Controlled compaction and decompaction of DNA by zwitterionic surfactants

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Abstract

Controlled compaction and decompaction of DNA by zwitterionic surfactants, alkyldimethylamine oxides ($C_n\text{DMAO}$, $n = 10, 12, \text{ and } 14$), were investigated by various analytical tools. It was found that DNA could effectively be compacted by cationic micelles of $C_n\text{DMAOH}^+$ which were produced by the protonation of $C_n\text{DMAO}$ in acidic media leading to the formation of water-insoluble $C_n\text{DMAOH}^+/\text{DNA}$ complexes. The DNA molecules were compacted at pH 4-5 when the concentration of $C_{10}\text{DMAOH}^+$, $C_{12}\text{DMAOH}^+$, and $C_{14}\text{DMAOH}^+$ reached 8.0, 1.6, and 0.9 $\text{mmol}\cdot\text{L}^{-1}$, respectively. Interestingly, the precipitates of $C_n\text{DMAOH}^+/\text{DNA}$ complexes can re-dissolve which indicated that the DNA molecules were released from the complexes by regulating the pH of the solution to ~ 4 and increasing the surfactant concentration to 40, 9.0, and 1.8 $\text{mmol}\cdot\text{L}^{-1}$ for $C_{10}\text{DMAOH}^+$, $C_{12}\text{DMAOH}^+$, and $C_{14}\text{DMAOH}^+$, respectively. This phenomenon was attributed to the hydrogen bonding formed between cationic $C_n\text{DMAOH}^+$ and zwitterionic $C_n\text{DMAO}$ species. These hydrogen-bonded species screen the electrostatic forces between the positively charged $C_n\text{DMAOH}^+$ micelles and the negatively charged backbones of DNA. Our results demonstrated that the release of DNA from the $C_n\text{DMAOH}^+/\text{DNA}$ precipitates depended on the concentration of cationic $C_n\text{DMAOH}^+$ and the pH of the solution. Compared with the conventional release of DNA by the addition of β -cyclodextrin, the present strategy allowed for a specific controlled release, which favored the penetration of DNA into cells and could protect the DNA from nucleases degradation.

Keywords: Controlled compaction and decompaction, DNA, zwitterionic surfactants, pH, β -cyclodextrin

1. Introduction

Gene therapy is a key research area, which relies on the principle of molecular biology and promises specific therapeutic approaches. It offers a new tool for patients with diseases which have a close causal relationship with genetic disorders. The strategy of gene therapy involves the transfer of typical genes into target cells for subsequent expression of DNA. However, the delivery of genes into cells remains the obstacles because the structure of DNA molecules bound to the carrier usually hampers its transfer. DNA possesses a rigid double helix structure and has to undergo a strong compaction process to fit within the limited available space. Hence, controlling DNA loading and release is a crucial step in gene delivery. Despite the progress achieved recently in this research field, the development of new methods which can effectively capture and release DNA molecules is still highly desired.

DNA-surfactant complexes have been studied as potential gene delivery carriers in last decades. There is a strong electrostatic attraction between the negatively-charged DNA and oppositely charged surfactants, i.e., cationic surfactants [1,2]. In these systems, when being mixed together, the phase separation usually occurs because of the formation of precipitates. The interactions between the DNA molecules and the nonionic surfactants mainly originate from the hydrophobic interactions and are thus relatively weak [3]. Lindman et al. and Wang et al. reported in two independent studies the condensation and precipitation of DNA controlled by the alkyldimethylamine oxide C_{12} DMAO micelles at acidic pH and low ionic strengths [4,5]. However, the researches of the two groups did not consider the compaction and decompaction of DNA molecules controlled only by C_n DMAOH⁺ micelles without the addition of other materials. Although a variety of surfactant-assisted DNA carriers

are reported since the past decades, the development of new systems to further optimize the properties of the DNA/surfactant complexes is crucial for our understanding on such systems. More specifically, carrier systems which are responsive to external stimuli such as pH are highly attractive, as the pH of the local environments in normal and cancer cells are different [6-8].

Recently, it has been reported that the precipitates formed in the DNA/CTABr (hexadecyltrimethylammonium bromide) complex system can be redissolved by the addition of an excess of cationic CTABr [9-11], which had been demonstrated to be impossible in previous studies [12,13]. But there were the controversial conclusions from the literatures. Carlstedt et al. concluded the phenomenon of redissolve was attributable to positively overcharged DNA/surfactant aggregates. Lopez-Cornejo et al. held the opinion that the phenomenon of redissolve was a consequence of the mismatch of DNA and new formed rod- or disklike micelles in geometry. Xu et al. concluded the phenomenon of redissolve was controlled by counterions of cationic surfactants.

To the best of our knowledge, the controlled compaction and decompaction of DNA by zwitterionic surfactants alone have not yet been reported. In this work, we focus on the compaction and decompaction of double-stranded DNA in the presence of C_n DMAO ($n = 10, 12$, and 14) in aqueous solution. The surfactants C_n DMAO are zwitterionic surfactants and can be transformed into cationic species in aqueous solution under acidic conditions [14]. We found that the redissolution phenomenon occurred with an excess of protonated surfactants, implying that dissociation of the $\text{DNA}/C_n\text{DMAOH}^+$ complexes and the release of DNA. These systems were investigated by phase behavior and a variety of techniques including UV-vis spectroscopy, circular dichroism (CD), zeta-potential measurements, dynamic light

scattering (DLS), agarose gel electrophoresis (AGE), atomic force microscopy (AFM), and fluorescence microscopy (FM).

For comparison, we have checked the DNA release induced by β -cyclodextrin. The redissolution phenomenon also occurred with the addition of an appropriate concentration of β -cyclodextrin, and is similar to that in DNA/cationic surfactant systems. DNA molecules were released from the water-insoluble DNA/C_nDMAOH⁺ complexes due to the formation of inclusion complexes by β -cyclodextrin and C_nDMAOH⁺. Compared to the β -cyclodextrin approaches, our strategy demonstrated the advantage of simplicity and stimuli-responsiveness. Also, the mild experimental conditions ensure the well protection of the double-stranded DNA from degradation. This work presents an understanding on surfactant-assisted DNA compaction and decompaction and widens the potential applications of DNA/surfactant complexes in gene delivery, gene therapy, and biomedicine.

2. Experimental section

2.1. Chemicals and materials

The surfactants C₁₂DMAO and C₁₄DMAO were purchased from Clariant Corp. (Frankfurt, Germany) and were recrystallized in acetone three times. The purity was determined by liquid chromatography-mass spectrometry and was superior to 99% as shown by the results summarized in the Figs. S1 and S2 and the Tables S1 and S2 from the Supporting Information. The chemical compounds C₁₀DMAO (purity > 98%), triethanolamine (TEA) (purity > 99%), acetic acid (purity > 99.8%) and β -cyclodextrin (purity > 98%) were purchased from J&K Chemical Ltd. (Shanghai, China) and used without further purification. Salmon testes double-strand DNA (dsDNA) sodium salt was purchased from ACROS (New Jersey, USA) and characterized

by AGE (500-600 bps) and absorption spectroscopy (molar extinction coefficient = $6600 \text{ mol}^{-1} \cdot \text{cm}^{-1}$). The DNA sample was not contaminated by proteins as the ratio of the intensities of the absorbance at 260 nm and 280 nm was 1.8-1.9. Thrice distilled water was used throughout the experiments.

2.2. Sample preparation

Protonated C_nDMAOH^+ solutions were prepared by adding an equimolar quantity of acetic acid to aqueous solutions of the zwitterionic C_nDMAO . The deprotonated C_nDMAO solutions were prepared by adding an excess of TEA into aqueous solutions of C_nDMAO with a molar ratio of TEA to C_nDMAO being 20:1. The DNA/ C_nDMAOH^+ solutions were prepared by mixing fixed volumes of the prepared solutions of the C_nDMAOH^+ and DNA, and water was added to obtain a final DNA concentration of 0.5 mmol L^{-1} in nucleotide units ($1.67 \text{ mg} \cdot \text{mL}^{-1}$). After overnight incubation at $25.0 \pm 0.1^\circ\text{C}$, aliquots were taken for turbidity measurements. The samples were centrifuged at 11000 rpm for 60 min and the supernatants were used for further characterization.

2.3. Measurements of pH values

The pH value was determined at $25 \pm 0.1^\circ\text{C}$ on a PHS-3C pH-meter (Shanghai Precision & Scientific Instrument Co., Ltd., China) using an E-201-C glass electrode.

2.4. Measurements of the critical micelle concentration (cmc)

The critical micelle concentrations at 25°C were determined by the well-established fluorescence probe method based on the pyrene probe [15]. The fluorescence emission of pyrene ($1 \times 10^{-6} \text{ mmol} \cdot \text{L}^{-1}$) in C_nDMAO and C_nDMAOH^+ solutions were respectively recorded with an excitation wavelength of 335 nm. The *cmc* was obtained by calculating the ratio of the intensities of the first peak at 373 nm

to the third peak at 384 nm (I_1/I_3) depending on the concentration of the C_nDMAO or C_nDMAOH⁺ solutions. The excitation and emission slits were fixed at 15.0 nm and 2.5 nm, respectively.

2.5. Turbidity and UV-vis measurements

Turbidity and UV-vis absorption were measured on a U-4100 UV-visible spectrometer (Hitachi High-Technologies Corp., Japan) with a 1 cm path-length quartz-cell. Turbidity measurements were conducted at a wavelength of 500 nm while the UV-vis absorption spectra were obtained in the range of 220-320 nm.

2.6. Zeta potential measurements

The zeta-potentials were measured using a Zeta PALS potential analyzer (Brookhaven Instruments Corp., USA) with parallel-plate platinum black electrodes spaced 0.5 cm apart and a 10 mm path-length rectangular organic-glass cell. All samples were measured using a sinusoidal voltage of 80 V with a frequency of 3 Hz. Each sample was measured three times.

2.7. Circular dichroism (CD) measurements

The CD spectra were recorded on a JASCO J-810 spectropolarimeter (Jasco analytical instruments Co., Ltd., City, Country). Samples were introduced in 1 cm path-length cells, and the scans were recorded in the range of 320-220 nm with a scanning rate of 50 nm min⁻¹, a bandwidth of 1.0 nm, and a response time of 8 s. Each sample was measured three times.

2.8. Agarose gel electrophoresis (AGE)

The agarose gel (1.0 g) was dissolved in TAE buffer (pH 7.4) to obtain a 1% w/v ratio. Electrophoresis was performed using a gel electrophoresis apparatus (HE-120, Tanon Science and Technology Inc., Beijing, China) under 115 V for 25 min with a

standard DNA ladder of 500 or 2000 bps as a reference. Then the gels were stained in $0.5 \mu\text{g mL}^{-1}$ ethidium bromide for 10 min before visualization by UV irradiation.

2.9. Dynamic light scattering (DLS) measurements

Dust-free solutions for DLS measurements were prepared by filtering the sample solutions through appropriate Millipore sterile membrane filters depending on the concentration and the size of the aggregates directly into the dust-free light-scattering cells. The cells, which were priorly rinsed in the inside and outside with acetone, were held in a brass thermostat block filled with refractive-index-matching silicone oil. A standard laboratory-built laser light-scattering spectrometer was equipped with a diode-pumped solid-state (DPSS) laser (Coherent Inc., 532 nm, 200 mW) and a correlator (BI-9000 AT, Brookhaven Instruments Corp., USA). The spectrometer could measure the angular dependence of the absolute integrated scattered intensity over a scattering angle range of 20° - 140° and also the intensity-intensity digital photon correlation over the same angular range (DLS and depolarized DLS). The temperature was controlled within $\pm 0.1^\circ\text{C}$.

2.10. Atomic force microscopy (AFM) observations

The conformation of the DNA structures was investigated by AFM in the presence of various concentrations of C_nDMAO . One drop of the sample was deposited on a mica surface and left to dry in air. The sample was then observed with a NanoScope III (Digital Instruments Inc., USA) in the tapping mode.

2.11. Fluorescence microscopic (FM) measurements

The sample was stained with 4',6-diamidino-2-phenylindole (DAPI) at $0.5 \text{ mmol}\cdot\text{L}^{-1}$. Images were recorded at room temperature with an Olympus IX81 microscope (Olympus Corp., Japan) equipped with a 60X oil immersion objective and a laser operating at 488 nm. The images were digitized on a computer through a

high-sensitivity electron multiplying charge coupled device (EMCCD) and treated with an image processor (Cascade 512B).

3. Results and discussion

3.1. Phase behavior

The self-assembly of cationic surfactants into micelles in the vicinity of a DNA backbone induces the conformational change of DNA molecules from an elongated coil structure to compacted globules [16-18]. The strong electrostatic interaction between a given surfactant and DNA molecules implies an effective critical micelle concentration called the critical association concentration (*cac*), which is considerably lower than the critical micelle concentration (*cmc*) of the given cationic surfactant alone in aqueous solution [19]. At high concentrations of compacted DNA, DNA/surfactant aggregates partially precipitate due to Coulomb attractive interaction. Thus, the observation of the macroscopic phase behavior is a convenient tool to understand the molecular interactions between DNA and surfactants.

The phase diagrams of the systems composed of $C_n\text{DMAOH}^+$ ($n = 10, 12, \text{ and } 14$) and DNA are summarized in Fig. 1a and indicated that the required amount of protonated surfactant able to induce a phase separation with the different chain length of the surfactant, specifically the concentrations of $C_{10}\text{DMAOH}^+$, $C_{12}\text{DMAOH}^+$, and $C_{14}\text{DMAOH}^+$ were 8.0, 1.0, and 0.4 mmol L⁻¹, respectively. This observed difference was probably due to the decrease of *cacs* of the $C_n\text{DMAOH}^+$ when the chain length increases as higher hydrophobic interactions occur at the alkyl moieties. Interestingly, the precipitates present at the phase separation of all the different mixtures redissolve when the concentrations of $C_{10}\text{DMAOH}^+$, $C_{12}\text{DMAOH}^+$, and $C_{14}\text{DMAOH}^+$ reached 40.0, 9.0, and 2.0 mmol·L⁻¹, respectively.

Fig. 1

The variations in the phase behavior of the systems with surfactants of different chain lengths can be explained due to different interactions at the molecular scale. Firstly, below the *cac*, the DNA molecules carried a net negative charge leading to the binding of the protonated molecules C_nDMAOH^+ to the phosphate groups of DNA. No precipitate was formed at this stage because the outnumbered C_nDMAOH^+ molecules cannot fully bind the large negatively charged DNA backbones. Secondly, when the *cac* was reached, micelles carrying multiple positive charges were formed. The binding of cationic C_nDMAOH^+ micelles reduced the repulsion between the negatively charged DNA molecules resulting in a strong association of the DNA/ C_nDMAOH^+ complexes and eventually to precipitation. Finally, upon further increase of the concentration of the surfactants, the C_nDMAOH^+ /DNA precipitates could be redissolved in solution.

In order to confirm that the phase separation was essentially caused by the interactions between C_nDMAOH^+ with DNA, we studied the phase behavior of systems composed of nonionic C_nDMAO and DNA. The pH value of the solutions was adjusted to $pH > 9$ by the addition of TEA for complete deprotonation of C_nDMAOH^+ . No phase separation was observed within the whole range of concentrations of C_nDMAO ($0.1-120 \text{ mmol}\cdot\text{L}^{-1}$) as shown in Fig. 1b. These results indicated that the interaction between the DNA and C_nDMAO molecules was weak and that the C_nDMAO micelles could not bind to the DNA backbones.

3.2. The stability of the double-stranded DNA structure

The compaction of DNA in the DNA/ C_nDMAOH^+ systems is directly related to the surfactant's protonation degree, which is governed by the pH value of the solution.

Therefore, appropriate pH values of the solution must be selected in order to stabilize the double-stranded structure of the DNA molecules. The pH in this work is between 4 and 7 according to the corresponding pH of DNA/C_nDMAOH⁺ complex solutions, as shown in Fig. S3.

We have firstly studied the structure and relative stability of the double-stranded DNA alone by measuring the UV absorption of a series of DNA solutions at different concentrations and at pH = 4. Indeed, double-stranded DNA exhibits a large characteristic absorption band between 220 nm and 300 nm with a maximum at 260 nm (OD₂₆₀) [20]. If the structure of the double-stranded DNA is broken, i.e. DNA is melting, the OD₂₆₀ value would rise sharply and no CD signal could be detected. The experimental OD₂₆₀ values were linearly correlated with the DNA concentration in the range of 0.025 mmol·L⁻¹ to 0.6 mmol·L⁻¹ and followed the regression equation $y (Abs) = 0.0997 + 6.8847c \text{ (mmol}\cdot\text{L}^{-1}\text{)}$, with a correlation coefficient $r = 0.9977$ (Fig. S4a).

Then, the OD₂₆₀ values were also recorded for samples at different pH values ranging from 4 to 7 and at a fixed concentration of DNA at 0.5 mmol·L⁻¹. The results are shown in the Fig. S4b of the supporting information and show no obvious change in the intensity of the OD₂₆₀ values upon pH variation. In addition, we have recorded the CD spectra of DNA solutions (0.5 mmol·L⁻¹) at different pH ranging (Fig. S5).

The experiments indicated a stable double-stranded DNA structure when the pH values of the DNA/C_nDMAOH⁺ systems solutions ranged from 4 to 7 and DNA concentration at 0.5 mmol·L⁻¹ (Fig.S3, S4 and S5 of the supporting information).

3.3. UV-vis spectra

The UV absorption measurements of DNA are important to determine whether π - π tacking of nucleobases occurs. DNA normally expresses a maximum optical density at $\lambda = 260$ nm (OD_{260}). Once DNA molecules precipitate from the solution, the reduction in the OD_{260} value was observed. To further determine the conformational changes and the phase behavior of DNA mixed with various amounts of C_nDMAOH^+ , UV-vis measurements were carried out and the results are collected in Fig. 2a. The critical association concentrations of C_nDMAOH^+ at which the decrease in the OD_{260} value of DNA took place were in accordance with those obtained on the phase diagrams (Fig. 1a). At the first interactions between DNA and C_nDMAOH^+ micelles, the OD_{260} value started to decrease at the concentrations of 1.0, 0.3, and 0.2 mmol·L⁻¹ for $C_{10}DMAOH^+$, $C_{12}DMAOH^+$, and $C_{14}DMAOH^+$, respectively. At this stage, the pH was 4.74, 5.73, and 6.44 for the solutions of DNA with $C_{10}DMAOH^+$, $C_{12}DMAOH^+$, $C_{14}DMAOH^+$, respectively (Fig. S3). Then, the OD_{260} values reached a minimum when the concentration of the surfactants increased to 8.0, 1.6, and 0.9 mmol·L⁻¹ for $C_{10}DMAOH^+$, $C_{12}DMAOH^+$, $C_{14}DMAOH^+$, respectively. The corresponding values of the pH of the DNA/ C_nDMAOH^+ solutions were 4.03, 4.63, and 4.98 for $n=10, 12$, and 14, respectively (Fig. S3). The experiment took place when the concentration of DNA was the lowest therefore all the DNA molecules were fully compacted. The values of the OD_{260} increased again with an increasing concentration of C_nDMAOH^+ . The samples turned clear and the OD_{260} value fully recovered to the original value of pure DNA when the C_nDMAOH^+ concentrations exceeded 40, 8.0, and 1.8 mmol·L⁻¹ for $n=10, 12$, and 14 respectively. The corresponding pH value of the DNA/ C_nDMAOH^+ solution were 3.9, 4.3, and 4.58 for $n=10, 12$, and 14, respectively

(Fig. S3). These observations were consistent with the results obtained in the phase behavior study (Fig. 1a).

Given all these observations, we can conclude that the concentration of $C_n\text{DMAOH}^+$ and the pH value are the two essential factors for the effective compaction and decompaction of DNA in the DNA/ $C_n\text{DMAOH}^+$ systems. Among the different alkyldimethylamine oxides, $C_{14}\text{DMAOH}^+$ required the lowest concentration to complex with DNA due to the higher chain length related to the low *cac*.

3.4. Turbidity measurements

The turbidity of the solution was closely related to the phase separation and formation of precipitates due to the strong attraction between DNA and oppositely charged surfactant aggregates. Turbidity was monitored by measuring the optical absorbance at 500 nm of the DNA/ $C_n\text{DMAOH}^+$ systems at different concentrations of surfactants.

As exhibited in Fig. 2b, the turbidity of the DNA/ $C_n\text{DMAOH}^+$ complex solutions remained the same at the initial concentrations of $C_n\text{DMAOH}^+$. However, when the concentration of $C_n\text{DMAOH}^+$ reached a critical value, the turbidity of the solution began to increase. The $C_n\text{DMAOH}^+$ critical concentrations were 1.2, 0.4, and 0.1 $\text{mmol}\cdot\text{L}^{-1}$ for $n = 10, 12$, and 14 , respectively. The increase in turbidity was due to the strong electrostatic interaction between the DNA and the $C_n\text{DMAOH}^+$ micelles. With an increasing concentration, more and more $C_n\text{DMAOH}^+$ molecules gathered in the vicinity of the backbone of the DNA molecules to form $C_n\text{DMAOH}^+$ micelles, which in turn bounded to the backbone of the DNA to balance the negative charges of the DNA, leading to the formation of insoluble aggregates and precipitates and an obvious increase of turbidity. These results also indicated that $C_n\text{DMAOH}^+$ with a

longer chain length had a stronger affinity to the DNA phosphate groups, leading to the formation of precipitates at lower surfactant concentration. Maximum turbidity was reached when all the compacted DNA molecules were bound to $C_n\text{DMAOH}^+$ micelles. For $C_{10}\text{DMAOH}^+$, $C_{12}\text{DMAOH}^+$ and $C_{14}\text{DMAOH}^+$, the maximum turbidity was observed at 8.0, 1.6, and 0.9 $\text{mmol}\cdot\text{L}^{-1}$, respectively. The values of the highest turbidity of the three mixtures were consistent with the corresponding lowest OD_{260} values, verifying the full compaction of the DNA molecules. As the concentrations of $C_n\text{DMAOH}^+$ increased continuously, the addition of the excess of the $C_n\text{DMAOH}^+$ molecules caused the decrease of the turbidity. Moreover, the turbid solutions turned back clear, indicating the release of DNA molecules from the $C_n\text{DMAOH}^+$ micelles. This observation was consistent with the observed macroscopic redissolution of the precipitates observed in the phase behavior study.

It should be noted that optical methods are more sensitive than macroscopic phase separation observations and explain the slight differences observed for the critical association concentrations (*cacs*) of $C_n\text{DMAOH}^+$ by the different methods.

Fig. 2

3.5. Zeta-potential measurements

Zeta potential (ζ) measurements indicate the changes of the surface charge density and evaluate the stability of colloid particles [21-23]. The surface charge density, σ , was obtained from the following formula:

$$\sigma = \frac{\zeta \epsilon \epsilon_0}{4\pi\tau} \quad (1)$$

where τ refers to the thickness of the electric double layer of the DNA/ $C_n\text{DMAOH}^+$ complexes; ϵ_0 is the vacuum dielectric constant; and ϵ is the solvent permittivity. A high absolute value of ζ suggests a high stability, while intermediate ζ value indicates

a thermodynamic instability and formation of aggregates. The Fig. 3 shows the variations of ζ when varying the concentrations of $C_n\text{DMAOH}^+$. The ζ of DNA measured in the absence of $C_n\text{DMAOH}^+$ was ca. -46 mV. This value underwent a significant increase when the concentration of $C_n\text{DMAOH}^+$ increased, indicating the binding of positively-charged $C_n\text{DMAOH}^+$ micelles on the DNA backbone. When the interaction between the DNA molecules and the surfactant micelles reached a binding saturation point, the surface charge density of the complexes was equal to zero. The charge neutralization limits of $C_{10}\text{DMAOH}^+$, $C_{12}\text{DMAOH}^+$, and $C_{14}\text{DMAOH}^+$ with DNA complexes were 8.0, 1.6, and 0.9 mmol·L⁻¹, respectively within the error range, where almost only compacted DNA molecules in the form of DNA/ $C_n\text{DMAOH}^+$ complex precipitates existed. Therefore, the zeta-potential measurements provided experimental evidences of the strong electrostatic attraction of the DNA molecules with the $C_n\text{DMAOH}^+$ micelles and that the DNA molecules were almost compacted into a globule state. Moreover, the charge neutralization point of the DNA/ $C_n\text{DMAOH}^+$ complexes decreased with the increasing chain length of $C_n\text{DMAO}$ used.

As the concentration of the surfactant exceeded the *cacs*, the value of ζ increased sharply. This phenomenon was brought by the excess positively-charged $C_n\text{DMAOH}^+$ micelles in solution, which favored the dissociation of the DNA/ $C_n\text{DMAOH}^+$ complexes and the efficient release of the DNA molecules. These results were in good agreement with the findings reported for the UV-vis and turbidity measurements.

Fig. 3

3.6. CD measurements

CD spectroscopy measurements were employed to study the conformational changes of DNA induced by $C_n\text{DMAOH}^+$ in the range of 220-320 nm. As an

illustration, the spectra of the DNA/C₁₄DMAOH⁺ complexes at a fixed DNA concentration with various C₁₄DMAOH⁺ concentrations are shown in Fig. 4. CD spectra of DNA typically presents a positive band at 275 nm due to the base stacking and a negative one at 249 nm brought by the helicity and a nodal point at 258 nm which indicates the β -form conformation of the DNA strands [23]. Once the DNA was compacted, apart from reduction in absorbance peak intensity, evident red shift of the two main opposite bands could be observed due to the conformational change in the DNA helix [24]. The Fig. 4 shows that the compaction started when the concentration of C₁₄DMAOH⁺ reached 0.1 mmol·L⁻¹ and that the CD spectra exhibited a continuous change together with the increase of the surfactant concentration. When the concentration of C₁₄DMAOH⁺ exceeded 1.8 mmol·L⁻¹, both the peak intensity and the position of the two main bands recovered those of pure DNA, indicating the occurrence of the decompaction. Again, these findings were in good agreement with the measurements obtained by the above-mentioned methods.

Fig. 4

3.7. DLS measurements

DLS measurements were used to investigate the interaction between DNA and the surfactant aggregates by providing the tiny change of the hydrodynamic radius (R_h). Each base pair (bp) of the double-stranded DNA had a size of 0.34 nm and the R_h of the individual DNA molecule was 97.7 nm (Fig. S6) [25]. The concrete change in R_h of the DNA/C₁₀DMAOH⁺ complexes are given in Fig. 5 as an example and the results for the complexes of DNA/C₁₂DMAOH⁺ and DNA/C₁₄DMAOH⁺ are shown in the Fig. S7 and S8 in the supporting information. Variation in the R_h of the DNA/C_nDMAOH⁺ complexes as a function of the C_nDMAOH⁺ concentration are summarized in Fig. 6. The R_h initially decreased with the addition of C_nDMAOH⁺,

and then reached a minimum value when the concentration of $C_n\text{DMAOH}^+$ reached 8.0, 1.6, and 0.9 $\text{mmol}\cdot\text{L}^{-1}$ for $C_{10}\text{DMAOH}^+$, $C_{12}\text{DMAOH}^+$, and $C_{14}\text{DMAOH}^+$, respectively. This behavior indicated that the DNA molecules structures changed from stretched coils to compacted globules. After this point, by adding excess amounts of $C_n\text{DMAOH}^+$, the R_h value increased gradually, and returned to the initial value. The recovery of the R_h demonstrated that the DNA molecules could be released from the $\text{DNA}/C_n\text{DMAOH}^+$ complexes under controlled $C_n\text{DMAOH}^+$ concentrations and at an appropriate pH value.

Figs. 5 and 6

3.8. AGE measurements

In order to further characterize the DNA decompaction of the $\text{DNA}/C_n\text{DMAOH}^+$ complexes, AGE studies were performed. AGE is a powerful technique for studying the size and charge of DNA. It can also be utilized to study liposome/DNA complexes because the mobility of the complex through the agarose gel reflects their net charge [26,27]. AGE images of a series of $\text{DNA}/C_n\text{DMAOH}^+$ complexes revealing the DNA binding affinity with various amounts of $C_n\text{DMAOH}^+$ are shown in Fig. 7. Lane 1 represents the DNA marker intercalated with EtBr. Lanes 2-4 represent the $\text{DNA}/C_n\text{DMAOH}^+$ complexes at low concentrations of $C_n\text{DMAOH}^+$ at which concentration the DNA molecules were partly in the uncondensed state as detected after migration. The cationic $C_n\text{DMAOH}^+$ micelles at this stage can only bind with some of the negatively-charged DNA molecules leaving a certain amount of DNA molecules carrying net negative charges as could be observed by the distinguishable signal of free DNA. In contrast, the DNA migration was blocked when the concentration of $C_n\text{DMAOH}^+$ increased (lanes 5-6) as the negative charges of the DNA were fully neutralized by the cationic $C_n\text{DMAOH}^+$ micelles. When the amount

of $C_n\text{DMAOH}^+$ was in excess (lanes 7-10), the migration of free DNA was detected again. The electrophoresis results further confirmed the release of DNA molecules in the presence of an excess of $C_n\text{DMAOH}^+$, which is in conjunction with the results discussed above.

Fig. 7

3.9. Conformational changes of DNA

AFM and FM provided direct observations on the structures of the complexes. Firstly, the structural changes of the DNA molecules were studied by AFM. In Fig. 8a, the native double-stranded DNA molecules displayed an extended coil conformation. When $C_{14}\text{DMAOH}^+$ was initially added, the elongated DNA molecule coils began to wind together (Fig. 8b). With a further increase of the concentration of $C_{14}\text{DMAOH}^+$, only compacted globular structures of DNA/ $C_{14}\text{DMAOH}^+$ complexes could be seen as shown in Fig. 8c. In addition, for FM observations, the samples were stained by a fluorescent dye, 4', 6'-diamidino-2- phenylindole (DAPI), which exhibited the conformational change of DNA between coils and globules in the presence of $C_n\text{DMAOH}^+$. The $C_{14}\text{DMAOH}^+$ micelles clearly induced the transition of the DNA structure from coils to globules as typically illustrated on Fig. 8 (d and e). However, the DNA compaction and decompaction processes with well-defined morphological changes could not be observed due to the short DNA used in our FM and AFM experiment.

Fig. 8

3.10. Mechanism

Based on our experimental observations, we discuss in this subsection the reasonable explanations of the close relation between the specificity of the zwitterionic $C_n\text{DMAO}$ molecules and the redissolution phenomenon with the pH

variation of the mixed solution. First, the pH of the three mixtures clearly dropped rapidly with an increase of C_nDMAO; and as the concentration of C_nDMAO (n = 10, 12, 14) reached 15, 1.2, 1.0 mmol·L⁻¹, respectively, the pH curve turned to level up (Fig. S3). The redissolution phenomenon occurred at a pH range of 4 to 5 and no obvious changes appeared beyond this point.

The cationic and nonionic forms of the surfactant, namely the cationic C_nDMAOH⁺ species (DH⁺) and the zwitterionic C_nDMAO species (D) are related to each other by the proton dissociation equilibrium [28], which is described in *Eq. (2)*.

When the concentration of C_nDMAO (n = 10, 12, 14) above 15, 1.2, 1.0 mmol·L⁻¹, respectively, and continued to rise, the nonionic C_nDMAO species in solution gradually increased as long as the pH did not radically rise. Studies have reported that for C_nDMAO systems, neutral micelles are formed at pH > 9, and cationic micelles are formed at pH < 3. When the pH is between 3 and 9, cationic-nonionic mixed micelles form [5]. Hence, in the three DNA/C_nDMAO systems, cationic-nonionic mixed micelles are probably present in the solutions. In order to validate this assumption, we determined the critical micelle concentrations of C_nDMAO and C_nDMAOH⁺ solutions by using the steady-state fluorescence probe pyrene, and the *cmc* of C_nDMAOH⁺ were 18, 2.4, and 0.65 mmol·L⁻¹ for C₁₀DMAOH⁺, C₁₂DMAOH⁺, and C₁₄DMAOH⁺, respectively (Fig. S9, Table S3 and S4). The results showed that when the redissolution phenomenon occurred, the concentrations of C_nDMAO in the DNA/C_nDMAO solutions are higher than the critical micelle concentrations of C_nDMAOH⁺, which confirmed the existence of mixed micelles in the solution. As reported, the hydrogen bonds between C_nDMAOH⁺ and C_nDMAO molecules or between two C_nDMAOH⁺ molecules in solutions (Fig. S10) [28,29] could be brought

by the disruption of the electrostatic interaction between negatively-charged DNA backbones and positively-charged C_nDMAOH^+ . As a consequence, the binding affinity of C_nDMAOH^+ micelles to the DNA molecules could decrease, permitting the DNA molecules to disengage from the DNA/ C_nDMAOH^+ complexes. On the macroscopic perspective, this hypothesis can be expressed by the phase separation disappearance and the redissolution of the precipitates of DNA/ C_nDMAOH^+ in solution. The schematic of this process is presented in Fig. 9.

Fig. 9

3.11. The redissolution phenomenon and release of DNA controlled by β -cyclodextrin

Based on the above discussion, the efficient compaction and decompaction of DNA molecules could be achieved by controlling the concentration of C_nDMAOH^+ and the pH of the solution. For comparison, traditional decompaction of DNA from DNA/ C_nDMAOH^+ complexes with the addition of β -cyclodextrin was also investigated. Although the decompaction and release of DNA from DNA/polycation complexes have been widely studied with an addition of anionic surfactants or cyclodextrin [30-39], similar researches are not as widely studied for DNA/protonated zwitterionic surfactant systems. As expected, the redissolution and release of DNA can be achieved through adding suitable amounts of β -cyclodextrin (Fig. S11). We monitored the change of the OD₂₆₀ value upon addition of β -cyclodextrin and the results are given in Fig. 10. When β -cyclodextrin was added, an increase in the OD₂₆₀ value was observed when the molar ratio of β -cyclodextrin to C_nDMAOH^+ exceeded 1. The association of β -cyclodextrin with C_nDMAOH^+ induced the dissociation of the C_nDMAOH^+ micelles in the vicinity of the DNA and thereby the release of DNA from the DNA/ C_nDMAOH^+ complexes. The conclusion drawn from the UV-vis

measurements was further confirmed by the results of AGE undertaken in the DNA/C_nDMAOH systems and addition of β -cyclodextrin (Fig. 11). The reappearance of the DNA bands in agarose gels indicated that the β -cyclodextrin molecules played an important role in reducing the electrostatic interaction between the C_nDMAOH⁺ micelles and the DNA backbone. The mechanism of the DNA decompaction from the DNA/C_nDMAOH⁺ complexes using cyclodextrins can be well explained by referring to Lindman et al.'s work [34,40]. The β -cyclodextrin molecules coated the surface of the micelles and shielded the hydrophobic interaction between C_nDMAOH⁺ and C_nDMAOH⁺ molecules. The structure of the micelles was disrupted and the DNA molecules released due to the lack of effective charge neutralization of the phosphate groups. A schematic diagram showing this process is given in Fig. 12. However, the concentrations of β -cyclodextrin necessary to decompact the DNA/C_nDMAOH⁺ complexes were found to be close to the solubility limit in water [41]. The weak water-solubility of most cyclodextrins would also restrict the corresponding applications in DNA decompaction.

Figs 10, 11 and 12

4. Conclusions

In this work, interactions between DNA molecules and the protonated zwitterionic surfactants C_nDMAOH⁺ (n=10, 12, 14) were investigated by several methods. Due to the electrostatic attraction, C_nDMAOH⁺ micelles could efficiently compact DNA at a low concentration, namely 8.0, 1.6, and 0.9 mmol·L⁻¹ for C₁₀DMAOH⁺, C₁₂DMAOH⁺ and C₁₄DMAOH⁺, respectively. DNA compaction was observable with an obvious phase separation phenomenon and appearance of water-insoluble DNA/C_nDMAOH⁺ complex precipitates. Interestingly, it was found that the complex precipitates

redissolve by regulating the pH of the solutions between 4 and 5 and applying a surfactant concentration of 40.0, 9.0, and 1.8 mmol·L⁻¹ for C₁₀DMAOH⁺, C₁₂DMAOH⁺ and C₁₄DMAOH⁺, respectively. This phenomenon was attributed to the hydrogen bonds formed between the cationic C_nDMAOH⁺ and the zwitterionic C_nDMAO or between the two cationic species. They reduced the electrostatic forces between the positively charged C_nDMAOH⁺ micelles and the negatively charged backbones of the DNA molecules. We provided experimental data from various techniques proving that the C_nDMAOH⁺ micelles could effectively compact and decompact DNA by regulating the pH and the concentration of C_nDMAOH⁺. We also demonstrated that the C_nDMAOH⁺ molecules with a longer alkyl chains could compact DNA at lower concentrations. Moreover, the decompaction of the DNA molecules could also occur by adding β -cyclodextrin to form inclusion complexes with C_nDMAOH⁺. By comparing the DNA/C_nDMAOH⁺ and the DNA/C_nDMAOH⁺/ β -cyclodextrin systems, the poor water-solubility of β -cyclodextrin could limit its use at the higher concentration. To conclude, a new strategy was provided to control the DNA compaction and decompaction only by regulating the concentration of C_nDMAOH⁺ at a proper pH. The current study could be potentially applied for controlling gene expression and in vitro and in vivo DNA delivery.

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Captions of Figures and Tables in Manuscript Text:

Fig. 1. Phase diagrams of (a) C_nDMAOH⁺/DNA and (b) zwitterionic C_nDMAO/DNA systems at 25 °C. The concentration of DNA is fixed to be 0.5 mmol·L⁻¹. Filled circles correspond to phase-separated samples while open circles denote clear solutions.

Fig. 2. (a) Absorbance at 260 nm and (b) turbidity measured at 500 nm for the C_nDMAOH⁺/DNA complexes with varying C_nDMAOH⁺ concentrations. The concentration of DNA was fixed at 0.5 mmol·L⁻¹.

Fig. 3. Variation of the zeta-potential (ζ) at a fixed DNA concentration of $0.5 \text{ mmol}\cdot\text{L}^{-1}$ with varying concentrations of C_nDMAOH^+ .

Fig. 4. Illustrative CD spectra of DNA aqueous solution and supernatants of incubated $\text{C}_{14}\text{DMAOH}^+/\text{DNA}$ complexes at a fixed DNA concentration of $0.5 \text{ mmol}\cdot\text{L}^{-1}$ with varying $\text{C}_{14}\text{DMAOH}^+$ concentrations: 0.1, 0.4, 0.9, 1.8, and $4 \text{ mmol}\cdot\text{L}^{-1}$, respectively.

Fig. 5. Size distributions of the aggregates made of $C_{10}\text{DMAOH}^+/\text{DNA}$ mixtures at a fixed DNA concentration of $0.5 \text{ mmol}\cdot\text{L}^{-1}$ with varying $C_{10}\text{DMAOH}^+$ concentrations: $1.2 \text{ mmol}\cdot\text{L}^{-1}$ with $R_h = 91.7 \text{ nm}$, $6 \text{ mmol}\cdot\text{L}^{-1}$ with $R_h = 60.8 \text{ nm}$, $8 \text{ mmol}\cdot\text{L}^{-1}$ with $R_h = 41.5 \text{ nm}$, and $30 \text{ mmol}\cdot\text{L}^{-1}$ with $R_h = 100.1 \text{ nm}$, respectively.

Fig. 6. Variations of the hydrodynamic radius (R_h) of the $C_n\text{DMAOH}^+/\text{DNA}$ complexes at a fixed DNA concentration of $0.5 \text{ mmol}\cdot\text{L}^{-1}$ with varying concentrations

of $C_n\text{DMAOH}^+$.

Fig. 7. Images of agarose gel electrophoresis for (a) $C_{10}\text{DMAOH}^+/\text{DNA}$, (b) $C_{12}\text{DMAOH}^+/\text{DNA}$ and (c) $C_{14}\text{DMAOH}^+/\text{DNA}$ complexes. The concentration of DNA was fixed at $0.5 \text{ mmol}\cdot\text{L}^{-1}$ while the concentration of $C_n\text{DMAOH}^+$ of lane 2 to lane 10 were for (a) 1, 2, 4, 10, 15, 20, 30, 40, and $50 \text{ mmol}\cdot\text{L}^{-1}$; (b) 0.2, 0.4, 0.8, 1, 2, 3, 4, 6, and $8 \text{ mmol}\cdot\text{L}^{-1}$; and (c) 0.1, 0.2, 0.3, 0.8, 1, 2, 4, 6, and $8 \text{ mmol}\cdot\text{L}^{-1}$. Lane 1 and lane 11 are 500 bp and 2000 bp DNA-markers, respectively.

Fig. 8. Images of $C_{14}\text{DMAOH}^+/\text{DNA}$ complexes at a fixed DNA concentration of $0.5 \text{ mmol}\cdot\text{L}^{-1}$ and at varying concentrations of $C_{14}\text{DMAOH}^+$; AFM images of (a)

DNA, (b) $0.2 \text{ mmol}\cdot\text{L}^{-1}$ and (c) $0.9 \text{ mmol}\cdot\text{L}^{-1}$ of $\text{C}_{14}\text{DMAOH}^+$; FM images of (d) DNA and (e) $0.9 \text{ mmol}\cdot\text{L}^{-1}$ of $\text{C}_{14}\text{DMAOH}^+$.

Fig. 9. Illustration of the decompaction and release of DNA triggered by C_nDMAOH^+ at high pH.

Fig. 10. Absorbance at 260 nm of the DNA/ C_nDMAOH^+ complexes upon the addition of β -cyclodextrin. The concentration of DNA was fixed at $0.5 \text{ mmol}\cdot\text{L}^{-1}$ while the concentrations of $\text{C}_{10}\text{DMAOH}^+$, $\text{C}_{12}\text{DMAOH}^+$ and $\text{C}_{14}\text{DMAOH}^+$ were 10, 1.2, and $0.9 \text{ mmol}\cdot\text{L}^{-1}$, respectively.

Fig. 11. Pictures of the agarose gel electrophoresis of (a) DNA/ $C_{10}\text{DMAOH}^+$, (b) DNA/ $C_{12}\text{DMAOH}^+$, and (c) DNA/ $C_{14}\text{DMAOH}^+$ complexes with increasing amount of β -cyclodextrin. The concentration of DNA was fixed at $0.5 \text{ mmol}\cdot\text{L}^{-1}$ while the concentrations of $C_{10}\text{DMAOH}^+$, $C_{12}\text{DMAOH}^+$, and $C_{14}\text{DMAOH}^+$ were 10, 1.2, and $0.9 \text{ mmol}\cdot\text{L}^{-1}$ respectively. The concentration of β -cyclodextrin (from lane 2 to lane 10) were for (a) 0, 1, 2, 5, 8, 10, 11, 12, 13, and $14 \text{ mmol}\cdot\text{L}^{-1}$; (b) 0, 0.4, 0.8, 1, 2, 3, 4, 5, 6, and $7 \text{ mmol}\cdot\text{L}^{-1}$; and (c) 0, 0.2, 0.6, 1, 2, 4, 5, 6 and $7 \text{ mmol}\cdot\text{L}^{-1}$. Lanes 1 and 11 were the 500 bp and 2000 bp DNA-markers, respectively.

Fig. 12. Illustration of the decompaction and release of DNA triggered by $C_n\text{DMAOH}^+$ and β -cyclodextrin.

GRAPHICAL ABSTRACT

Compaction and decompaction of DNA by zwitterionic surfactants with adding acids and β -CD were investigated.