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Interaction of a fluorescent cationic surfactant bearing a coumarin derivative with DNA

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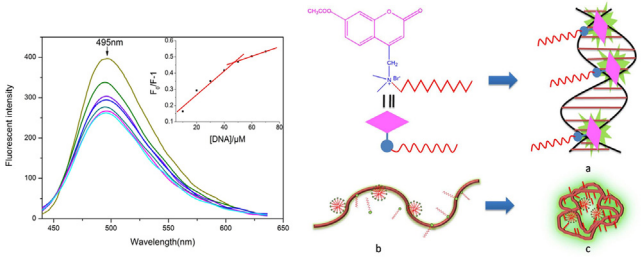
HIGHLIGHTS

- DNA condensation induced by Br-Mac-12 was identified and its mechanism is discussed.
- The stronger DNA affinity of Br-Mac-12 than DTAB shows the role of groove binding.
- Br-Mac-12 has the potential to be fluorescence marker without an extra label.

GRAPHICAL ABSTRACT



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ABSTRACT

A fluorescent cationic surfactant with a coumarin derivative (Br-Mac-12) has been designed, synthesized and characterized. The critical association concentration (CAC) and critical micelle concentration (CMC) of Br-Mac-12 was 1.0×10^{-4} and 1.6×10^{-3} mol/L, respectively. The interaction between Br-Mac-12 and DNA has been investigated using UV–vis and fluorescence spectroscopy as well as gel electrophoresis and atomic force microscopy (AFM). Both linear and plasmid DNA were condensed to nanoparticles by Br-Mac-12 at a concentration of 320 μM with incubation at 37 °C for 4 h. Br-Mac-12 has a more efficient binding capacity with DNA than the corresponding DTAB without the fluorophore. The groove binding between the coumarin derivative and DNA plays an important role in addition to the electrostatic and hydrophobic interaction for effective DNA condensation inductions. In addition, the condensation process was reversible, and the residual fluorescence of Br-Mac-12 upon binding with DNA remains at 37.5% of the initial value. The results suggest that Br-Mac-12 combined with a fluorophore has the potential for applications in tracing membrane transport in gene delivery and transfection due to its excellent fluorescence property and DNA binding affinity.

1. Introduction

The gene therapy has attracted much attention as a promising method for both genetic and acquired diseases. DNA condensation induced by a gene vector plays an important role in the across membrane transport involved in gene transfection [1,2]. The condensing agents that have been used as nonviral vectors include multivalent ions [3], cationic surfactants [4–6], cationic lipids [7], polymer [8], dendrimers [9,10], peptides [11], metal complexes [12] and nanoparticles [13].

In recent years, DNA condensation by cationic surfactants has been extensively investigated due to their multifunctional structures that include positive charges to neutralize charges on the DNA phosphate backbone and hydrophobic tails to interact with DNA bases and the cell membrane. A clear understanding of these

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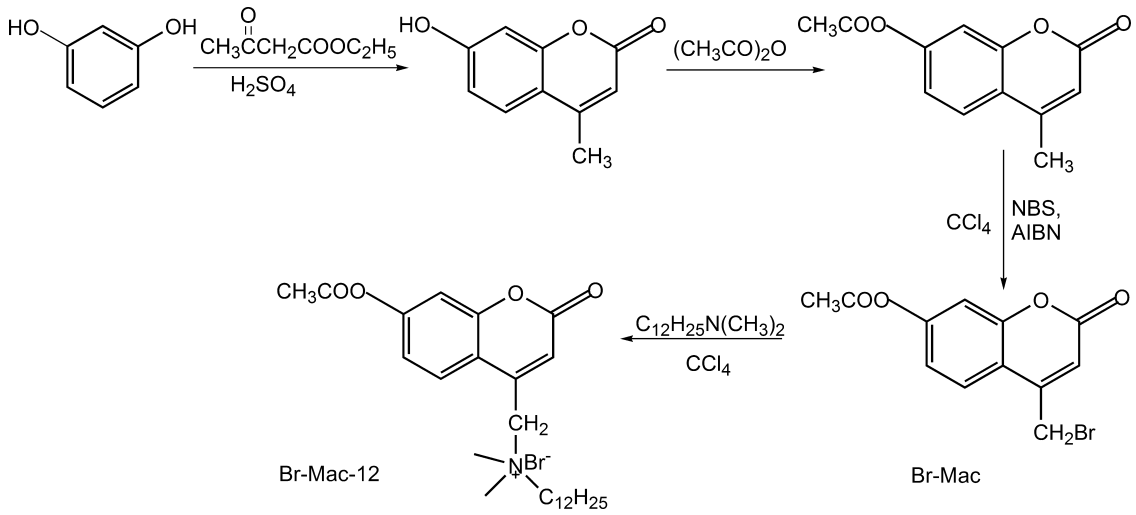

Scheme 1. Synthetic route for Br-Mac and Br-Mac-12.

driving forces is important for exploring and predicting biological application of cationic surfactants. The CTAB-DNA aggregate, which is representative of cationic surfactants/DNA system, has been widely investigated for decades due to its simple structure and low cost [14–18]. The morphology of the DTAB/DNA complex and interaction mechanism between DTAB and DNA have been studied [19,20]. These studies shed light on the application and design of novel surfactants as DNA condensation agents.

Recently, fluorescent labeling techniques have been developed as an efficient strategy for probing inter/intramolecular interactions. Many novel fluorescent probes have been constructed using diversified technologies [21–25]. For example, coumarin derivatives [26–30] have frequently been explored for the construction of fluorescent probes due to their high fluorescence quantum yields, large Stokes shifts, good photostability and cell compatibility. These derivatives may enable visualization of molecules inside living cells as new extrinsic and intrinsic fluorescent probes for biologically active molecules.

There have been many studies on the interaction between surfactants with a complicated structure and DNA. However, a surfactant with a simple architecture may be more favorable as an ideal model. Therefore, we introduce a coumarin derivative into the N terminal of a long chain of DTAB, which plays dual roles as a fluorescent label and a DNA binding site.

In the current work, we have synthesized and characterized a novel fluorescent cationic surfactant (called [Br-Mac-12]). It is anticipated that the coumarin derivative aid in stronger binding of quaternary ammonium cations bind to DNA via groove binding. The surface activity of the surfactant has been characterized. The interaction between Br-Mac-12 and DNA has been systematically investigated by UV–Vis spectroscopy, fluorescence spectroscopy, gel electrophoresis and atomic force microscopy. Because the relative fluorescence of Br-Mac-12/DNA complexes remains 37.5% of their initial value, it may be readily used to follow the interaction of Br-Mac-12/DNA complexes in cells without an extra label.

2. Experiment

2.1. Materials

Calf thymus DNA (Ct-DNA) was purchased from Sigma and used as received. The ratio of the absorbance of the DNA stock solution at 260 nm to that at 280 nm was determined to be 1.9, which indicated that the DNA solution was protein-free [5]. pUC18 DNA was purchased from the Solarbio Company. All of the solvents and reagents were of analytical grade and used as received. Ultrapure milli-Q water (18.25 MX) was used in all of the DNA condensation assays. The DNA stock solution was prepared by dissolving an appropriate amount of the solid in Tris buffer and stored at 4 °C for more than 72 h to achieve homogeneity. The concentration of the DNA solution was calculated by measuring the absorbance of the DNA solution according to Beer-Lambert equation and the molar extinction coefficient of the phosphate group at 260 nm (6600 M^{-1} cm^{-1}) [31]. The stock solutions of the surfactants were prepared by simple dissolution.

2.2. Synthetic routes

Br-Mac-12 was synthesized in four steps, as shown in Scheme 1.

2.2.1. Synthesis of 7-hydroxy-4-methyl coumarin

To a solution of resorcinol (2.75 g, 0.025 mol) in ethanol (5 mL), concentrated sulfuric acid (3 mL) was added at room temperature under vigorous stirring. After cooling, ethyl acetoacetate (3.2 mL, 0.025 mol) was added and the mixture was refluxed at room temperature for 2 h. Then ice water (25 mL) was added to ensure complete precipitation. The product was filtered, dried and recrystallized with ethanol to yield 7-hydroxy-4-methyl coumarin as a white acicular crystal. Yield 61.0%.

2.2.2. Synthesis of 4-methyl-7-acetoxy coumarin

7-Hydroxy-4-methyl coumarin (2 g, 0.012 mol) was dissolved in an aqueous solution of NaOH (8 mL, 10%), and then, 4 g of crushed ice and acetic anhydride (2.45 g, 0.024 mol) were added. The reaction proceeded for 10 min at room temperature. The mixture was filtered and recrystallized three times with ethanol to afford 4-methyl-7-acetoxy coumarin as a white crystal with a melting point of 150–152 °C. Yield 73.0%.

2.2.3. Synthesis of 4-(bromomethyl)-7-acetoxycoumarin (Br-Mac)

To a solution of 4-methyl-7-acetoxy coumarin (1 g, 0.00336 mol) in CCl_4 (70 mL), NBS (4.5 g, 0.025 mol) and AIBN (0.1 g) were added. The reaction was heated to reflux for 20 h followed by cooling to room temperature. The solvent was removed under reduced pressure, and the residue was washed with distilled water, filtered, dried and recrystallized with ethyl acetate and cyclohexane