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The DNA–DNA spacing in gemini surfactants–DOPE–DNA complexes

Petra Pullmannová [a,*], Sergio S. Funari [b], Ferdinand Devínsky [c], Daniela Uhríková [a]

[a] Department of Physical Chemistry of Drugs, Faculty of Pharmacy, Comenius University, Odbojárov 10, SK-832 32 Bratislava, Slovakia
[b] HASYLAB at DESY, Notkestr. 85, D-22607 Hamburg, Germany
[c] Department of Chemical Theory of Drugs, Faculty of Pharmacy, Comenius University, Odbojárov 10, SK-832 32 Bratislava, Slovakia

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ABSTRACT

Gemini surfactants from the homologous series of alkane-α,ω-diyl-bis(dodecyldimethylammonium bromide) (CnCS12, number of spacer carbons $n = 2-12$) and dioleoylphosphatidylethanolamine (DOPE) were used for cationic liposome (CL) preparation. CLs condense highly polymerized DNA creating complexes. Small-angle X-ray diffraction identified them as condensed lamellar phase L_α^C in the studied range of molar ratios CnGS12/DOPE in the temperature range 20–60 °C. The DNA–DNA distance (d_{DNA}) is studied in dependence to CnGS12 spacer length and membrane surface charge density. The high membrane surface charge densities (CnGS12/DOPE = 0.35 and 0.4 mol/mol) lead to the linear dependence of d_{DNA} vs. n correlating with the interfacial area of the CnGS12 molecule.

1. Introduction

Gemini surfactants (GS) were synthesized as promising amphiphilic compounds due to their excellent self-assemble properties. They contain two polar headgroups and two aliphatic chains linked by a spacer [1,2]. GS show greatly enhanced surfactant properties relative to the corresponding monomeric (single chain, single head group) compounds—their surface activity can be increased 1000-fold. This makes them interesting for biological and especially biomedical research.

Generally, cationic surfactants can create complexes with polyanions including nucleic acids, giving rise to various periodical structures of great interest for biomedical or industrial applications [3]. For example, DNA–cetyltrimethylammonium bromide–hexanol aggregates express complex phase behavior in dependence on the increasing hexanol content. The addition of hexanol leads to simultaneous lowering of the charge density and bare rigidity of the bilayers. The same effect can, in principle, be independently achieved by adding a neutral lipid [4]. GS carrying a positive charge condense DNA and can act as agent in nucleic acid transfection [5,6] and bacterial transformation [7].

Within this study we focus on the simple type of GS alkane-α,ω-diyl-bis(alkyldimethylammonium bromide) (CnGSm, where $n = 2$–12 is the number of spacer carbons and $m = 12$ is the number of carbons in the alkyl tails) (Fig. 1). The physico-chemical and micellar properties of CnGSm have attracted extensive scientific interest, focusing particularly on the role of the spacer. For example, the critical micelle concentration of homologous series CnGS12 with a hydrophobic polymethylene spacer express a maximum at $n = 4$–6 [8–10]. The surface occupied by one surfactant at air/solution interface reaches a maximum at $n = 10$–12 [10–13]. At $n > 10$ the spacer becomes too hydrophobic to remain in contact with water and adopts a looped (wicket-like) conformation [12].

The CnGSm can interact with DNA either as monomers, micelles [14–17] or as a dispersion of CnGSm–phospholipid liposomes forming complexes with regular inner microstructure [18–20]. Neutral lipid used together with cationic components moderates the structural properties and the membrane surface charge density of the complex. Neutral lipid also facilitates the transport of the complex through the cell's membrane during transfection [21,22]. Several types of CnGSm-phospholipid–DNA complexes arrangement have been observed: condensed lamellar phase L_α^C with ordered DNA monolayers intercalated between lipid bilayers [18,20,23], condensed inverted hexagonal phase H_{II}^C with linear DNA molecules surrounded by lipid monolayers forming inverted cylindrical micelles arranged on a hexagonal lattice [24–26] and also a cubic phase [27] were reported. The membrane surface charge density was found to be a key parameter for the transfection efficiency of L_α^C phase-forming complexes. Lin et al. [28] defines the optimal membrane surface charge density $\sigma_m^* \approx 0.0104\ e^-/Å^2 \approx 1.04\ e^-/1\ nm^2$ for stable complexes allowing successful escape of DNA from the complexes through their fusion with endosomal membranes.

Complexes CnGS12–dioleyphosphatidylethanolamine (DOPE)–DNA have been tested as gene delivery carriers for somatic cells *in vitro* [20]

Abbreviations: A_{CnGS12}, CnGS12 surfactant headgroup area; C12TMA, dodecyltrimethylammonium bromide; CL, cationic liposomes; CnGS12, alkane-α,ω-diyl-bis(dodecyldimethylammonium bromide); DNA, deoxyribonucleic acid; DOPE, dioleoylphosphatidylethanolammine; GS, gemini surfactant; H_{II}^C, condensed inverse hexagonal phase; L_α^C, condensed lamellar phase; R_4N^+, quaternary ammonium group

* Corresponding author. Tel.: +421 250117289.
E-mail address: pullmannova@fpharm.uniba.sk (P. Pullmannová).

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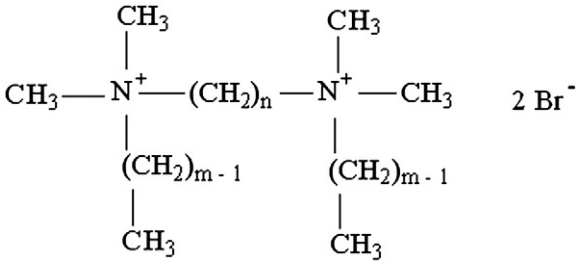

Fig. 1. The scheme of the structure of alkane-α,ω-diyl-bis(dodecyldimethylammonium bromide) molecule (CnCS12, $n=2$–12 is the number of spacer carbons, $m=12$ is the number of alkyl carbons).

and *in vivo* [29]. The character of the spacer has been found to play a role in the transfection efficiency of DNA complexes based on GS [20,30,31]. The highest transfection activity *in vitro* was detected at CnGSm compounds having the spacer of three carbons (C3GS12, C3GS16) [20]. Generally, GS with short spacer length were reported regarding their enhanced transfection activity. For example C2GS14/cholesterol/DOPE = 2:1:1 mol/mol/mol [32] and derivatives of *N,N*-bisdimethyl-1,2-ethanediamine with 2 carbons in the spacer and 12 carbons in alkyl chains, in a mixture with DOPE (1:2 mol/mol) have shown significantly higher tranfection activity in comparison to a standard commercial transfection reagent [33].

In spite of published results in transfection efficiency of CnGSm–neutral phospholipid carriers for DNA, information about the particle's microstructure, their polymorphic behavior and the role of CnGSm spacer length in DNA packing is rather scarce in literature. We used small-angle X-ray diffraction (SAXD) to study the microstructure of complexes formed due to DNA interaction with cationic liposomes (CL) prepared as a mixture of CnGS12 with various spacer $n=2$–12 and DOPE. In this study we focus our attention on a role of the spacer's length in the DNA packing in L_α^C phase.

2. Materials and methods

DOPE (1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine) was obtained from Avanti Polar Lipids, Inc., USA; highly polymerized calf thymus deoxyribonucleic acid (sodium salt) type I (DNA, average Mr of nucleotide 308) from Sigma Chemicals Co., USA. Alkane-α,ω-diyl-bis(dodecyldimethylammonium bromide) (CnCS12, number of spacer carbons $n=2$–12) were prepared as described in ref. [1] and purified by manyfold crystallization from a mixture of acetone and methanol. Dodecyltrimethylammonium bromide (C12TMA) was purchased from Fluka Chemie AG, Buchs, Switzerland. Sodium chloride (NaCl) was obtained from Lachema, Brno, Czech Republic. The chemicals were of analytical grade and were used without further purification. The aqueous solution of NaCl was prepared with redistilled water, pH ~ 6, at concentration 150 mM. The solution of DNA was prepared by dissolving DNA in 150 mM NaCl solution, the precise value of DNA concentration was determined spectrophotometrically (Hewlett Packard 8452A Diode array spectrophotometer), according to: $c_{DNA}=A_{260}.\ 47\times10^{-6}$ [g/ml], where A_{260} is the absorbance at wavelength $\lambda=260$ nm. The concentration of DNA is referred as molar concentration of DNA base pairs (mol bp). The purity of DNA was checked by measuring the absorbance A_λ at $\lambda=260$, 230 and 280 nm. We have obtained the values of $A_{260}/A_{230}=2.23$ and $A_{260}/A_{280}=1.76$. DOPE, CnGS12 and C12TMA were dissolved in organic solvent (mixture of chloroform and methanol at volume ratio = 3:1). The appropriate amounts of organic stock solutions were added to obtain a lipid mixture with the desired ratio cationic surfactant/DOPE. The solvent was evaporated under a stream of gaseous nitrogen and its residue removed by vacuum. The dry mixture was hydrated by 150 mM NaCl solution for 12 hours and afterwards homogenized (by vortexing, freezing-thawing cycles or sonication in ultrasound bath) until an opalescent liposome dispersion was created. A fully hydrated DOPE was used as a control sample.

The samples were prepared at the calculated isoelectric point CnCS12/DNA = 1 [mol/mol bp]. The DNA solution was added to the liposome dispersion at once and the sample was shortly mixed. A precipitate was created spontaneously during the mixing. To exclude the possibility of an incomplete binding of DNA in DNA–CL, we measured the residual DNA concentration in the sample's supernatant spectrophotometrically for CnGS12–DOPE–DNA and C12TMA–DOPE–DNA complexes. The average DNA condensation efficiency was 98.1 ± 1.9% reporting to an effective binding of DNA. The samples were stored at 2–6 °C or in a freezer at approx. −20 °C.

Before the measurement the samples were shortly centrifuged. The sedimented precipitate with a few drops of bulk solution was enclosed between two Kapton (Dupont, France) windows of a sample holder for X-ray diffraction. Small- (SAXD) and wide-angle (WAXD) synchrotron radiation diffraction experiments were performed at the soft condensed matter beamline A2 at HASYLAB at the Deutsches Elektronen Synchrotron (DESY) in Hamburg (Germany), using a monochromatic radiation of wavelength $\lambda=0.15$ nm. The sample was equilibrated at selected temperature (20 or 60 °C) before exposure to radiation. The evacuated double-focusing camera was equipped with a linear position sensitive detector for WAXD and a 2D MarCCD detector or a linear position sensitive detector for SAXD. The raw data were normalized against the incident beam intensity. The SAXD patterns were calibrated using Ag behenate [34] or rat tail collagen [35] and the WAXD patterns by tripalmitin or polyethylene terephthalate [36,37]. Each diffraction peak of SAXD region was fitted with a Lorentzian above a linear background using the Origin software. The WAXD pattern of all measured samples exhibited one wide diffuse scattering in the range $s\sim1.8$–$3.2\ nm^{-1}$, characteristic for liquid-like carbon chains of phospholipid and CnGS12 molecules. We do not show WAXD patterns because we observed no qualitative change in WAXD patterns in the temperature range 20–60°.

3. Results and discussion

3.1. The effect of surface charge density

The structure of complexes C3GS12–DOPE–DNA was investigated throughout the range of molar ratios $0.1\le$ C3GS12/DOPE ≤0.5 mol/mol (at 20 °C). We determined the structural parameters of complexes with the aim to find out the appropriate range of molar ratios to study the spacer's effect. Representative SAXD patterns are shown in Fig. 2A. At the molar ratios C3GS12/DOPE ≥0.15 we observed two sharp reflections L(1) and L(2) having the origin in a lamellar structure of lipid bilayers. The repeat distance d was evaluated as $d=1/s_1$, where s_1 is the first order reflection's maximum. The DNA exhibits a broad small peak at $s_{DNA}=1/d_{DNA}$, where d_{DNA} is the average distance of periodically spaced DNA strands. The repeat distance d involves the thickness of phospholipids bilayer d_L and the water thickness containing a monolayer of hydrated DNA strands d_W, so $d=d_L+d_W$. The thickness of hydrated DNA monolayer d_W is assumed to be not less than $d_W\sim2.45$ nm [38]. The observed SAXD patterns are typical for a condensed lamellar phase L_α^C of complexes DNA–CL. At low molar ratios C3GS12/DOPE ≤0.12, the coexistence of L_α^C phase and condensed hexagonal phase H_{II}^C is observed. We have shown earlier [26], that the heating of complexes CnGS12–DOPE–DNA leads to the increase in the fraction of H_{II}^C phase due to a phase transition $L_\alpha^C\rightarrow H_{II}^C$. The lattice parameter of the H_{II}^C phase in complexes at C3GS12/DOPE = 0.12 mol/mol is $a=2/\sqrt{3}\,s_1^{'}=7.14$ nm, where $s_1^{'}$ is the first order reflection's maximum of H_{II}^C phase. The fully hydrated DOPE used as a control sample formed an inverted hexagonal phase H_{II} with the lattice parameter $a=7.58$ nm (at 20 °C). DOPE has an inverted truncated cone-shaped molecule and confers a negative spontaneous curvature to membranes. Although hydrated DOPE forms inverted hexagonal phase, the sufficient content of C3GS12 surfactant stabilizes the