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Bis-quaternary gemini surfactants as components of nonviral gene delivery systems: A comprehensive study from physicochemical properties to membrane interactions

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ABSTRACT

Gemini surfactants have been successfully used as components of gene delivery systems. In the present work, a family of gemini surfactants, represented by the general structure $[C_mH_{2m+1}(CH_3)_2N^+(CH_2)_sN^+(CH_3)_2C_mH_{2m+1}]2Br^-$, or simply *m–s–m*, was used to prepare cationic gene carriers, aiming at their application in transfection studies. An extensive characterization of the gemini surfactant-based complexes, produced with and without the helper lipids cholesterol and DOPE, was carried out in order to correlate their physico-chemical properties with transfection efficiency. The most efficient complexes were those containing helper lipids, which, combining amphiphiles with propensity to form structures with different intrinsic curvatures, displayed a morphologically labile architecture, putatively implicated in the efficient DNA release upon complex interaction with membranes. While complexes lacking helper lipids were translocated directly across the lipid bilayer, complexes containing helper lipids were taken up by cells also by macropinocytosis. This study contributes to shed light on the relationship between important physico-chemical properties of surfactant-based DNA vectors and their efficiency to promote gene transfer, which may represent a step forward to the rational design of gene delivery systems.

1. Introduction

Gemini surfactants are amphiphilic molecules composed of two sets of a polar head group plus an hydrocarbon chain, linked by a spacer at the level or close to the head group (Menger and Littau, 1991). Interest in these molecules originally sparkled from the work of Menger and Littau in 1991 (Menger and Littau, 1991), and, over the last 20 years, several families of gemini surfactants were produced and extensively studied in terms of their aggregation and surface properties (Alami et al., 1993; Buijnsters et al., 2002; Silva et al., 2012). Gemini surfactants have been shown to present several relevant biological activities, namely as antimicrobial (Murguía et al., 2008; Badr et al., 2010; Colomer et al., 2011; Hoque et al., 2012; Obłąk et al., 2014) and antifungal (Murguía et al., 2008; Obłąk et al., 2013) agents. Their application in drug delivery has also been reported as a promising approach. In fact, recent studies have shown that gemini surfactants are able to facilitate drug delivery by enhancing the drug load and the cellular entry of a peptide-based drug delivery system (Ding et al., 2011), and a cyclodextrin-modified gemini surfactant was used to successfully deliver anticancer drugs (Singh et al., 2012). However, the most extensively studied application of cationic gemini surfactants regards their use as nucleic acid delivery systems (Rosenzweig et al., 2001; Bombelli et al., 2005a; Badea et al., 2005, 2007; Wang and Wettig, 2011; Damen et al., 2010; Donkuru et al., 2010; Cardoso et al., 2011; Mohammed-Saeid et al., 2012; Grigoriev et al., 2012; Wang et al., 2013).

The therapeutic potential of DNA depends on the development of efficient and safe vehicles that can overcome the potential bottle-neck for intracellular gene delivery. Due to the propensity of gemini surfactants for structure modulation (Menger and Littau, 1991; Rosenzweig et al., 2001; Wang et al., 2007), these compounds have been designed in order to promote low toxicity

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and immunogenicity, high stability in biological fluids and biodegradability, which are essential requirements for safe gene delivery systems (Kirby et al., 2003).

In the case of bis-quat gemini surfactants, the presence of two quaternary ammonium groups per surfactant molecule, which increases their strength of interaction with DNA (Rosenzweig et al., 2001), as well as the hydrophobic contribution from the spacer, which increases surfactant tendency to self-assemble (Menger and Keiper, 2000), contribute to enhance the formation of stable gemini surfactant-DNA complexes (Karlsson et al., 2002; Bombelli et al., 2005b), when compared to the monomeric counterparts of the gemini. The length and corresponding conformational flexibility of the spacer (Luciani et al., 2007), and the hydrophobicity of the two main hydrocarbon chains (Wang et al., 2007), influence the type of self-assembled structures formed *a priori*, as well as their complexation with DNA. Thus, gemini surfactants present a rich mesomorphism in aqueous solution, with the ability to form inter alia non-lamellar structures (Zana, 2002b; In and Zana, 2007), regarded as an important feature for transfection competence (Zuhorn et al., 2002; Wasungu et al., 2006; Koynova et al., 2006). In this context, cationic gemini surfactants exhibit suitable features for the design of promising gene delivery systems.

In several cases, however, gemini surfactants were found to be unable to efficiently mediate gene delivery *per se*, benefiting from the addition of other components, such as helper lipids, to perform this task (Badea et al., 2005). The lipid 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) has been reported to enhance transfection efficiency (Hui et al., 1996; Cardoso et al., 2011) by facilitating the formation of non-bilayer structures (Siegel and Epand, 1997), and the enhancing effect of cholesterol on transfection has been assigned to its ability tostabilize a fluid, yet ordered, lamellar phase (liquid-ordered phase, Lo) (Feigenson, 2006), which may guide a lipid/DNA arrangement that combines stability with lability, two essential features of an efficient gene delivery system. The incorporation of helper lipids in the surfactant-based system has the potential to promote structural alterations and increase the susceptibility for DNA exposure in the presence of model membranes containing anionic lipids (Cardoso et al., 2011). On the other hand, in complexes formulated with three components – gemini surfactant, helper lipids, and DNA – the order of component addition is decisive to the achievement of the maximal performance of the systems (Badea et al., 2005), as has been described for other types of gene delivery vectors, such as those formulated with cationic lipids (Penacho et al., 2008) or cell-penetrating peptides (Trabulo et al., 2008; Cardoso et al., 2013).

In the present study, the main purpose is to unveil the properties of gemini surfactant-based delivery systems that promote high transfection efficiency, in parallel with low cytotoxicity, with the ultimate goal of paving the way to a more rational design of DNA delivery systems. In a previous work (Cardoso et al., 2011), a gemini surfactant of the alkanediyl-α,ω-bis (alkyldimethylammonium bromide) family, or *m–s–m* (Fig. 1), containing 14 carbon atoms-long hydrocarbon chains and a two carbon atom-long spacer (14–2–14) showed that, in the presence of helper lipids, was able to protect DNA from degradation and to efficiently transfect cultured mouse mammary adenocarcinoma cells. In the present work, we aimed at further investigating this approach, by broadening the study to five bis-quaternary gemini surfactants of the *m–s–m* family differing in the length of their spacer or main hydrocarbon chains. These surfactants were used to assess their ability to mediate transfection, either *per se* or in a mixture with the helper lipids DOPE and cholesterol, kept at constant molar ratio and hence treated here as single helper lipid component. Efforts have, thus, been made to unveil the properties that underlie the most suitable gene delivery systems. With this purpose, an extensive characterization of gemini-based complexes was performed in terms of their physicochemical properties (size, surface charge, and colloidal stability), surfactant/lipid mesomorphic behavior, and protection conferred to the carried nucleic acids. Complex-membrane interaction, membrane association/binding, and cellular internalization, regarded as major events for an effective gene delivery, were also addressed.

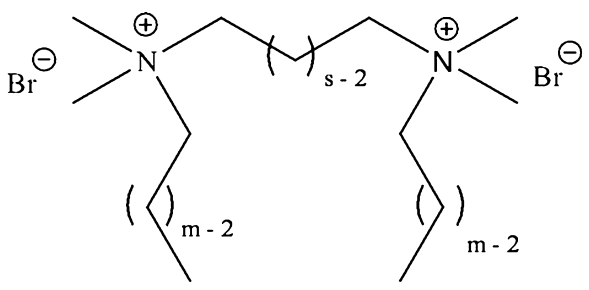

Fig. 1. Schematic representation of the general structure of the bis-quaternary gemini surfactants used in this work (*s* = 2, 5, or 10 and *m* = 12, 14, or 16).

2. Materials and methods

2.1. Materials

The gemini surfactants were synthesized by the method reported by Menger (Menger and Littau, 1993) and purified by recrystallization. The purity of the compounds was evaluated by NMR and mass spectrometry and further confirmed by the cmc values, obtained by surface tension measurements, which were all in very good agreement with those already reported in the literature (Burrows et al., 2007; Zana, 2002a). The lipids 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), cholesterol, 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), and 1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-L-serine (POPS) were purchased from Avanti Polar Lipids (Alabaster, AL). All the other chemicals were of the highest grade.

2.2. Cells

HeLa cells (human epithelial cervical carcinoma cell line) were maintained in culture at 37 °C, under 5% CO_2, in Dulbecco's modified Eagle's medium–high glucose (DMEM–HG; Sigma, St. Louis, MO, USA), supplemented with 10% (v/v) heat inactivated fetal bovine serum (FBS; Sigma, St. Louis, MO, USA), penicillin (100 U/ml) and streptomycin (100 μg/ml). The cells were grown in monolayer and detached by treatment with 0.25% trypsin solution (Sigma, St. Louis, MO, USA).

2.3. Complex preparation

Plain complexes of surfactant/DNA or ternary complexes of surfactant/helper lipid/DNA were prepared using two different methods (A and B). Method A was previously reported (Cardoso et al., 2011) and consisted of dissolving gemini surfactants or their mixture with DOPE and cholesterol in chloroform, at the desired molar ratios (3:2:1 and 3:4:2). Solutions were then dried under vacuum in a rotatory evaporator, and the resulting lipid films were hydrated with deionised water to a final lipid concentration of 2 mM. Surfactant or surfactant plus lipid dispersions were then sonicated for 3 min, extruded 21 times through two stacked polycarbonate filters of 50 nm pore diameter, using a Liposofast device (Avestin, Toronto, Canada), and after a three-fold dilution with deionised water, they were filter-sterilized utilizing 0.22 μm pore-diameter filters (Schleicher & Schuell, BioScience, Germany). Plain complexes