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Research paper

New serine-derived gemini surfactants as gene delivery systems

Ana M. Cardoso [a,b], Catarina M. Morais [a,b], A. Rita Cruz [a,c], Sandra G. Silva [d], M. Luísa do Vale [d], Eduardo F. Marques [d], Maria C. Pedroso de Lima [a,b], Amália S. Jurado [a,b,*]

[a] CNC – Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal
[b] Department of Life Sciences, University of Coimbra, Coimbra, Portugal
[c] Faculty of Pharmacy, University of Coimbra, Coimbra, Portugal
[d] Centro de Investigação em Química, Department of Chemistry and Biochemistry, University of Porto, Porto, Portugal

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ABSTRACT

Gemini surfactants have been extensively used for *in vitro* gene delivery. Amino acid-derived gemini surfactants combine the special aggregation properties characteristic of the gemini surfactants with high biocompatibility and biodegradability. In this work, novel serine-derived gemini surfactants, differing in alkyl chain lengths and in the linker group bridging the spacer to the headgroups (amine, amide and ester), were evaluated for their ability to mediate gene delivery either *per se* or in combination with helper lipids. Gemini surfactant-based DNA complexes were characterized in terms of hydrodynamic diameter, surface charge, stability in aqueous buffer and ability to protect DNA. Efficient formulations, able to transfect up to 50% of the cells without causing toxicity, were found at very low surfactant/DNA charge ratios (1/1–2/1). The most efficient complexes presented sizes suitable for intravenous administration and negative surface charge, a feature known to preclude potentially adverse interactions with serum components. This work brings forward a new family of gemini surfactants with great potential as gene delivery systems.

1. Introduction

New biocompatible materials, such as gemini surfactants mimicking natural amphiphilic structures, featured by low toxicity and high biodegradability and thus overcoming environmental concerns and suiting pharmaceutical applications, have recently emerged [1]. Gemini surfactants composed of two sets of an ionic group and an hydrocarbon chain, linked by a spacer at the level of or close to the head group [2], have shown to exhibit special surface and aggregation properties, which allow a decrease of the concentration needed for a therapeutic effect and thus minimize their potential toxicity [3–5]. The inclusion of aminoacid moieties in these structures has shown to influence their intermolecular behavior, due to the chiral centers that allow the formation of aggregates with a variety of morphologies [5–7], and to enhance their biodegradability and thus their safe use in biological applications [8,9]. The presence of various functional groups in the amino acid offers the possibility of a great structural variability in the resulting surfactants, which can be finely tuned in order to obtain an effective control of their aggregation properties and biological activity. This approach may be of utmost relevance in biomedical applications, for example to modulate pH sensitivity of surfactant-containing formulations, through the introduction of amide/ester bonds, which may be employed in gene delivery to promote DNA cytoplasmic release in response to the low pH of the endosomal compartment.

Serine-derived gemini surfactants have been recently synthesized for the first time [5]. These surfactants, composed by two N-alkylated serine amino acid residues, linked to each other through a spacer, bear one positive charge at the amine functional group of each serine residue. This new family of serine-derived gemini surfactants comprises three different series of compounds, designated, according to the nature of the spacer linkage to the headgroup, as the amine series (Fig. 1A), the ester and the amide series (Fig. 1B). For the amine series, compounds with different alkyl chain lengths were prepared. The impact of this new family of gemini surfactants on the viability of HeLa cells has been studied aiming at their application in the pharmaceutical field [10]. With respect to their monomeric counterparts as well as to conventional *bis-quaternary* gemini surfactants with similar hydrocarbon chains and spacer length, serine derivatives show the advantage of exerting lower cytotoxic effects.

* Corresponding author. Department of Life Sciences, Faculty of Sciences and Technology, University of Coimbra, Calçada Martim de Freitas, 3000-456 Coimbra, Portugal. Tel.: +351 239 853 600; fax: +351 239 853 607.
E-mail address: asjurado@bioq.uc.pt (A.S. Jurado).

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In the present work, six members of this new family of serine-derived gemini surfactants were studied toward their application as gene delivery systems in gene therapy. For this purpose, serine-derived gemini surfactants were complexed with a reporter gene and evaluated for their capacity to mediate transfection, while inducing low cytotoxicity. Complexes were characterized in terms of physico-chemical properties relevant for their *in vivo* application, such as their hydrodynamic diameter, surface charge and stability in an aqueous buffer. Furthermore, the ability of the complexes to condense and protect DNA was assessed using a DNA-intercalating agent, as an approach to predict the degree of DNA protection from nuclease degradation. However, in order to promote an efficient gene delivery, the complexes should be able to dissociate upon interaction with cell membrane structures. In this context, membrane-mimicking lipid models were used to evaluate complex cell interactions. In an attempt to unravel the intracellular traffic of the complexes (leading to the endolysosomal degradative route or bypassing this obstacle), the cellular internalization pathways used for the uptake of the complexes able to efficiently mediate transfection were also evaluated.

2. Materials and methods

2.1. Materials

The gemini surfactants were synthesized by the method reported by Silva et al. [5] and purified by column chromatography. The purity of the compounds was assessed 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 [5]. 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.

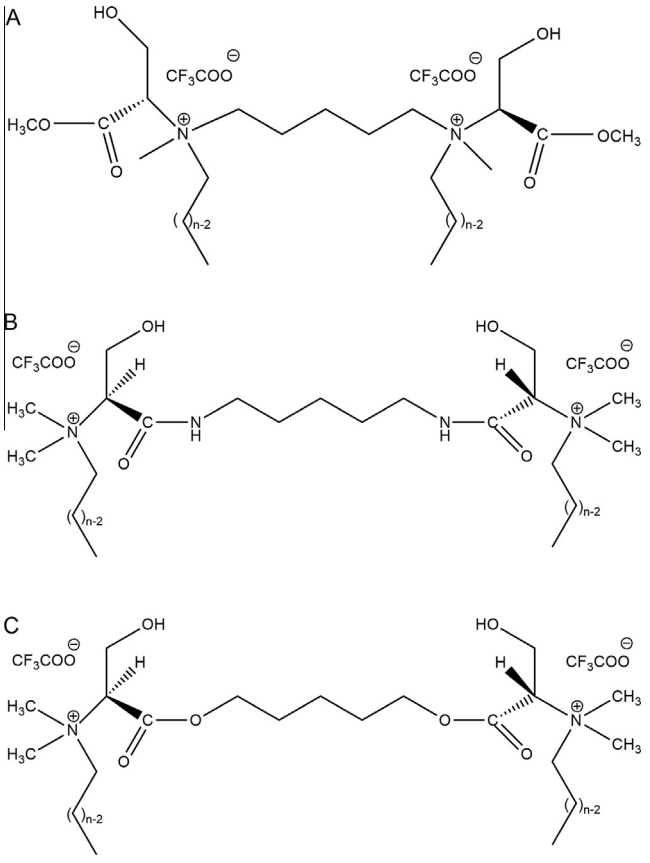

Fig. 1. Schematic representation of serine-derived gemini surfactants: (A) amine series, herein designated $(n\text{Ser})_2\text{N}m$, (m = 5 and n = 12, 14, 16 and 18); (B) amide, $(12\text{Ser})_2\text{CON5}$, and ester, $(12\text{Ser})_2\text{COO5}$, series ($n$ = 12). All the surfactants contain fully saturated alkyl chains.

2.2. Preparation of DNA complexes

Complexes were prepared using the method described by Badea et al. [11] with a few modifications [12]. Aqueous solutions (0.5 mM) of gemini surfactants were filtered through 0.22 μm pore-diameter filters (Schleicher & Schuell, BioScience, Germany). Plain complexes were prepared by mixing 100 μL of HBS containing 0.5 μg of pEGFP-C1 plasmid DNA encoding GFP with aliquots of the aqueous gemini surfactant solution, to obtain a gemini/DNA charge ratios in the range of 1/1–8/1 thereafter incubated at room temperature for 15 min. To produce helper lipid/surfactant/DNA complexes, aliquots of DOPE:Chol vesicles (see below) were added to surfactant/DNA complexes to obtain surfactant/helper lipids molar ratios in the range of 1/1–1/4, followed by 30 min incubation at room temperature. The DOPE-Chol vesicles were prepared as follows. A solution of 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) and cholesterol (Chol) (Avanti Polar Lipids, Alabaster, AL, USA) in chloroform was dried under vacuum in a rotatory evaporator, the resulting lipid films being hydrated with HBS (pH 9.0) to a final lipid concentration of 0.5 mM. The resulting multilamellar vesicles (MLV) of DOPE plus cholesterol were then sonicated for 3 min and filtered through 0.22 μm pore-diameter filters (Schleicher & Schuell, BioScience, Germany).

2.3. 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).

2.4. Cell viability

Cell viability was assessed by a modified Alamar Blue assay [13]. This assay takes into account the redox capacity of cells and measures the extent of the produced metabolites (resorufin) as a result of cell growth [14]. Briefly, 48 h after transfection 0.3 mL of 10% (v/v) resazurin dye in complete DMEM-HG medium was added to each well. After 45 min of incubation at 37 °C, 150 μL of the supernatant was collected from each well and transferred to 96-well plates. The absorbance at 570 and 600 nm (information provided by the supplier) was measured in a SPECTRAmax PLUS 384 spectrophotometer (Molecular Devices, Union City, CA) and cell viability was calculated according to the equation:

$$\text{Cell viability } (\%\text{ of control}) = [(A_{570} - A_{600})/(A'_{570} - A'_{600})] \times 100$$

where A_{570} and A_{600} are the absorbances of the samples, and A'_{570} and A'_{600} are those of the control (non-treated cells), at the indicated wavelengths.