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Conformational changes of DNA in the presence of 12-*s*-12 gemini surfactants (*s* = 2 and 10). Role of the spacer's length in the interaction surfactant-polynucleotide

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ABSTRACT

A multifaceted study on the interaction of calf-thymus DNA with two different cationic gemini surfactants alkanediyl-α-ω-bis(dodecyldimethyl-amonium)bromide, 12-*s*-12,2Br$^-$ (with *s* = 2, G2, and 10, G10) was carried out. The measurements were done at different molar ratios *X* = [surfactant]/[DNA]. Results show two different conformational changes in DNA: a first compaction of the polynucleotide corresponding to a partial conformational (not total) change of DNA from an extended coil state to a globular state that happens at the lower molar ratio *X*. A second change corresponds to a breaking of the partial condensation, that is, the transition from the compacted state to a new more extended conformation (for the higher *X* values) different to the initial extension. According to circular dichroism spectra and dynamic light scattering measurements, this new state of DNA seems to be similar to a ψ-phase. Measurements confirm that interactions involved in the compaction are different to those previously obtained for the analog surfactant CTAB. *X* values at which the conformational changes happen depend on the length of the spacer in the surfactant along with the charge of the polar heads.

1. Introduction

Gene therapy can be defined as the treatment of a disease in which genetic material is placed inside the cells of a patient. The genetic material is driven into diseased cells to produce proteins of a therapeutic character [1]. During this procedure, modified DNA must enter a large number of cells in order to produce an effective pharmacological treatment. DNA crosses the cell membrane and adjusts its structure to tiny places of living organisms and, because of this, it has to undergo a compaction process. The collapse of extended polynucleotide chains into compact particles, containing one or few molecules, is called DNA condensation.

The study of DNA condensation *in vitro* is considered an important model system and working tool to understand what happens in the cells (*in vivo*). The condensation phenomenon can be reproduced *in vitro* in several manners. Therefore, the addition to a DNA solution of different agents such as alcohol [2,3], positively charged polypeptides [4–6], histones [7,8], nanoparticles [9,10], or multivalent ions in general [11–14], provokes conformational changes in the DNA such as compaction of the polynucleotide.

It is known that DNA is a polyelectrolyte with a high negative charge that shows a stable B-DNA conformation, under physiological conditions, as a consequence of electrostatic and hydrophobic interactions between the different residues of the polynucleotide. A structural change in its conformation uses a considerable quantity of metabolic energy. However, the condensation process occurs spontaneously upon adding species such as ions of high charge or cationic surfactants [15,16]. The structural change experienced by DNA in the presence of cationic surfactants has been studied for decades [17–20]. According to Dias et al. [21], attractive interactions between different parts of the DNA produce the compaction of the polymer by ion-ion correlation effects arising from the presence of multivalent ions, for example, leading to the formation of a nucleation center in the DNA strands that grows along the molecule chain.

Cationic surfactants form micellar structures at the surface of the nucleic acid from a surfactant concentration called critical association concentration (cac). Hydrophobic interactions among tails of the surfactant molecules provoke their self-assembly. Cationic surfactants act, therefore, as multivalent ions inducing DNA compaction.

One of the most widely studied processes is the condensation of DNA with the cationic surfactant bromide of cetyltrimethyl ammonium, CTAB [18,21–25]. Results show that, after the addition of a

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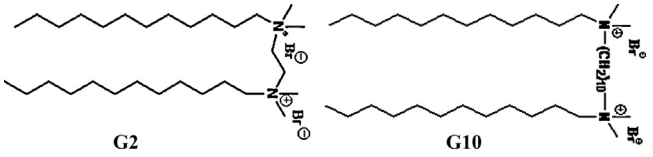

Fig. 1. Structures of the surfactants G2 (12-2-12) and G10 (12-10-12).

certain quantity of surfactant (the cac), the surfactant molecules will associate in aggregates in the polymer's surface. The interaction among the polynucleotide and the micellar aggregates, the latter acting as multivalent ions, provokes the compaction of the polynucleotide. The working conditions (temperature, ionic strength, pH, *etc.*) influence the condensation process.

An important type of surfactant is the gemini (dimeric) surfactants. It is made of two hydrophobic chains and two polar head groups covalently linked through a spacer group, which modifies and improves the properties of these amphiphilic molecules with respect to those of conventional single-chain surfactants [26–30].

Effects that the latter surfactant produces on DNA structure have been studied by different authors in the last few years [31–35]. The vast majority of their work was done by using dimeric surfactants of spacers with an odd number of methylenes. Taking into account that dimeric surfactants with odd spacers show different properties to those with even numbers [36], a thorough study of the interaction of two gemini surfactants alkanediyl-α-ω-bis(dodecyldimethyl-amonium)bromide, 12-*s*-12,2Br$^-$ (with $s = 2$, G2, and 10, G10), with calf thymus DNA was carried out at a distinct molar ratio [surfactant]/[DNA].

Results show the partial condensation of the polynucleotide. They also show that the size of the spacers influences the conformational change of the polynucleotide. It is demonstrated that kinetic measurements can be used to obtain qualitative information about structural changes in polymers.

2. Materials and methods

2.1. Materials

Sodium cacodylate and ethidium bromide were from Sigma–Aldrich. Calf thymus DNA sodium salt and sodium persulfate, purchased from Fluka, were used without further purification. Polynucleotide concentration, given by phosphate groups, was determined spectrophotometrically from molar absorptivity (6600 mol^{-1} dm^3 cm^{-1} at 260 nm) [37]. An agarose gel electrophoresis test using ethidium bromide indicated that the average number of base pairs per DNA molecule is above 10,000 bp [38]. The ratios in absorbance at 260 and 280 nm of the solutions were found to be between 1.7 and 1.8, which suggested the absence of proteins [39]. Other chemicals were of reagent grade and used without further purification.

The synthesis of the gemini surfactants G2 and G10 (see Fig. 1) were done as described in Ref. [40]. The surfactants were characterized by ^{1}H NMR, ^{13}C NMR, and elemental analysis (CITIUS, University of Seville), with the results being in agreement with those previously reported.

The complex [Fe(bpy)$_3$](ClO$_4$)$_2$ (bpy = 2,2′bipyridine, see Figure I in Supplementary data) was prepared and purified according to procedure described in the literature [41].

Measurements were done at a fixed pH using a buffer solution ([cacodylate] = 0.01 mol dm^{-3}, pH = 7) and at a fixed temperature of 298.2 K.

2.2. Methods

2.2.1. Fluorescence measurements

Intensity measurements were carried out in a Hitachi-f-2500 spectrofluorimeter interfaced to a PC for the recording and handling of the spectra. A standard quartz cell of 10 mm path length was used. Fluorescence titrations were performed at a fixed ethidium bromide probe concentration of 5×10^{-7} mol dm^{-3} and different DNA concentrations: 5×10^{-6} and 1×10^{-5} mol dm^{-3}. The surfactant quantity added to the solution depended on the polynucleotide concentration in order to study the whole range of the molar ratio X from 0 to 4 (X = [surfactant]/[DNA]). Higher X values were not done because of solubility problems in the systems. The excitation and emission wavelengths used were 480 and 590 nm, respectively.

2.2.2. Absorption spectroscopy

Absorbance spectra were performed to prove the stability of the different DNA/surfactant solutions (as well as to determine concentration of the polynucleotide, as was previously mentioned) using a CARY 500 SCAN UV-vis-NIR spectrophotomer (Varian). Data were collected every 2 nm and the spectra were recorded in the wavelength range from 800 to 200 nm. A standard quartz cell of 10 mm path length was used.

2.2.3. Viscosity measurements

Viscosity measurements were carried out using an Ostwald viscosimeter immersed in a thermostated water bath at a temperature of 298.2 ± 0.1 K. The viscosimeter was calibrated with water and ethanol. The measurements were repeated at least ten times.

The viscosity behavior of the DNA-surfactant solutions, for the two gemini surfactant studied, was characterized through the relative viscosity η_r, defined as η/η_o, that is, the ratio between the viscosity of the suspension and the viscosity of the pure solvent (water at pH = 7 with a cacodilate buffer). The relative variation of this parameter is directly related to the viscosity of the DNA solution, η_{DNA}, and of the DNA-surfactant solution, $\eta_{complex}$, as follows:

$$\frac{\Delta\eta_r}{\eta_r} = 1 - \frac{\eta_{DNA}}{\eta_{complex}} \quad (1)$$

$\Delta\eta_r$ being the difference between the relative viscosity of the DNA/surfactant solutions and the relative viscosity of DNA aqueous solution (that is, $\Delta\eta_r = \eta_{complex} - \eta_{DNA}$).

The dependence that the ratio $\Delta\eta_r/\eta_r$ shows with the molar ratio [surfactant]/[DNA] gives information about structural changes in the polynucleotide.

2.2.4. Circular dichroism spectra

Electronic circular dichroism (CD) spectra were recorded in a Biologic Mos-450 spectropolarimeter. A standard quartz cell of 10 mm path length was used. Scans were taken from 220 to 310 nm. Each spectrum was obtained from an average of 10 runs at a fixed temperature of 298.2 K with a 5 min equilibration before each scan. The spectra obtained were expressed in terms of molar ellipticity, [θ].

Spectra were performed at a fixed DNA concentration and in the presence of varying amounts of gemini surfactants in order to obtain the appropriate molar ratio X (in the range 0–3 for G2 and 0–2 for G10). The concentration of DNA used was 2×10^5 mol dm^{-3} for the two surfactants.

2.2.5. Zeta potential experiments

Zeta potential experiments were carried out with a Zetasizer Nano ZS Malvern Instrument Ltd. (UK), which measures the electrophoretic mobility of the sample from the velocity of the particles using a Laser Doppler velocimeter (LDV). A DTS1060 polycarbonate capillary cell was used at 298.2 K. Samples were prepared