Thermochimica Acta 590 (2014) 219–225

Contents lists available at ScienceDirect

Thermochimica Acta

journal homepage: www.elsevier.com/locate/tca

Effect of biocompatible gluconamide-type cationic surfactants on thermotropic phase behavior of phosphatidylcholine/cholesterol bilayers

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ARTICLE INFO

Article history:
Received 27 May 2014
Received in revised form 4 July 2014
Accepted 6 July 2014
Available online 8 July 2014

Keywords:
Saccharide-derived cationic surfactants
Membrane phase transition by DSC
DPPC/cholesterol membrane
Cytotoxic activity
Computational modeling

ABSTRACT

A series of soft cationic surfactants, containing a saccharide-derived moiety in their hydrophilic grouping – 2-(alkyldimethylammonio) ethylgluconamide bromides, denoted as C_nGAB, n = 10, 12, 14 and 16 – were analyzed with respect to their influence on the thermotropic phase behavior of phosphatidylcholine (DPPC)/cholesterol bilayers. The compounds were found to be uniformly distributed in the studied lipid bilayer. As usual, the longer the chain of C_nGAB, the stronger was the effect on T_m and ΔH_m, except C_{16}GAB. Having the longest chain, C_{16}GAB reduces T_m to the smallest extent and does not affect ΔH_m until the molar ratio of surfactant/DPPC is 0.15. The interactions with model cells in vitro were determined by cytotoxicity evaluation with respect to two cell lines – mouse fibroblast cell line L929 and human lung cancer cell line A549. The toxicity of C_nGABs is much lower than a classical DTAB.

1. Introduction

There is currently a growing interest in a variety of functionalized surfactants produced by clean and sustainable technologies from natural resources, not only to improve the quality of life, but also to obtain products as a result of modern technological demands with specific properties for targeted biomedical applications in the area of engineering nanostructures such as biosensors and functional bioactive surfaces, gene- and drug-delivery carriers [1–3]. It should be emphasized that the pathway to surface-active products with desired physicochemical properties and application potential involves the pursuit to obtain surfactants with more daring structural variations in their molecular structures, in which even slight structural modifications with respect to conventional linear surfactants allow one to create a wide spectrum of new functionalities [4–6]. One of the most profound examples is the incorporation of carbohydrate-based species into the polar building blocks of amphiphilic molecules, leading to the creation of new, non-toxic, and custom-designed derivatives [7–9]. The grafting of the saccharide-derived moiety into the hydrophilic part of the surfactant structure provides opportunities to achieve biocompatible products with added new capabilities related to their tunable molecular geometry and aggregation potential at the interfaces. Among many known such soft surfactants, functionalized quaternary ammonium salts, comprising the sugar-derived entity in their hydrophilic grouping, form a very interesting group of studied compounds and their popularity keeps growing [5,9–12]. They mostly exhibit lower toxicity, higher biodegradability and environmental compatibility, and they can be considered as one of the most promising candidates for gene delivery. Additionally, like other cationic surfactants they have the ability to bind and compact DNA molecules and the amenable compact state of DNA enables its delivery to cells [2,11,13,14].

An attempt to develop new gluconamide-type cationic surfactants – 2-(alkyldimethylammonio) ethylgluconamide bromides (abbreviated as C_nGAB; n = 10, 12, 14, 16) – interacting with DNA and lipid membranes, along with determination of their thermodynamic properties in aqueous solution, was recently made by our research group [15,16]. These compounds were found to be able to

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http://dx.doi.org/10.1016/j.tca.2014.07.005
0040-6031/

bind and compact DNA. In particular, C_{16}GAB performed this action at concentrations as low as 1:1 surfactant-to-nucleotide ratio, and only very slightly disturbed the 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylcholine (DPPC) lipid membrane structure. It was suggested that C_{16}GAB is a promising candidate for the main component of gene-delivery carrier systems. As a continuation of our studies on soft structures and their potential for possible biomedical applications we describe here the influence of the C_nGAB surfactants on thermotropic phase behavior of phosphatidylcholine (DPPC)/cholesterol bilayers and their toxicity with respect to living cells. The chemical structure of all studied C_nGAB compounds (n = 10, 12, 14, 16), DPPC and cholesterol is shown in Fig. 1.

It should be recalled that cholesterol is a major and essential component of the plasma membrane in many higher organisms [17]. Therefore, DPPC/cholesterol systems resemble the biological environment more than DPPC liposomes without cholesterol. Besides, the DNA transfection efficiency is increased when cholesterol is solubilized in cationic liposomes [18]. As a consequence, we performed our studies with DPPC/cholesterol bilayers containing 10 mol% of cholesterol. This content seems to be especially interesting. At cholesterol content of approximately 1–2 mol% to 20–25 mol%, the main transition of DPPC/cholesterol can be considered as consisting of superimposed sharp and broad components [19]. The sharp component corresponds to the transition in cholesterol-poor domains and the broad component to the melting of cholesterol-rich domains. Cholesterol-rich domains in DPPC/cholesterol systems can be treated as a model for rafts in natural membranes [20].

To test biological interactions of C_nGAB with model cells in vitro, we performed the cytotoxicity evaluation of C_nGAB with respect to two cell lines – mouse fibroblast cell line L929 and human lung cancer cell line A549 – and compared the obtained findings with the cytotoxicity of the commercial 12-carbon surfactant dodecyltrimethylammonium bromide (DTAB). Low toxicity of a molecule toward animal cell lines is crucial for noble applications such as gene delivery. Besides, the cytotoxicity of C_nGAB is also very interesting from a theoretical point of view. Comparing toxicity of C_{12}GAB with DTAB, a compound with the same alkyl chain, the effect of the gluconamide moiety, as a part of the hydrophilic moiety, on the cytotoxicity can be evaluated. In this way the paper may contribute to understanding of the structure-toxicity relationship.

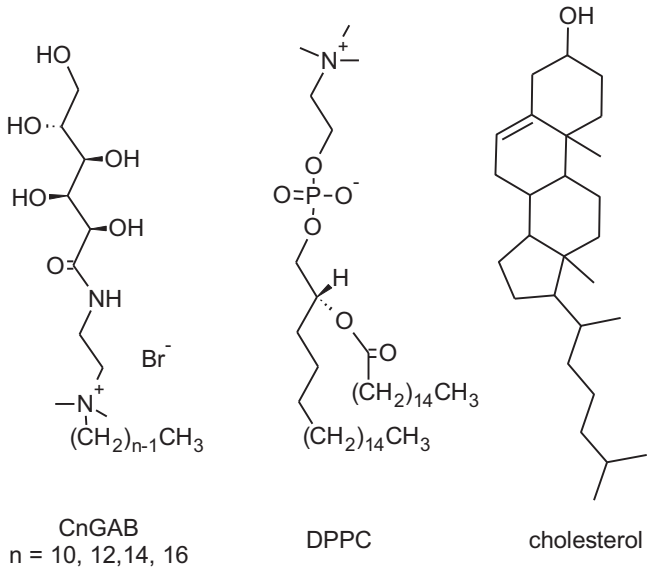

Fig. 1. Chemical structure of the compounds C_nGAB (n = 10, 12, 14, 16), DPPC and cholesterol.

2. Materials and methods

All the starting materials and solvents were of commercial grade and were not additionally purified before use. The gluconamide surfactants, i.e. 2-(alkyldimethylammonio) ethylgluconamide bromides C_nGAB (n = 10, 12, 14, 16), were synthesized using a method described in our previous paper [16] (Fig. 1 for the structures and abbreviations). Dodecyltrimethylammonium bromide (DTAB) was purchased from Sigma–Aldrich Chemie (Germany). The water used in all the experiments was doubly distilled and purified using the Millipore (Bedford, MA) Milli-Q purification system. 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylcholine (DPPC) and cholesterol were purchased from Sigma–Aldrich, Steinheim, Germany. Lipid purity was greater than 99%. Dulbecco's modified Eagle's medium essential medium (DMEM) and Eagle's minimum essential medium (EMEM) were from Cytogen, Germany, and MTT-93-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide was from Sigma Chemicals Co., USA. Fetal calf serum was from Gibco, Grand Island, NY, USA. Sodium dodecyl sulfate (SDS), 2 mM l-glutamine and 1.0 mM sodium pyruvate were all purchased from Sigma–Aldrich, Germany. All culture medium was supplemented with penicillin and streptomycin from Polfa Tarchomin S.A., Poland.

2.1. Sample preparation for DSC

Samples for differential scanning calorimetry (DSC) were prepared on multilamellar vesicles (MLVs). A mixture of DPPC with cholesterol dissolved in chloroform was evaporated. Traces of chloroform were removed with a stream of dry nitrogen under vacuum. The lipid film was then dispersed by adding water solutions of the surfactants of appropriate concentrations. The suspension was intensively mixed at 60 °C for 15 min, loaded into a crucible and incubated at 5 °C for 5 days. DSC studies were performed according to the protocol described earlier [21–23] using the Mettler Toledo Thermal Analysis System D.S.C. 821e. The cycles were performed three times. The experimental error in temperature was ±0.2 °C.

2.2. Biological evaluation

2.2.1. Cell lines

A549 cells (American Type Culture Collection Certified Cell Line–ATCC CCL 185), a human adenocarcinoma lung cells line, were maintained in DMEM supplemented with 10% fetal calf serum, antibiotics (100 U/ml penicillin and 100 μg/ml streptomycin), L-glutamine, sodium pyruvate, and 2-mercaptoethanol.

L929 cells (ATCC CCL1), a murine fibroblast-like cell line, were maintained in EMEM supplemented with 10% fetal calf serum, antibiotics (100 U/ml penicillin and 100 μg/ml streptomycin), L-glutamine, sodium pyruvate, and 2-mercaptoethanol.

All lines were obtained from ATCC (Rockville, Maryland, USA) and are being maintained at the Institute of Immunology and Experimental Therapy, Wrocław, Poland.

2.2.2. Cytotoxicity assay in vitro

Cytotoxicity was investigated in vitro according to the Polish standard (PN-EN ISO 10993-5; 2009), where the human lung cancer A549 cell line and mouse fibroblast L929 were used. Cells were exposed to substances for 72 h, in concentrations of 1.90–250.00 μg/ml. Cell growth, cell morphology and cell viability were examined as the parameters to determine the