

ITC XIX - Lipid-Nucleic Acid interactions

Goncalves E., Debs R. J., and Heath T. D. (2004) The effect of liposome size on the final lipid/DNA ratio of cationic lipoplexes. *Biophys J* **86**, 1554-1563.

Abstract: Several studies have demonstrated that lipoplexes are two-phase systems over most mixing lipid/DNA charge ratios. Because these studies have focused on small unilamellar vesicles (SUV), they leave open the question as to whether a similar pattern is followed by other liposome types. The main purpose of this work is to examine the question further by characterizing the assembly of cationic lipoplexes prepared from 1-[2-(oleoyloxy)ethyl]-2-oleyl-3-(2-hydroxyethyl)imidazolium chloride (DOTIM)/dioleoylphosphatidylethanolamine (DOPE) (1:1) liposomes of various types. Sedimentation in sucrose density gradients reveals that large unilamellar vesicles (LUV) and sedimented multilamellar vesicles (sMLV), as opposed to SUV, form lipoplexes that exist as a single phase over a relatively broad range of mixing (+/-) ratios. This is indicated by observing that most of the LUV and sMLV become involved in the assembly reaction up to mixing (+/-) ratios of 4 and 9, respectively, while only a small and constant fraction of SUV associates with DNA at all mixing (+/-) ratios tested. Consequently, while maximal (+/-) ratios of approximately 4.5 and 9 are found in LUV and sMLV lipoplexes, respectively, a final (+/-) ratio of only approximately 2 is determined in SUV lipoplexes. Isothermal titration calorimetry shows that this is the lowest possible charge ratio achieved when liposomes are titrated with DNA. Based on these observations and on the size differences of the liposomes used, a model of lipoplex formation is proposed.

Keller M., Jorgensen M. R., Perouzel E., and Miller A. D. (2003) Thermodynamic aspects and biological profile of CDAN/DOPE and DC-Chol/DOPE lipoplexes. *Biochemistry* **42**, 6067-6077.

Abstract: The DNA complexation and condensation properties of two established cationic liposome formulations, CDAN/DOPE (50:50, m/m; Trojene) and DC-Chol/DOPE (60:40, m/m), were investigated by using a combination of isothermal titration calorimetry (ITC), circular dichroism (CD), photon correlation spectroscopy (PCS), and turbidity assays. Plasmid DNA (7528 bp) was titrated with extruded liposomes (90 +/- 15 nm) and a thermodynamic profile established. ITC data revealed that the two liposome formulations differ substantially in their DNA complexation characteristics. Equilibrium dissociation constants for CDAN/DOPE ($K_d = 19 \pm 3 \mu\text{M}$) and DC-Chol/DOPE liposomes ($K_d = 2 \pm 0.5 \mu\text{M}$) were obtained by fitting the experimental data in a one-site binding model. Both CDAN/DOPE and DC-Chol/DOPE binding events take place with a negative binding enthalpy (ΔH degrees = -0.5 and -1.7 kcal/mol, respectively) and increasing system entropy ($T\Delta S = 6 \pm 0.3$ and 6.2 ± 0.3 kcal/mol, respectively). Interestingly, CDAN/DOPE liposomes undergo substantial rehydration and protonation prior to complexation with pDNA, which is observed as two discrete exothermic signals during titration. No such biphasic effects are seen with respect to the binding between DC-Chol/DOPE and pDNA that appears to be otherwise instantaneous with no rehydration effects. The rehydration and protonation characteristics of CDAN/DOPE liposomes in comparison with those of DC-Chol/DOPE cationic liposomes are confirmed by ITC; CDAN/DOPE liposomes have strongly exothermic dilution characteristics and DC-Chol/DOPE liposomes only mildly endothermic characteristics. Furthermore, analysis of cationic liposome-pDNA binding by CD spectroscopy reveals that CDAN/DOPE-pDNA lipoplexes are more structurally fluid than DC-Chol/DOPE-pDNA lipoplexes. CDAN/DOPE liposomes induced considerable fluctuation in the DNA structure for at least 60 min, whereas liposomes obtained from DC-Chol/DOPE lack the same effect on the DNA structure. Turbidity studies show that DC-Chol/DOPE lipoplexes exhibit greater resistance to serum than CDAN/DOPE lipoplexes, which showed substantial precipitation after incubation for 100 min with serum. Transfection studies on HeLa and Panc-1 cells reveal that CDAN/DOPE lipoplexes are superior in efficacy to DC-Chol/DOPE lipoplexes. CDAN/DOPE liposomes tend to transfect best in normal growth medium (including 10% serum and antibiotics), whereas DC-Chol/DOPE lipoplexes transfect best under serum free transfection conditions.

Kennedy M. T., Pozharski E. V., Rakhmanova V. A., and MacDonald R. C. (2000) Factors governing the assembly of cationic phospholipid-DNA complexes. *Biophys J* **78**, 1620-1633.

Abstract: The interaction of DNA with a novel cationic phospholipid transfection reagent, 1,2-dioleoyl-sn-glycero-3-ethylphosphocholine (EDOPC), was investigated by monitoring thermal effects, particle size, vesicle rupture, and lipid mixing. By isothermal titration calorimetry, the heat of interaction between large

unilamellar EDOPC vesicles and plasmid DNA was endothermic at both physiological and low ionic strength, although the heat absorbed was slightly larger at the higher ionic strength. The energetic driving force for DNA-EDOPC association is thus an increase in entropy, presumably due to release of counterions and water. The estimated minimum entropy gain per released counterion was 1.4 cal/mole- degrees K (about 0.7 kT), consistent with previous theoretical predictions. All experimental approaches revealed significant differences in the DNA-lipid particle, depending upon whether complexes were formed by the addition of DNA to lipid or vice versa. When EDOPC vesicles were titrated with DNA at physiological ionic strength, particle size increased, vesicles ruptured, and membrane lipids became mixed as the amount of DNA was added up to a 1.6:1 (+:-) charge ratio. This charge ratio also corresponded to the calorimetric end point. In contrast, when lipid was added to DNA, vesicles remained separate and intact until a charge ratio of 1:1 (+:-) was exceeded. Under such conditions, the calorimetric end point was 3:1 (+:-). Thus it is clear that fundamental differences in DNA-cationic lipid complexes exist, depending upon their mode of formation. A model is proposed to explain the major differences between these two situations. Significant effects of ionic strength were observed; these are rationalized in terms of the model. The implications of the analysis are that considerable control can be exerted over the structure of the complex by exploiting vectorial preparation methods and manipulating ionic strength.

Lobo B. A., Davis A., Koe G., Smith J. G., and Middaugh C. R. (2001) Isothermal titration calorimetric analysis of the interaction between cationic lipids and plasmid DNA. *Arch Biochem Biophys* **386**, 95-105. **Abstract:** The effects of buffer and ionic strength upon the enthalpy of binding between plasmid DNA and a variety of cationic lipids used to enhance cellular transfection were studied using isothermal titration calorimetry at 25.0 degrees C and pH 7.4. The cationic lipids DOTAP (1,2-dioleoyl-3-trimethyl ammonium propane), DDAB (dimethyl dioctadecyl ammonium bromide), DOTAP:cholesterol (1:1), and DDAB:cholesterol (1:1) bound endothermally to plasmid DNA with a negligible proton exchange with buffer. In contrast, DOTAP: DOPE (L-alpha-dioleoyl phosphatidyl ethanolamine) (1:1) and DDAB:DOPE (1:1) liposomes displayed a negative enthalpy and a significant uptake of protons upon binding to plasmid DNA at neutral pH. These findings are most easily explained by a change in the apparent pKa of the amino group of DOPE upon binding. Complexes formed by reverse addition methods (DNA into lipid) produced different thermograms, sizes, zeta potentials, and aggregation behavior, suggesting that structurally different complexes were formed in each titration direction. Titrations performed in both directions in the presence of increasing ionic strength revealed a progressive decrease in the heat of binding and an increase in the lipid to DNA charge ratio at which aggregation occurred. The unfavorable binding enthalpy for the cationic lipids alone and with cholesterol implies an entropy-driven interaction, while the negative enthalpies observed with DOPE-containing lipid mixtures suggest an additional contribution from changes in protonation of DOPE.

Lobo B. A., Koe G. S., Koe J. G., and Middaugh C. R. (2003) Thermodynamic analysis of binding and protonation in DOTAP/DOPE (1:1): DNA complexes using isothermal titration calorimetry. *Biophys Chem* **104**, 67-78.

Abstract: A better understanding of the nature of the interaction between various cationic lipids used for gene delivery and DNA would lend insight into their structural and physical properties that may modulate their efficacy. We therefore separated the protonation and binding events which occur upon complexation of 1:1 DOTAP (1,2-dioleoyl-3-trimethylammonium propane):DOPE (1,2-dioleoylphosphatidylethanolamine) liposomes to DNA using proton linkage theory and isothermal titration calorimetry (ITC). The enthalpy of DOPE protonation was estimated as -45.0 ± 0.7 kJ/mol and the intrinsic binding enthalpy of lipid to DNA as $+2.8 \pm 0.3$ kJ/mol. The pK(a) of DOPE was calculated to shift from 7.7 ± 0.1 in the free state to 8.8 ± 0.1 in the complex. At physiological ionic strength, proton linkage was not observed upon complex formation and the buffer-independent binding enthalpy was $+1.0 \pm 0.4$ kJ/mol. These studies indicate that the intrinsic interaction between 1:1 DOTAP/DOPE and DNA is an entropy-driven process and that the affinities of cationic lipids that are formulated with and without DOPE for DNA are controlled by the positive entropic changes that occur upon complex formation.

Matulis D., Rouzina I., and Bloomfield V. A. (2002) Thermodynamics of cationic lipid binding to DNA and DNA condensation: roles of electrostatics and hydrophobicity. *J Am Chem Soc* **124**, 7331-7342.

Abstract: Alkylammonium binding to DNA was studied by isothermal titration calorimetry. Experimental data, obtained as functions of alkyl chain length, salt concentration, DNA concentration, and temperature,

provided a detailed thermodynamic description of lipid-DNA binding reactions leading to DNA condensation. Lipid binding, counterion displacement, and DNA condensation were highly cooperative processes, driven by a large increase in entropy and opposed by a relatively small endothermic enthalpy at room temperature. Large negative heat capacity change indicated a contribution from hydrophobic interactions between aliphatic tails. An approximation of lipid-DNA binding as dominated by two factors—ionic and hydrophobic interactions—yielded a model that was consistent with experimental data. Chemical group contributions to the energetics of binding were determined and could be used to predict energetics of other lipid binding to DNA. Electrostatic and hydrophobic contributions to Gibbs free energy, enthalpy, entropy, and heat capacity could be distinguished by applying additivity principles. Binding of lipids with two, three, and four aliphatic tails was investigated and compared to single-tailed lipid binding. Structurally, the model suggests that lipid cationic headgroups and aliphatic tails distribute evenly and lay down on DNA surface without the formation of micelles.

Pector V., Backmann J., Maes D., Vandenbranden M., and Ruyschaert J. M. (2000) Biophysical and structural properties of DNA-diC(14)-amidinium complexes. Influence of the DNA/lipid ratio. *J Biol Chem* **275**, 29533-29538.

Abstract: Cationic liposomes are used as vectors for gene delivery both in vitro and in vivo. Comprehension of both DNA/liposome interactions on a molecular level and a description of structural modifications involved, are prerequisites to an optimization of the transfection protocol and, thus, successful application in therapy. Formation and stability of a DNA/cationic liposome complex were investigated here at different DNA:lipid molar ratios (ρ). Isothermal titration calorimetry (ITC) of cationic liposomes with plasmid DNA was used to characterize the DNA-lipid interaction. Two processes were shown to be involved in the complex formation. A fast exothermic process was attributed to the electrostatic binding of DNA to the liposome surface. A subsequent slower endothermic reaction is likely to be caused by the fusion of the two components and their rearrangement into a new structure. Fluorescence and differential scanning calorimetry confirmed this interpretation. A kinetic model analyzes the ITC profile in terms of DNA/cationic liposome interactions.

Pozharski E. and MacDonald R. C. (2002) Thermodynamics of cationic lipid-DNA complex formation as studied by isothermal titration calorimetry. *Biophys J* **83**, 556-565.

Abstract: The detailed analysis of the cationic lipid-DNA complex formation by means of isothermal titration calorimetry is presented. Most experiments were done using 1,2-dioleoyl-sn-glycero-3-ethylphosphocholine (EDOPC), but basic titrations were also done using DOTAP, DOTAP:DOPC, and DOTAP:DOPE mixtures. Complex formation was endothermic with less than 1 kcal absorbed per mole of lipid or DNA charge. This enthalpy change was attributed to DNA-DNA mutual repulsion within the lamellar complex. The exception was DOTAP:DOPE-containing lipoplex for which the enthalpy of formation was exothermic, presumably because of DOPE amine group protonation. Experimental conditions, namely, direction and titration increment as well as concentration of titrant, which dictate the structure of resulting lipoplex (whether lamellar complex or DNA-coated vesicle), were found to affect the apparent thermodynamics of complex formation. The structure, in turn, influences the biological properties of the lipoplex. If the titration of lipid into DNA was carried out in large increments, the ΔH was larger than when the injection increments were smaller, a finding that is consistent with increased vesicle disruption under large increments and which is expected theoretically. Cationic lipid-DNA binding was weak in high ionic strength solutions, however, the effective binding constant is within micromolar range because of macromolecular nature of the interaction.

Pozharski E. and MacDonald R. C. (2003) Lipoplex Thermodynamics: Determination of DNA-Cationic Lipid Interaction Energies. *Biophys J* **85**, 3969-3978.

Abstract: An experimental study of the cationic lipid-DNA binding affinity is presented. The binding free energy was determined by monitoring lipoplex dissociation under conditions of increasing salt concentration. The primary procedure was based on the extent of quenching by energy transfer of fluorophores on DNA molecules by fluorophore on a lipid as these molecules came into close association in the lipoplex. Titration calorimetry on the Dickerson dodecamer was also done, with results that were in agreement with the fluorescence data. Measurements on short oligonucleotides allowed estimation of the binding energy per nucleotide. The binding free energy is approximately 0.6 kcal/mole nucleotide for the Dickerson dodecamer and declines for longer oligonucleotides. The entropy gained upon complex

formation is approximately 1 entropy unit per released counterion. The method was applied to long DNA molecules (herring and lambda-phage DNA) and revealed that complete dissociation occurs at 750 mM NaCl. Likely contributions of macromolecular desolvation and DNA flexibility to the binding energy are discussed.

Rajan R., Wisler J.W., and Bell C.E. (2006) Probing the DNA sequence specificity of Escherichia coli RECA protein. *Nucleic Acids Res.* **34**, 2463-71.

Abstract: Escherichia coli RecA protein catalyzes the central DNA strand-exchange step of homologous recombination, which is essential for the repair of double-stranded DNA breaks. In this reaction, RecA first polymerizes on single-stranded DNA (ssDNA) to form a right-handed helical filament with one monomer per 3 nt of ssDNA. RecA generally binds to any sequence of ssDNA but has a preference for GT-rich sequences, as found in the recombination hot spot Chi (5'-GCTGGTGG-3'). When this sequence is located within an oligonucleotide, binding of RecA is phased relative to it, with a periodicity of three nucleotides. This implies that there are three separate nucleotide-binding sites within a RecA monomer that may exhibit preferences for the four different nucleotides. Here we have used a RecA coprotease assay to further probe the ssDNA sequence specificity of E.coli RecA protein. The extent of self-cleavage of a lambda repressor fragment in the presence of RecA, ADP-ALF4 and 64 different trinucleotide-repeating 15mer oligonucleotides was determined. The coprotease activity of RecA is strongly dependent on the ssDNA sequence, with TGG-repeating sequences giving by far the highest coprotease activity, and GC and AT-rich sequences the lowest. For selected trinucleotide-repeating sequences, the DNA-dependent ATPase and DNA-binding activities of RecA were also determined. The DNA-binding and coprotease activities of RecA have the same sequence dependence, which is essentially opposite to that of the ATPase activity of RecA. The implications with regard to the biological mechanism of RecA are discussed.

Rungsardthong U., Ehtezazi T., Bailey L., Armes S. P., Garnett M. C., and Stolnik S. (2003) Effect of polymer ionization on the interaction with DNA in nonviral gene delivery systems. *Biomacromolecules* **4**, 683-690.

Abstract: The optimization of DNA-cationic polymer complexation is crucial for nonviral gene delivery. Although physicochemical characterization of the interaction between DNA and cationic polymers has recently attracted more attention in the nonviral DNA delivery field, the literature on the effect of varying polycation charge density on DNA-cationic polymer complexation is still scarce. Thus, the aim of this study was to systematically assess the influence of the degree of ionization of a weak cationic polyelectrolyte (poly[2-(dimethylamino)ethyl methacrylate] or DMAEMA homopolymer) on its ability to form complexes with DNA. This was achieved by varying the solution pH from 4.0 to 8.0 and analyzing the resulting effects on the binding affinity, thermodynamic properties, complex size, and morphology. Lowering the solution pH led to higher degrees of ionization for the cationic polymer and hence greater binding affinities with DNA, as judged by the increased propensity of the former to displace ethidium bromide from DNA and also by relatively low monomer:nucleotide molar ratio (0.8:1) required to retard the migration of free DNA. Isothermal titration microcalorimetry studies further confirmed that a stronger interaction occurred at low pH than at high pH. By decreasing the pH from 8.0 to 6.6, K_{obs} increased from 7.8×10^5 to $20.4 \times 10^5 \text{ M}^{-1}$. More efficient condensation at low pH was demonstrated by the reduction of ethidium bromide fluorescence in the loading wells from gel electrophoresis, decreased complex sizes without agglomeration occurring at high polymer/DNA ratios, together with discrete and dense spherical complexes observed in TEM studies. This may be attributed to the presence of electrostatic stabilization from excess cationic polymer chains, which provide a repulsive shell around the polymer/DNA complex. The physicochemical data indicate that the increased degree of ionization for the DMAEMA homopolymer at lower pH results in higher binding affinity, smaller and more compact complexes, and more efficient condensation. These findings therefore highlight the importance of the degree of ionization on DNA complex formation for weak cationic polyelectrolytes.