Available online at www.sciencedirect.com
SCIENCE DIRECT®
Colloids and Surfaces B: Biointerfaces 42 (2005) 59–68
COLLOIDS AND SURFACES B
www.elsevier.com/locate/colsurfb

Interaction of gemini surfactants butane-1,4-diyl-bis (alkyldimethylammonium bromide) with DNA

Daniela Uhríková [a,*], Igor Zajac [a], Martina Dubničková [a], Martin Pisárčik [a], Sérgio S. Funari [b], Gert Rapp [c], Pavol Balgavý [a]

[a] *Faculty of Pharmacy, Comenius University, Odbojárov 10, 832 32 Bratislava, Slovak Republic*
[b] *Max-Planck-Institute of Colloids and Interfaces, c/o HASYLAB, DESY, D-22603 Hamburg, Germany*
[c] *Rapp OptoElectronic GmbH, Gehlenkamp 9a, D-22559 Hamburg, Germany*

Received 10 November 2004; received in revised form 21 January 2005; accepted 3 February 2005

Abstract

The size and structure of aggregates formed by interaction of DNA with homologous series of cationic gemini surfactants butane-1,4-diyl-bis(alkyldimethylammonium bromide) (C*n*GS, *n* = 10–16 is the number of alkyl carbons) were investigated using UV–vis turbidity, dynamic light scattering and small-angle synchrotron X-ray (SAX) diffraction. The detailed analysis of turbidity in the range of λ = 450–600 nm indicates an anomaly in the growth of C*n*GS + DNA aggregates with increasing concentration of C*n*GS, possibly involving changes of structure and size of aggregates. Using dynamic light scattering, changes of the effective diameter of C*n*GS + DNA (*n* = 12 and 16) aggregates formed in the C*n*GS concentration range 0.002–0.140 mmol/l were observed. SAX diffractograms show the presence of long-range organization of C*n*GS + DNA (*n* = 12, 13, 14 and 16) aggregates due to DNA interaction with C*n*GS above the critical micellar concentration. The C*n*GS + DNA (*n* = 12, 13 and 14) aggregates at 25 °C are packed in a lattice of two-dimensional hexagonal symmetry. With increasing C14GS:DNA molar ratio the changes of the lattice parameter in the range of 4.80–5.27 nm are observed at 25 °C. The aggregates undergo structural changes induced by temperature in the range 60–95 °C, which are accompanied by changes of the diffraction patterns, namely in the region of reciprocal spacing $s = 0.15–0.30\ \mathrm{nm}^{-1}$.

Keywords: Gemini surfactant; DNA; Turbidity; Dynamic light scattering; Small-angle X-ray diffraction

1. Introduction

The importance of the study of aggregate formation between DNA and cationic surfactants become evident recently due to growing interest in the use of cationic lipids [1–3], and particularly quaternary ammonium surfactants [4–7] for the construction of liposomal genetic delivery systems. The basic requirements for effective transfection vectors are the ability to compact DNA, in order to protect it against degradation and to deliver it to the cell membrane with efficiency and specificity and, finally, to facilitate the DNA transport through the cell membrane [8]. Three types of condensed organized microstructures of cationic surfactant/lipid/DNA systems were identified [9–11] and the relationship structure of aggregate-transfection activity is intensively investigated for different novel synthesized cationic lipid molecules [12,13]. The mechanism of interaction of DNA with cationic surfactants, the physicochemical characteristics of the resulting surfactant–DNA aggregates [14–18], and also the relationship between their properties and transfection efficiency [19–22] are also intensively studied with the aim to obtain a detailed knowledge of the process of aggregate growth and the possibility to find an effective DNA delivery system with minimal toxicity. Hayakawa et al. [23] reported the binding isotherms of *N*-alkyl-*N*,*N*,*N*-trimethylammonium bromides with DNA and suggested that this type of cationic surfactants binds to the negatively charged DNA molecule in a cooperative manner. This phenomenon is analogous to the binding of sur-

* Corresponding author. Tel.: +421 2 50117 289; fax: +421 2 50117 100.
E-mail address: daniela.uhrikova@fpharm.uniba.sk (D. Uhríková).

0927-7765/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.colsurfb.2005.02.002

factants to synthetic polymers [24,25]. Interactions between ionic surfactants and polyions with opposite charge lead to the formation of polymer–surfactant aggregates, in which the polyelectrolyte chain binds with surfactant molecules through Coulomb attractive interaction, and the hydrophobic moieties of the surfactant molecules stabilize the aggregates due to the hydrophobic interactions in aqueous solution. Some theoretical work has been done by Kuhn et al. [26–28] in formulating a theory for polyelectrolyte-ionic-surfactant solutions based on electrostatics and hydrophobicity. The structure of the aggregates depends on the length of polymer fragments, on the size of both the polar head and the hydrophobic part of the surfactant, on the concentrations and molar ratio of both components and on ionic strength, pH of solution, etc. The different structures of DNA–cationic surfactant aggregates were observed with increasing concentration of cationic surfactant in DNA solution. At very low concentrations of positively charged surfactant, i.e. circa two orders of magnitude smaller than the critical micellar concentration (cmc) of individual surfactant in polyelectrolyte-free solution, isolated DNA chains undergo a discrete coil–globule transition as it was shown by Mel'nikov et al. [29–31]. The presence of a structure with long-range organization of surfactant molecules was identified by X-ray diffraction experiments when DNA interacts with surfactants above their cmc. A hexagonal symmetry of DNA–cationic surfactant aggregates packing was reported [31–34]. The lamellar structure of aggregates formed due to DNA interaction with cationic surfactants [6,31] and also due to DNA interaction with positively charged catanionic vesicles [15] has been observed. Dynamic light scattering experiments [35] have shown that the size of DNA–cationic surfactant aggregates is smaller then the size of DNA–cationic liposome aggregates which increases their chance for successful transport of aggregate across the cell membrane.

The gemini bis(quaternary ammonium) surfactants in which two cationic surfactant moieties are connected by a polymethylene chain, referred to as a spacer, are attracting increasing interest. Results of DSC, polarizing optical microscopy, X-ray diffraction and neutron scattering experiments confirmed their ability to form lyotropic mesophases with long-range organization at higher concentrations [36,37]. These surfactants show a very powerful bactericidal activity [38,39], much larger than that of conventional monoquaternary ammonium surfactants. Several papers deal with the correlation between structure, activity and critical micellar concentration of gemini surfactants [40,41], their interactions with lipid membrane [42–47], their ability to inhibit photosynthetic activity [48], and to eliminate plasmids from bacterial host cells [49]. Their aggregates with DNA were tested for transformation [50,51] and transfection [5,7,52].

In the present paper, we study the interaction of homologous series of butane-1,4-diyl-bis(alkyldimethylammonium bromide) (C*n*GS, $n = 10$–16, where n is the number of carbons in the alkyl chain) with DNA from calf thymus. Preliminary experiments have shown, that these gemini surfactants interact cooperatively with DNA forming aggregates, seen by light scattering [42]. The addition of NaCl to the preformed C*n*GS–DNA aggregates leads to their dissociation; the stability of aggregates towards NaCl dissociation depends critically on the alkyl chain length [42]. Fluorescence experiments have shown, that C*n*GS with $n \geq 12$ form supramolecular structures with DNA in a condensed state [53]. In the presence of synthetic dilauroylphosphatidylcholine (DLPC), the aggregate C12GS + DNA + DLPC forms a condensed lamellar phase with ordered DNA monolayers intercalated between lipid bilayers [54,55].

In the present paper, the interaction of C*n*GS with DNA and the formation of C*n*GS–DNA aggregates is followed by the changes in the turbidity of DNA solution with increasing concentration of C*n*GS below and above cmc. We intend to clarify the structural changes in the process of C*n*GS binding to DNA polyanion with increasing concentration of C*n*GS in solution. The growth of aggregates is followed by changes in an effective diameter of aggregates determined from dynamic light scattering experiments in the low concentration range of C*n*GS ($n = 12$ and 16). Small angle X-ray diffraction (SAXD) experiments at different temperatures were performed with the aim to determine the structure of DNA–C*n*GS aggregates formed due to the interaction of DNA with C*n*GS above cmc.

2. Materials and methods

Gemini surfactants butane-1,4-diyl-bis(alkyldimethylammonium bromide), C*n*GS ($n = 10$–16) were prepared as described in [38] and purified by manifold crystallization from a mixture of acetone and methanol. The DNA from calf thymus was purchased from Sigma, Germany. The NaCl of analytical purity was obtained from Lachema, Czech Republic.

2.1. UV–vis transmittance

DNA and C*n*GS ($n = 10$, 11, 12, 13, 14 and 16) were dissolved in 2 mmol/l NaCl stock solution, pH 5.7. Redistilled water was used for the preparation of all solutions. The interaction of C*n*GS with DNA was followed according to the changes of transmittance in the C*n*GS–DNA solution using the Hewlett-Packard 845A UV–vis spectrophotometer in the range of wavelengths 190–820 nm. Concentrations of C*n*GS increased in the range of 0–0.5 mmol/l and the DNA concentration was kept constant (0.5 mmol/l) during the experiment.

2.2. Dynamic light scattering

The samples for light scattering experiments were made up of DNA solution and C*n*GS ($n = 12$ and 16) in the concentration range from 8×10^{-6} to 1.25×10^{-4} mol/l for C12GS and from 2×10^{-6} to 1.4×10^{-4} mol/l for C16GS. Final concentration of DNA (0.5 mmol/l) was kept constant in all sam-