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Structural, biocomplexation and gene delivery properties of hydroxyethylated gemini surfactants with varied spacer length

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ABSTRACT

Gemini surfactants with hexadecyl tails and hydroxyethylated head groups bridged with tetramethylene (**G4**), hexamethylene (**G6**) and dodecamethylene (**G12**) spacers were shown to self-assemble at the lower critical micelle concentration compared to their conventional m-s-m analogs. The lipoplex formation and the plasmid DNA transfer into different kinds of host cells were studied. In the case of eukaryotic cells, high transfection efficacy has been demonstrated for DNA–gemini complexes, which increased as follows: **G6** < **G4** < **G12**. Different activity series, i.e., **G6** > **G4** > **G12** has been obtained in the case of transformation of bacterial cells with plasmid DNA–gemini complexes, mediated by electroporation technique. Solely **G6** shows transformation efficacy exceeding the control result (uncomplexed DNA), while the inhibitory effect occurs for **G4** and **G12**. Analysis of physico-chemical features of single surfactants and lipoplexes shows that compaction and condensation effects change as follows: **G6** < **G4** ≤ **G12**, i.e., agree with the order of transfection efficacy, which is supported by membrane tropic properties of **G12**. On the other hand, gel retardation assay and docking study testify low electrostatic affinity in **G12**/DNA pair, thereby indicating that hydrophobic effect probably plays important role in the lipoplex formation. Two factors are assumed to be responsible for the inhibition effect of gemini in the case of transformation of bacterial cells. They are (i) an unfavorable influence of cationic surfactants on the electroporation procedure due to depressing the electrophoretic effect; and (ii) antibacterial activity of cationic surfactants that may cause the disruption of integrity of cell membranes.

1. Introduction

The interactions of cationic compounds with DNA result in the formation of biochemical constructs that are of significant importance in gene diagnostic and therapy [1–4]. Cationic surfactants are in the focus of modern biotechnological applications as relatively simple and effective carriers of therapeutic agents that assist in transfer of genetic material. The use of the so-called non-viral vectors [4] makes it possible to solve problems connected with the condensation and compaction of giant DNA polyanions, with such limitations of viral vectors as immune response avoided. Although the application of synthetic vectors including those based on cationic surfactants and lipids is well documented [5–7], it is yet a challenging problem due to their relatively lower transfection efficacy compared to viral carriers. One of the main trends in the development of current protocols is the design of carriers with multicentered interactions with phosphate anions of DNA, including those based on gemini surfactants [8–11]. However, publications available demonstrate that DNA delivery is typically mediated by the binary surfactant/lipid compositions or by the use of additional protective polymer, such as polyethylene glycol. Few studies were devoted to the application of monocationic and gemini surfactants functionalized by fragments with H-bonding capacity [12]. Recently, fundamental research activity have focused on the elucidation of the molecular mechanisms of the DNA–surfactant interactions [13,14], which may contribute to the purposeful design

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of nonviral vectors based on the structure–activity relations. Taking into account these trends, we focused our study on the investigation of hydroxyethylated gemini surfactants as cationic agents for the DNA complexation. The DNA–surfactant complexation was testified by several complementary experimental methods such as AFM, DLS, DNA transfer, molecular docking, and gel retardation assay. In addition, we have undertaken the turbidimetry research of the interaction of gemini surfactants with lipid bilayer to probe the ability of lipoplexes of penetrating through cell membranes.

Gemini surfactants demonstrate much lower critical micelle concentrations (cmc) and different morphological behavior as compared to their single-head analogs [15–19]. Besides, surfactants under study vary in their spacers, namely, head groups are bridged by four (**G4**), six (**G6**) or twelve (**G12**) methylene groups. Recent publications have reported on different effects of spacer nature on size and morphology of gemini surfactant aggregates for various types of amphiphiles, with role of spacer fragment thoroughly discussed in Ref. [20]. The cmc of *m-s-m* geminis with polymethylene spacer was documented to have maximum at $s = 5–6$ for different m values. They tend to form small spherical micelles with aggregation numbers of 30 for monomethylene spacer and 50 for tetramethylene spacer [21]. In the case of rigid spacer containing C═C or benzene fragment small micelle-like aggregates with hydrodynamic diameter $D_H = 2$ nm can occur [22]. In Ref. [23] effects of spacer length and rigidity was compared. Authors concluded that alkyl chain length of polymethylene spacer influenced aggregation capacity more markedly than the variation in spacer rigidity. Noteworthy, geminis with natural fragments attract special attention of researchers. For arginine-based dimeric surfactants, micelles with $D_H = 3$ nm are observed in the case of hexamethylene spacer, while bimodal size distribution with $D_H = 100$ nm and 700 nm is reported for longer spacer fragment [24]. In Ref. [25] amino-acid based geminis with polymethylene spacer of different length ($s = 2, 5, 8$) were studied, with positively or negatively charged wormlike micelles revealed.

While the role of the spacer architecture in the DNA–surfactant complexation is exemplified in literature [26–29], there is some disagreement in these data. Superior results were obtained for ethanediyl- and propanediyl versus the longer $(—CH_2—)_5$ and $(—CH_2—)_{12}$ polymethylene spacers in Refs. [30,24], while the gemini with pentanediyl spacer appeared the most effective in work [31]. Complicated dependence of transfection efficacy on the spacer length was obtained in Ref. [32], where an increase in gene delivery occurred with the transition from propanediyl to pentanediyl chain, while further increase in spacer length resulted in worse activity. Probably, the most common trend summarized in Ref. [29] is that the efficient DNA compaction is promoted by gemini surfactants having either short (<C4) or long (>C10) spacers. This is due to the fact that a distance of ca. 5 Å between surfactant charged groups is suitable for their interaction with adjacent phosphate groups of DNA. On the other hand, the much longer flexible spacers can coil up, thereby reducing the distance between head groups. Importantly the effect of spacer length can be different in the case of molecular and aggregated forms of surfactants [26].

Another advantage of the studied surfactants is the presence of hydroxyethyl fragments, since the possibility of additional H-bonding may contribute to peculiar properties of their interaction with DNA. The introduction of OH—group in both spacer and head groups may affect the structural behavior of single surfactant solution and its complexes with DNA [12,27,33]. This influence is probably mediated through solvation of head groups, changes in their size and the surface curvature of aggregates, which in turn modify the coverage of DNA molecules. The favorable effect of hydroxyalkyl fragments in gemini surfactants is reported in respect of aggregation and solubilization capacity, catalytic activity, complexation with DNA and specifically of transfection efficiency [34]. Meanwhile, these studies are mainly exemplified by canonical single-head surfactant cetylhydroxyethyl dimethylammoniumbromide (CHAB). Importantly $CH_2—CH_2—OH$ fragments are similar to the monomeric units of polyethylene glycols which are widely used for the so-called PEGylation procedure aimed at the toxicity decrease of therapeutic formulations [11]. Therefore, one can expect that hydroxyethyl groups of suggested nonviral agents may decorate the lipoplexes formed, thus improving their biocompatibility and toxic characteristics.

The present work is devoted to the structural behavior, biocomplexation and gene delivery properties of gemini surfactants with hexadecyl tails and hydroxyethylated head groups bridged with tetramethylene (**G4**), hexamethylene (**G6**) and dodecamethylene (**G12**) spacers (Fig. S1) namely (i) comprehensive characteristics of these surfactants in point of their interaction with DNA molecules are determined by means of atomic force microscopy (AFM), gel retardation assay and molecular docking; (ii) their influence on the artificial membrane structures is examined with the help of turbidity measurements (ii) the DNA transferring into prokaryotic and eukaryotic cells were carried out to estimate potential properties of hydroxyethylated gemini surfactants as gene delivery agents.

2. Materials and methods

2.1. Materials

For transfection activity study we used the *pEGFP-N1* plasmid (Clontech, United States) which is 4700 bp in length and expresses the green fluorescent protein and the *pCI-NEO* plasmid-based vector (Promega, United States), which is 5500 bp in length and does not contain the green fluorescent protein gene. Cell line 293 T (human embryonic kidney epithelium cells), which was used as a producer of pseudo lentiviruses and as a model target cell line, was maintained by the standard procedure. *pEGFP-N1* plasmid was also used for AFM. Standard growth medium DMEM contains 10% heat-inactivated fetal bovine serum (FBS), 2 mM L-glutamine, 100 U of penicillin/ml and 100 μg of streptomycin/ml. The *pK18* plasmid DNA (2661 bp) was used for the transformation of bacterial cells. Dipalmitoylphosphatidylcholine (DPPC) (Sigma) was used as received for construction of artificial liposomes.

2.2. Synthesis

Hydroxyethylated geminis, including **G4** and **G6** are reported in refs [35–39], while gemini **G12** is described herein. Synthetic details and quantification of properties by NMR, IR spectroscopy and elemental analysis are given in Supplementary data section.

2.3. AFM

Intermittent contact or tapping mode AFM was performed with a Multimode V (Veeco, USA). The 250–350 kHz cantilevers (Veeco, USA) with silicone tips were used in all measurements. Tip curvature radius is of 10–13 nm. The microscopic images were obtained by means of 8279JV scanner with a 512 × 512 resolution. The scanning rate was 1 Hz. The antivibrational system (SG0508) was used to eliminate external distortions. The aqueous dispersions of the sample was placed on mica surface with the roughness no more than 1–2 nm. The AFM imaging was performed after water evaporation.

2.4. Turbidity measurements

The phase transition of surfactant/DPPC mixtures was studied using Lambda 25 (PerkinElmer) and Specord 250 Plus double-beam spectrophotometers, equipped with a Peltier thermostated cell holder, using quartz cells of 1 cm path length. Before turbidity