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Complexation of DNA with cationic gemini surfactant in aqueous solution

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Abstract

Interactions between DNA and the cationic gemini surfactant trimethylene-1,3-bis(dodecyldimethylammonium bromide) (12-3-12) in aqueous solution have been investigated by UV–vis transmittance, zeta potential, and fluorescence emission spectrum. Complexes of DNA and gemini surfactant are observed in which the negative charges of DNA are neutralized by cationic surfactants effectively. The DNA-induced micelle-like structure of the surfactant due to the electrostatic and hydrophobic interactions is determined by the fluorescence spectrum of pyrene. It is found that the critical aggregation concentration (CAC) for DNA/12-3-12 complexes depends little on the addition of sodium bromide (NaBr) because of the counterbalance salt effect. However, at high surfactant concentration, NaBr facilitates the formation of larger DNA/surfactant aggregates. Displacement of ethidium bromide (EB) by surfactant evidently illustrates the strong cooperative binding between surfactant and DNA. In contrast to that in the absence of surfactant, the added NaBr at high surfactant concentration influences not only the binding of surfactant with DNA, but also the stability of DNA/EB complex.

Keywords: DNA; Gemini surfactant; Electrostatic interaction; Hydrophobic interaction; Fluorescence

1. Introduction

DNA is not only an important biological material with a unique double helical rodlike structure, but also an interesting anionic polyelectrolyte. Numerous studies reveal that DNA can act as an ideal candidate for future nanodevices and also can serve as a good component for the fabrication of ordered or hierarchical DNA-based molecular assemblies [1–16]. In the past decades, the interactions between DNA and cationic surfactants have attracted immense interest in the separation and purification of DNA [17]. Recently it has been shown that cationic surfactants can induce DNA to cross the cell or nuclear membrane and sequentially interact with the target. This is closely relevant to gene therapy, in which an inherited disease is treated by introducing a correct duplicate of the defective gene into the cell [18]. Studies have shown that the binding of surfactant to DNA is cooperative based on the binding isotherm, and is similar to the interaction of surfactant with a synthetic polymer [19–23]. Kuhn et al. [24–26] have proposed a theoretical framework in terms of the electrostatic and hydrophobic forces in polymer/surfactant system and predicted that the aggregate structure depends on the polymer length, the size of polar head and hydrophobic tail of the surfactant, the concentrations of both components, the ionic strength, the pH, etc.

While a large number of studies have been dedicated to DNA/conventional surfactants [27–31], far less clear is the interaction of DNA with gemini surfactants [32–36]. Gemini surfactants composed of two hydrocarbon chains covalently attached by a spacer group close at head groups were first synthesized by Menger [37] and have attracted increasing interest. Compared to conventional surfactant with a single chain and a single head, gemini surfactants have unique physicochemical properties, including lower critical micelle concentration (CMC) and better wetting properties [38,39]. Gemini surfactants allow expanded structural diversity by adjustment of the length of hydrophobic chains, the polarity of head groups, the structure of spacer, and the counterions.

Among various types of gemini surfactants, the set of dicationic quaternary ammonium compounds (often expressed as *m-s-m*, where *m* and *s* denote the numbers of carbon atoms in the free alkyl chains and the spacer, respectively) have been

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probably the most widely studied. The spacer length plays an important role in determining the properties of monolayers at the air–water interface formed by the DNA/gemini surfactant complex [33]. Due to the changes of hydration during the association, the counterion has a marked influence on both micellization and aggregation of gemini surfactant in the presence of DNA [36]. In addition, the interaction of DNA with dissymmetric gemini surfactants with two hydrophobic chains of different length has also been reported, which provides another way to control the aggregation process by varying the dissymmetry degree [35].

To better explore the detailed interaction mechanism between DNA and gemini surfactants, in this work we have investigated the complexation of DNA with the cationic gemini surfactant trimethylene-1,3-bis(dodecyldimethylammonium bromide) $C_{12}H_{25}N\ (CH_3)_2–(CH_2)_3–(CH_3)_2NC_{12}H_{25}\cdot 2Br$ (12-3-12) in aqueous solutions. Fluorescence emission spectroscopy using two different probes and UV–vis transmittance were measured. The zeta potential change of DNA/surfactant complex induced by increasing surfactant concentration was also determined. The effect of ionic strength has been examined by changing the concentration of NaBr. To compare with conventional surfactants, we also carried out similar experiments using DTAB to replace gemini surfactant.

2. Materials and methods

2.1. Materials

Gemini surfactant 12-3-12 was prepared as described in Ref. [40]. *n*-Dodecyltrimethylammonium bromide (DTAB) (Alfa Aesar, 97%) was purchased and used without further purification. Salmon sperm DNA was purchased from Sigma, and the concentration of DNA phosphate groups in the solution was determined by UV absorbance at 260 nm; the corresponding molar extinction coefficient was 6600 $M^{-1}\,cm^{-1}$. Pyrene and ethidium bromide (EB) were obtained from Aldrich Chemicals and Sigma, respectively. The AR NaBr was received without further treatment before use. Redistilled water was used for preparation of all solutions.

2.2. Methods

2.2.1. UV–vis transmittance

DNA and gemini surfactant were dissolved in NaBr stock solutions at 0.01, 0.05, and 0.1 mol/L and then mixed to obtain various DNA/surfactant samples with constant DNA concentration and pH of 6.0. Transmittance of the mixed solutions was measured using a UV spectrophotometer (UV-2450, Shimadzu) at room temperature. All measurements were made in a quartz cuvette (1 cm in width) in the range of wavelengths 260–700 nm.

2.2.2. Zeta potential

Nano-ZS (Malvern) using laser Doppler velocimetry and phase analysis light scattering was used for zeta potential measurement. The temperature of the scattering cell was controlled at 25 °C. A light scattering angle of 17° was combined with the reference beam, and the data were analyzed with the software supplied for the instrument. The samples were prepared like those for transmittance.

2.2.3. Measurement of micropolarity

Samples for fluorescence spectroscopy measurements were prepared like those for transmittance except that the solvent was pyrene stock solution, which was obtained by dissolving pyrene in pure hot water up to saturation, then cooling until at room temperature, and finally filtering. The fluorescence spectrum of the mixed solution was recorded by an F4500 fluorospectrophotometer (Hitachi) at room temperature, 1 hour after the samples were prepared. A typical emission spectrum ($\lambda_{EX} = 335$ nm) of the mixed solution with pyrene as a probe has five peaks at 373, 379, 384, 390, and 397 nm, and the ratio of the first to the third vibronic peak, I_1/I_3, is sensitive to the local environment (micropolarity) of pyrene [41,42].

2.2.4. Ethidium bromide (EB) exclusion

DNA solution was directly mixed with EB with a composition of [EB]/[P] = 0.25, where [P] is the molar concentration of DNA phosphate groups. This ratio corresponds to one intercalated EB molecule per two pairs of DNA bases. The maximum EB fluorescence intensity was observed at this ratio [43]. Complexes of DNA with 12-3-12 were obtained by mixing DNA/EB solution with 12-3-12 solution. The fluorescence intensity of the solutions was measured using F4500 fluorospectrophotometer (Hitachi) at room temperature. The excitation and emission wavelengths were 535 and 595 nm, respectively.

3. Results and discussion

3.1. UV–vis transmittance

Fig. 1A shows the transmittance as a function of wavelength λ for DNA/surfactant solutions with different 12-3-12 concentrations. The transmittance has a minimum in the range of 190 nm < λ < 300 nm corresponding to the absorption band of DNA (as shown in Fig. 1B), and approaches approximately 99.5% of the maximum magnitude when λ > 300 nm. With the addition of surfactant, the transmittance decreases. The values of transmittance at λ = 450 nm (T^{450}), far from the absorption band of DNA, were taken from the measured spectra to examine the surfactant effect [32]. Fig. 1C shows the surfactant concentration (C_s) dependence of T^{450} for DNA/gemini surfactant 12-3-12 and DNA/DTAB, respectively. With increasing C_s, the transmittance initially keeps almost constant and then decreases rapidly because of the formation of large DNA/surfactant aggregates. The binding of surfactant molecules to DNA and the formation of DNA/surfactant aggregates are the consequence of electrostatic and hydrophobic interactions. In this work, the pH of all the solutions is about 6, and hence DNA appears as a polyion with considerable negative charges. Therefore, positively charged surfactants bind onto the phosphate fragments of DNA due to electrostatic attraction together with strong nearest neighbors' hydrophobic interaction of surfactant alkyl chains.