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# The ionic strength effect on the DNA complexation by DOPC — gemini surfactants liposomes

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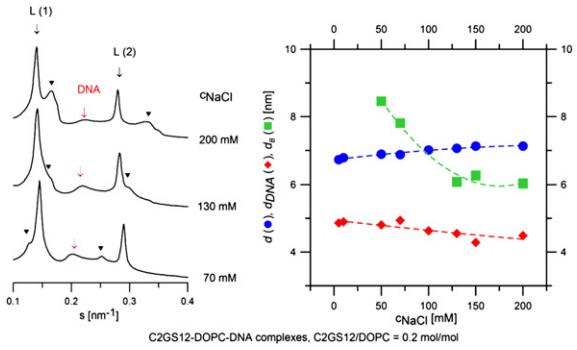
## HIGHLIGHTS

- Gemini surfactant – DOPC liposomes condense DNA at physiologically relevant ionic strength.
- In addition to the condensed lamellar $L_\alpha^c$ phase, a coexisting lamellar phase $L_B$ was also identified in formed complexes.
- Sufficiently high surface charge density and method of complex preparation are key parameters to avoid the phase coexistence.

## GRAPHICAL ABSTRACT



C2GS12-DOPC-DNA complexes, C2GS12/DOPC = 0.2 mol/mol

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## ABSTRACT

Liposome dispersions obtained from the mixture of gemini surfactants of the type alkane-α,ω-diyl-bis(alkyldimethylammonium bromide) and helper lipid DOPC create complexes with DNA showing a regular inner microstructure, identified by small angle X-ray diffraction as condensed lamellar phase ($L_\alpha^c$). In addition to the $L_\alpha^c$ phase, a coexisting lamellar phase $L_B$ was also identified in the complexes formed, with periodicities in the range ~8.8–5.7 nm, at ionic strengths corresponding to 50–200 mM NaCl. The periodicities of $L_B$ phase did not correspond to those identified in liposome dispersion without DNA using small angle neutron scattering. The observed phase separation is shown to depend on the interplay between the surface charge density of cationic liposomes, ionic strength and method of complex preparation. The effect of ionic strength on complex formation was studied by isothermal titration calorimetry and zeta potential measurements. High ionic strength reduces the fraction of bound DNA in the complexes, and the isoelectric point is attained at a ratio of DNA/gemini surfactant which is lower than the one that can be estimated by calculation based on nominal charges of CLs and DNA.



*Abbreviations:* CLs, cationic liposomes; CnGSm, gemini surfactant alkane-α,ω-diyl-bis(alkyldimethylammonium bromide); DOPC, dioleylphosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine; CTDNA, calf thymus DNA; HTDNA, DNA from herring testes; $L_\alpha^c$, condensed lamellar phase; $L_B$, additional lipid phase with lamellar structure; SAXD, small angle X-ray diffraction; WAXD, wide angle X-ray diffraction; SANS, small angle neutron scattering; ITC, isothermal titration calorimetry.

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## 1. Introduction

Cationic liposomes (CLs) condense nucleic acids into complexes with a regular inner microstructure, capable of passing through somatic cell's membrane. As gene delivery carriers, they were used for the first time by Felgner et al. [1]. Since the first study, many cationic lipids have been synthesized, as potential candidates for gene delivery vectors. Physico-chemical properties, structure, transfection efficiency and relations between them have been intensively investigated. To design complexes with the desired microstructure and enhanced transfection efficiency, the interplay of the electrostatic, elastic, and entropic-mixing forces has to be considered [2–6]. The helper neutral lipid used together with cationic components moderates the colloidal and structural properties of the complex and facilitates the transport through the cell's membrane [7,8]. Fluidity, hexagonal-phase-forming propensity and fusogenic potential of the helper lipid play the major role in the enhanced transfection efficiency [4,9]. DNA – cationic liposome complexes create three types of organized microstructures: i) condensed lamellar phase ($L_{\alpha}^{c}$) with ordered DNA monolayers intercalated between lipid bilayers [10,11]; ii) condensed columnar inverted hexagonal phase ($H_{II}^{c}$) with linear DNA molecules surrounded by lipid monolayers forming inverted cylindrical micelles arranged on a hexagonal lattice [12]; iii) condensed columnar hexagonal phase ($H_{I}^{c}$) with cylindrical micelles arranged in an hexagonal lattice, and DNA strands filling the interspace between micelles, forming a honeycomb structure [13].

Kirby et al. [14] introduced gemini surfactants as possible gene delivery carriers. These amphiphilic compounds attract the scientific interest because they represent a useful group of surfactants for applications where precisely designed aggregation properties are required [15]. The complexes based on gemini surfactants alkane-α,ω-diyl-bis(alkyldimethylammonium bromide) (CnGSm, Fig. 1) have shown good transfection activity *in vitro* [16] and *in vivo* [17]. CnGSm, with two alkyl chains (where *m* is the number of carbons in the alkyl chain) and two quaternary ammonium groups, connected by a polymethylene chain referred to as a spacer (*n* is the number of carbons in the spacer) were used to build a simple model system for our study.

We have used small and wide angle X-ray diffraction (SAXD and WAXD) to characterize the microstructure of complexes prepared from the CnGS12 surfactant and DNA, using dioleyphosphatidylcholine (DOPC) as a helper lipid, mixed at a ratio corresponding to the calculated isoelectric point. In this study we focused on the structural diversity induced by the liposome composition or bulk solution's conditions. The dependence of the process of DNA condensation on the DNA/C2GS12 molar ratio and bulk solution's conditions was monitored by isothermal titration microcalorimetry (ITC) and zeta potential measurements. Small angle neutron scattering (SANS) was used to characterize the C2GS12–DOPC dispersion.

Aiming to adopt physiologically relevant conditions in the study we decided to work initially at the ionic strength corresponding to a NaCl concentration of 150 mM. However, the preliminary experiments have alerted the need of a more detailed investigation of the high salt concentration's effects. Until now the studies of the effect of ionic strength on DNA – cationic liposomes complexes (DNA-CLs) have shown that the increase in ionic strength reduces the amount of DNA which the CLs are able to bind [3,18,19]. At sufficiently high salt concentration the charge screening precludes the interaction of DNA with CLs. Other study reported that both under optimum medium conditions for routine transfection as well as in water, the formed DNA-CLs complexes carry negative charges [20]. It has also been reported that the zeta potential of the complexes formed in water can be significantly lowered by the addition of NaCl [21].

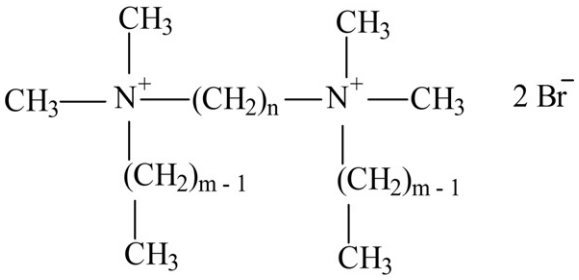


**Fig. 1.** The structure of alkane-α,ω-diyl-bis(alkyldimethylammonium bromide) (CnGSm).

DNA was found to induce microscopic lateral phase segregation in mixed lipid membranes [22–24]. The segregation is seen as the formation of lipid microdomains having different composition. In the presence of electrostatic interactions between the lipid membrane and the polymer, the segregation could be induced by the attraction between the oppositely charged components leading to spatial changes in the distribution of the charged compounds in the membrane [25].

## 2. Materials and methods

### 2.1. Chemicals

Neutral phospholipid DOPC (1,2-dioleoyl-*sn*-glycero-3-phosphocholine, $M_r = 786.15$) was purchased from Avanti Polar Lipids, Inc., USA, highly polymerized calf thymus DNA (sodium salt) type I (CTDNA, average Mr of nucleotide = 308) and DNA (sodium salt) type XIV from Herring testes (HTDNA, average Mr of nucleotide = 308) from Sigma Chemicals Co., USA. Alkane-α,ω-diyl-bis (dodecyldimethylammonium bromide) surfactants (CnCS12, $n = 2$, 3, 4) were prepared as described in [26] and purified by manifold crystallization from a mixture of acetone and methanol. The NaCl and NaOH of analytical purity were obtained from Lachema, Brno, Czech Republic. $^2H_2O$ (99.9%) was purchased from Merck, Germany. The chemicals were of the analytical grade and were used without further purifications. The aqueous solutions were prepared with redistilled water, pH ~ 6 except the solutions for isothermal titration microcalorimetry, which were prepared using Millipore water.

### 2.2. Preparation of cationic liposomes

DOPC and CnGS12 were dissolved in organic solvent (mixture of chloroform and methanol at volume ratio = 3:1). The appropriate amounts of organic stock solutions were mixed to obtain the desired ratio (CnGS12/DOPC = 0.15–0.5 mol/mol). The solvent was evaporated under a stream of gaseous nitrogen and its residue removed by a vacuum. The dry mixture was hydrated by the NaCl solution of appropriate concentration for 12 h. Afterwards the mixture was homogenized (by vortexing, freezing–thawing cycles or sonication in an ultrasound bath) until an opalescent dispersion was created. We tested the size and polydispersity of prepared cationic liposomes (CLs) by the dynamic light scattering measurements using the Nano ZetaSizer 5000. We obtained the values of diameter ~250 nm for the CLs at ratio C2GS12/DOPC = 0.3 in 5 mM NaCl and ~1000 nm in 70 mM NaCl. Both dispersions have shown low polydispersity in diameter. The control samples of pure DOPC were prepared in the same manner and we obtained a milky dispersion of multilamellar liposomes. Liposome dispersions for SANS experiments were prepared at phospholipid concentration 10 mg/ml in the same manner except the usage of $^2H_2O$ instead of $H_2O$.

### 2.3. Preparation of DNA solutions

The stock solutions were prepared by dissolving CTDNA or HTDNA at concentration 2 mg/ml or 3 mg/ml, respectively, both in 5 mM NaCl or 200 mM NaCl solution. To obtain the intermediate values of NaCl concentration of DNA solution, we mixed the stock solutions of DNA in 5 mM NaCl and 200 mM NaCl at appropriate ratios. The