a lower charge ratio (+/-) of 1/4. A small amount of alkylated chitosans play the same shielding role as chitosan in protecting DNA from DNase hydrolysis. Differential scanning calorimetry (DSC) and atomic force microscopy (AFM) were employed separately to investigate the thermodynamic behavior of dipalmitoyl-sn-glycero-3-phosphocholine (DPPC)/CS and DPPC/ACS mixtures and the variation in topological structure of DPPC membrane induced by CS and ACS. It is shown that CS and ACS can cause the fusion of DPPC multilamellar vesicles as well as membrane destabilization. In contrast, the perturbation effect induced by

ACS is more evident due to the hydrophobic interaction. CS and ACS were used to transfer plasmid-encoding CAT into C(2)C(12) cell lines. Upon elongating the alkyl side chain, the transfection efficiency is increased and levels off after the number of carbons in the side chain exceeds 8. It is proposed that the higher transfection efficiency of ACS is attributed to the increasing entry into cells facilitated by hydrophobic interactions and easier unpacking of DNA from ACS carriers due to the weakening of electrostatic attractions between DNA and ACS.

Lobo B. A., Rogers S. A., Choosakoonkriang S., Smith J. G., Koe G., and Middaugh C. R. (2002) Differential scanning calorimetric studies of the thermal stability of plasmid DNA complexed with cationic lipids and polymers. *J Pharm Sci* **91**, 454-466.

Abstract: The thermal stabilities of supercoiled (SC) and linear/open circular (LIN/OC) forms of plasmid DNA when complexed with cationic lipids or cationic polymers used for cellular transfection were assessed using differential scanning calorimetry. Differences in the stability of SC DNA produced by the cationic lipids DOTAP (1,2-dioleoyltrimethyl ammoniumpropane chloride), DSTAP (1,2-distearyltrimethyl ammoniumpropane chloride), and DDAB (dimethyldioctadecylammonium bromide) upon complexation suggest possible effects of headgroup structure on the stability of SC DNA and minimal effects of lipid acyl chain saturation/unsaturation. Complexation of DNA with the cationic polymers polyethylenimine (PEI) or poly-L-lysine (PLL) (but not poly-L-arginine) resulted in a decreased stability of SC DNA when the DNA was in charge excess, although all polymers stabilized SC DNA when the polymer was in charge excess. The effects of these cationic polymers on the stability of SC DNA can be explained by changes produced in the tertiary structure of SC DNA upon binding and may reflect the importance of the topological constraint of supercoiling upon the stability of the resulting complexes.

Mrevlishvili G. M., Kankia B. I., Mdzinarashvili T. J., Brelidze T. I., Khvedelidze M. M., Metreveli N. O., and Razmadze G. Z. (1998) Liposome-DNA interaction: microcalorimetric study. *Chem Phys Lipids* **94**, 139-143.

Abstract: The authors applied differential scanning calorimetry (DSC) for studying the thermodynamic characteristics of DNA-liposome interactions. At the first stage, the melting curves of the 'order-disorder' thermal transition for lipid component and of the 'helix-coil' transition for DNA were obtained. At the second stage, the phase behavior of the DNA-lipid mixture as a function of both components (lipid/DNA ratio) was obtained. The liposome-DNA interaction was investigated comparing the melting curves of the pure components and the mixture.

Ryhanen S. J., Saily M. J., Paukku T., Borocci S., Mancini G., Holopainen J. M., and Kinnunen P. K. (2003) Surface charge density determines the efficiency of cationic gemini surfactant based lipofection. *Biophys J* **84**, 578-587.

Abstract: The efficiencies of the binary liposomes composed of 1,2-dimyristoyl-sn-glycero-3-phosphocholine and cationic gemini surfactant, (2S,3R)-2,3-dimethoxy-1,4-bis(N-hexadecyl-N,N-dimethylammonium)butane dibromide as transfection vectors, were measured using the enhanced green fluorescent protein coding plasmid and COS-1 cells. Strong correlation between the transfection efficiency and lipid stoichiometry was observed. Accordingly, liposomes with $X(\text{SR-1}) \geq 0.50$ conveyed the enhanced green fluorescent protein coding plasmid effectively into cells. The condensation of DNA by liposomes with $X(\text{SR-1}) > 0.50$ was indicated by static light scattering and ethidium bromide intercalation assay, whereas differential scanning calorimetry and fluorescence anisotropy of diphenylhexatriene revealed stoichiometry dependent reorganization in the headgroup region of the liposome bilayer, in alignment with our previous Langmuir-balance study. Surface charge density and the organization of positive charges appear to determine the mode of interaction of DNA with (2S,3R)-2,3-dimethoxy-1,4-bis(N-hexadecyl-N,N-dimethylammonium)butane dibromide/1,2-dimyristoyl-sn-glycero-3-phosphocholine liposomes, only resulting in DNA condensation when $X(\text{SR-1}) > 0.50$. Condensation of DNA in turn seems to be required for efficient transfection.

Saunders M., Taylor K. M., Craig D. Q., Palin K. and Robson H. (2007) High sensitivity differential scanning calorimetry study of DNA-cationic liposome complexes. *Pharm. Res* **24**, 1954-1961.

Abstract: PURPOSE: To investigate plasmid DNA interactions with liposomes prepared from dimyristoylglyceroethylphosphocholine (EDMPC) and DOPE using high sensitivity differential scanning calorimetry (HSDSC). MATERIALS AND METHODS: Large unilamellar liposomes of EDMPC with

DOPE (mol ratio 0-50%) were prepared. Plasmid DNA was added to give a final DNA/lipid (-/+) charge ratio of 0.5. Samples were placed into an HSDSC and cooled to 3 degrees C, held isothermally for 30 min and then the temperature was ramped to 120 degrees C at a rate of 1 degree C/min. RESULTS: On heating EDMPC liposomes, the main phase transition occurred at 21.2 degrees C, with a low temperature shoulder on the endothermic peak. At low DOPE concentrations the main phase transition temperatures and enthalpies of transition were lower than for pure EDMPC, with a peak corresponding to a pure EDMPC phase occurring at DOPE concentrations of 12-17 mol%. At 50 mol%, no main transition endotherm was observed. DNA solution produced two endothermic peaks with numerous 'satellite' peaks indicating thermal denaturation. DNA binding to EDMPC changed the shape of the thermogram, indicating alteration in lipid packing within the bilayer. DNA induced demixing in the bilayers of DOPE-containing liposomes. CONCLUSION: HSDSC provided information for characterizing liposome formulations and DNA interactions with such vesicles.

Subramanian M., Holopainen J. M., Paukku T., Eriksson O., Huhtaniemi I., and Kinnunen P. K. (2000) Characterisation of three novel cationic lipids as liposomal complexes with DNA. *Biochim Biophys Acta* **1466**, 289-305.

Abstract: Cationic lipids (CLs) are being increasingly exploited as transfection vectors for the delivery of DNA into eukaryotic cells. To obtain further insight to the complex formation and interactions between cationic liposomes and DNA, we characterised three novel cationic lipids, viz. bis[2-(11-phenoxyundecanoate)ethyl]-dimethylammonium bromide, N-hexadecyl-N- inverted question mark10-[O-(4-acetoxy)-phenylundecanoate]ethyl inverted question mark- dimethylammonium bromide, and bis[2-(11-butyloxyundecanoate)ethyl]dimethylammonium bromide. These lipids bear the same charged headgroup yet have different hydrophobic parts. Accordingly, we may anticipate their electrostatic interactions with DNA to be similar while differing in both thermal phase behaviour and physicochemical properties of their complexes with DNA. In keeping with the above all three lipids formed complexes with DNA as evidenced by light scattering, fluorescence spectroscopy and Langmuir film balance. Differential scanning calorimetry revealed very different phase behaviours for the binary mixtures of the three CLs with dimyristoylphosphatidylcholine and also provided evidence for DNA-induced lipid phase separation. These data were confirmed by compression isotherms and fluorescence microscopy of monolayers residing on an aqueous buffer, recorded both in the presence and absence of DNA. Importantly, binding to cationic liposomes appears to prevent thermal denaturation of DNA upon heating of the complexes. Likewise, renaturation of heat-treated DNA complexed with the cationic liposomes appears to be abolished as well.

Suleymanoglu E. (2006) Phospholipid-nucleic acid recognition: energetics of DNA-Mg²⁺-phosphatidylcholine ternary complex formation and its further compaction as a gene delivery formulation. *PDA J Pharm Sci Technol* **60**, 218-231.

Abstract: Thermodynamic features related to the preparation and use of self-assemblies formed between multilamellar and unilamellar zwitterionic liposomes and polynucleotides with various conformation and sizes are presented. The divalent metal cation-induced adsorption, aggregation, and adhesion between single- and double-stranded polyribonucleotides and phosphatidylcholine vesicles was followed by differential adiabatic scanning microcalorimetry. Nucleic acid condensation and compaction mediated by Mg²⁺ was followed, with regard to interfacial interaction with unilamellar vesicles. Microcalorimetric measurements of synthetic phospholipid vesicles and poly(ribo)nucleotides and their ternary complexes with inorganic cations were used to build the thermodynamic model of their structural transitions. The increased thermal stability of the phospholipid bilayers is achieved by affecting their melting transition temperature by nucleic acid-induced electrostatic charge screening. Measurements give evidence for the stabilization of polynucleotide helices upon their association with liposomes in the presence of divalent metal cations. Such an induced aggregation of vesicles leads either to heterogeneous multilamellar DNA-lipid arrangements or to DNA-induced bilayer destabilization and lipid fusion. The further employment of these polyelectrolyte nanostructures as improved formulations in therapeutic gene delivery trials, as well as in DNA chromatography, is discussed.

Tarahovsky T. S., Khusainova R. S., Gorelov A. V., Nicolaeva T. I., Deev A. A., Dawson A. K., and Ivanitsky G. R. (1996) DNA initiates polymorphic structural transitions in lecithin. *FEBS Lett* **390**, 133-136.

Abstract: The inverted micellar phase, obtained by treating lecithin and Ca(2+)-DNA complex with

chloroform, was used as an intermediate step in the preparation of DNA-Ca(2+)-lecithin complex. DSC analysis demonstrated the involvement of a large fraction of lipid in the interaction with DNA. Freeze-fracture electron microscopy revealed (i) rod-like structures on the hydrophobic fracture surface of membranes and (ii) regular bundles of fibrils with a repeat distance of about 6 nm, which were located free in solution. Similar regular bundles of fibrils were also revealed by staining the samples with uranyl acetate. According to the suggested model, the observed structures are hexagonally packed inverted lipid tubes, with DNA located in their central cores. The possible biological relevance of the capability of Ca(2+)-DNA to initiate polymorphic phase transitions of lecithin is discussed.

Tarahovsky Y. S., Rakhmanova V. A., Epan R. M., and MacDonald R. C. (2002) High temperature stabilization of DNA in complexes with cationic lipids. *Biophys J* **82**, 264-273.

Abstract: The influence on the melting of calf thymus and plasmid DNA of cationic lipids of the type used in gene therapy was studied by ultraviolet spectrophotometry and differential scanning calorimetry. It was found that various membrane-forming cationic lipids are able to protect calf thymus DNA against denaturation at 100 degrees C. After interaction with cationic lipids, the differential scanning calorimetry melting profile of both calf thymus and plasmid DNA revealed two major components, one corresponding to a thermolabile complex with transition temperature, $T(m(\text{labile}))$, close to that of free DNA and a second corresponding to a thermostable complex with a transition temperature, $T(m(\text{stable}))$, at 105 to 115 degrees C. The parameter $T(m(\text{stable}))$ did not depend on the charge ratio, $R(+/-)$. Instead, the amount of thermostable DNA and the enthalpy ratio $\Delta H((\text{stable}))/\Delta H((\text{labile}))$ depended upon $R(+/-)$ and conditions of complex formation. In the case of O-ethylidoleoylphosphatidylcholine, the cationic lipid that was the main subject of the investigation, the maximal stabilization of DNA exceeded 90% between $R(+/-) = 1.5$ and 3.0. Several other lipids gave at least 75% protection in the range $R(+/-) = 1.5$ to 2.0. Centrifugal separation of the thermostable and thermolabile fractions revealed that almost all the transfection activity was present at the thermostable fraction. Electron microscopy of the thermostable complex demonstrated the presence of multilamellar membranes with a periodicity 6.0 to 6.5 nm. This periodic multilamellar structure was retained at temperatures as high as 130 degrees C. It is concluded that constraint of the DNA molecules between oppositely charged membrane surfaces in the multilamellar complex is responsible for DNA stabilization.

Wang J., Guo X., Xu Y., Barron L., and Szoka F. C., Jr. (1998) Synthesis and characterization of long chain alkyl acyl carnitine esters. Potentially biodegradable cationic lipids for use in gene delivery. *J Med Chem* **41**, 2207-2215.

Abstract: A series of alkyl acyl carnitine esters (alkyl 3-acyloxy-4-trimethylammonium butyrate chloride) were synthesized as potential biocompatible cationic lipids for use in gene transfer. The physicochemical properties of the lipids, liposomes prepared from them, and their complexes with DNA were characterized by differential scanning calorimetry (DSC), particle size, zeta potential, and surface monolayer measurements. The transition temperatures and behavior at an air-water interface for this series are similar to phosphatidylcholines with the same hydrocarbon chain length. The physical properties of the l derivatives were not significantly different from the dl derivatives. At 70 degrees C, the acyl chains were readily hydrolyzed at pH 7. The influence of the aliphatic chain length ($n = 12-18$) on transfection efficiency in vitro was determined using cationic liposomes prepared from these lipids or their mixtures with the helper lipids, dioleoylphosphatidylethanolamine (DOPE), dioleoylphosphatidylcholine, monooleoylglycerol, and cholesterol (Chol). The mixture of myristyl 3-myristoyloxy-4-trimethylammonium butyrate chloride (MMCE, 4d) with DOPE at a 1:1 molar ratio mediated the highest transfection efficiency in cell culture. The mixture of oleyl 3-oleoyloxy-4-trimethylammonium butyrate chloride (OOCE, 4f) with Chol at a 1:1 molar ratio gave the highest transfection efficiency after intravenous administration in mice. In vivo gene expression using 4f was comparable to values obtained with the best cationic lipids reported to date.