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Phase behavior of DNA in the presence of cetyltrimethylammonium bromide/alkyl polyglucoside surfactant mixture

Received: 3 July 2003
Accepted: 27 November 2003
Published online: 16 January 2004
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Abstract Interaction of DNA with a CTAB/APG mixture was studied by determining its phase behavior. Results showed that, at low DNA concentration, the addition of APG could lead to earlier turbidity, indicating that APG can strengthen the interaction between CTAB and DNA. At high DNA concentration, however, APG had little influence on the turbidity boundary. We found that addition of salt could also lead to such an asymmetry of the phase map. Two mechanisms were presented to account for the asymmetry of the phase behavior. At the lower DNA concentration, it was assumed that the surfactant was bound to DNA in a way similar to that of micelle formation. That is, the

binding is dominated by hydrophobic association processes. APG can facilitate this process by forming a surfactant mixture with CTAB, and salt facilitates this process by “salting out” effects. At high DNA concentrations, surfactants bind to DNA mainly through random electrostatic interaction. Addition of salt screens this interaction and therefore delays the turbidity. APG, however, exerts little influence on this process. Viscosity measurements at low DNA concentration showed that the complex with APG is more compact than that of CTAB alone with DNA.

Keywords DNA · APG · Surfactant mixture · Precipitation · Turbidity

Introduction

Polymer/surfactant mixtures are present in a large number of systems in nature and industry, in foods, pharmaceutical formulations, cosmetics, detergents, and paints; their interactions form a challenging blend of two well-established scientific fields [1, 2, 3, 4]. Special attention has been paid to the interactions between amphiphilic molecules and biologically active polyelectrolytes for both their physiochemical and biomedical importance. Within this group, DNA/cationic surfactant systems are of special importance for scientific research because, for example, the precipitation of various DNA by cationic surfactants has been used for DNA extraction, concentration, and counting [5, 6, 7]. More

importantly, it has been found that some cationic surfactants can act as non-viral vectors for controlled gene delivery due to their ability to change DNA from elongated coils into compact globules, which is an essential step required for gene transportation into cells [8, 9, 10, 11].

Among all of the synthetic surfactants studied, CTAB was found to be the most effective. However, CTAB cannot satisfactorily fulfill the criterion for effective genetic transfection owing to its relatively high cytotoxicity and low stability of the resultant complex to changes in the environment [12]. It was reported, however, that CTAB can be used in small amounts, for positive charging of neutral liposomes in order to improve their transfection efficiency, considering that the cell

surface is often negatively charged [13]. This has inspired us to select a cationic/nonionic surfactant mixture for gene delivery in which the nonionic surfactant is biologically safe and can interact with DNA. We found that alkyl polyglucoside (APG) was a good candidate, on account of its excellent biodegradability and the absence of toxicity [14, 15, 16, 17]. More importantly, we found that APG itself can interact with DNA via hydrogen bonding and, to some extent, change its conformation [18].

To our knowledge, only one paper on the phase behavior of the DNA/surfactant system has been reported [19]. In that paper, phase behavior for DNA and quaternary ammonium surfactant series was determined and the hydrophobic and electrostatic effects were discussed [19]. In this paper, CTAB was chosen because its interaction with DNA has been fully studied and its mechanism was well-understood. In addition, the phase behavior for the DNA and CTAB/APG mixture was studied to examine the effect of the addition of nonionic surfactant on the phase behavior.

It has been reported that the addition of nonionic surfactant, CnEm, weakens the interaction between SDS and protein (another biologically active polyelectrolyte) [20]. In contrast to the surfactant/DNA system, the addition of SDS unfolds the protein chain instead of packing it. Considering that mixtures of cationic and nonionic surfactant exist in living organisms, such as in cellular membrane and karyotheca [21], it is of biological and physicochemical importance to justify these observations.

Experimental

Materials

Cetyltrimethylammonium bromide (CTAB, from Beijing Chemical Co.) was used after being recrystallized twice from acetone. DNA from salmon testes (300–500 b.p.) was used for phase behavior determination and DNA from calf thymus (5000–10000 b.p.) for viscosity measurements. Both types of DNA (from Sigma) were used without further purification. The concentration of DNA was spectroscopically determined (molar extinction coefficient $\epsilon_{260} = 6600 \text{ L mol}^{-1} \text{ cm}^{-1}$ at 260 nm). For salmon testes DNA, the ratio of UV adsorption at 260 and 280 nm, $A_{260}/A_{280} = 1.8$, and for calf thymus DNA, $A_{260}/A_{280} = 1.85$. The water was twice distilled over potassium permanganate.

Table 1 Composition and molecular weight of APG samples

dAPG	Monoglucoside	Diglucoside	Triglucoside	Quanterglucoside	residua	M (g/mol)
$\text{C}_8\text{G}_{1.34}^a$	72.32	19.59	7.38	—	0.71	331.13
$\text{C}_{10}\text{G}_{1.45}^a$	70.12	17.45	5.90	5.61	0.92	368.73
$\text{C}_{10}\text{G}_{1.86}^b$	45.33	32.91	11.09	10.41	0.26	417.01
$\text{C}_{12}\text{G}_{1.42}$	67.92	18.25	12.59	—	1.24	401.12

^a Subscripts of G denote average degree of polymerization; ^b $\text{C}_{10}\text{G}_{1.86}$ was obtained by partial precipitation of the monoglucoside in $\text{C}_{10}\text{G}_{1.45}$

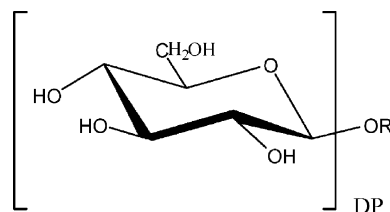


Fig. 1 Structure of APG. R = alkyl chain, DP = average degree of polymerization

APG was synthesized via Fischer glycosidation, followed by removal of the remaining alcohol under vacuum at 110 °C. The composition was determined by gas chromatography (Table 1). APG's structure is shown in Fig. 1.

For better comparison, APG concentration was expressed in terms of the molar concentration of the alkyl chain instead of the average molecular weight. The calculated molecular weights of APG were also listed in Table 1. Note that this molecular weight is different from the usually used average molecular weight for APG.

Sample preparation

For the phase diagram determination, all samples were initially prepared in the monophasic region at high concentrations. The samples were then diluted with twice distilled water to obtain the points at lower concentration and the final volumes were precisely adjusted to 5 ml. Teflon was used to seal the tubes, which were stored at 25 °C and regularly examined visually and between crossed polaroids. Initial inspection was made after a few minutes and the final assessment after at least two weeks. To determine the turbidity points, samples were measured on a Perkin-Elmer UV/vis 1600 spectrometer twice at 350 and 400 nm.

Measurements

Light scattering and zeta potential measurement

Observations were performed on the Malvern Instrument Zetasizer 3000. The zeta potential of DNA/surfactant complexes in water were determined using an electrophoretic light scattering technique. For each sample, the mean electrophoretic mobility was measured at 25 °C and a frequency of 1000 Hz [22]. After precipitation in the DNA/surfactant system, the precipitate was centrifuged and the zeta potential of the particles in the supernatant were measured. The size of the particles in solution was obtained by light scattering with a scattering angle of 90°.

Critical micelle concentration measurements

The cmc was measured with a Krüss K12 tensiometer, employing the Wilhelmy plate method. The platinum plate used was sand-blasted to ensure a contact angle of zero degrees at the three-phase

line, then cleaned with chromic acid and distilled water. All cmc values were obtained at 25 °C.

Viscosity measurements

Viscosity measurements were performed with an Ostwald capillary viscometer at 25 °C. The efflux time always succeeded 100 s, so that kinetic corrections were not necessary.

Results and discussion

The phase behavior of DNA with CTAB/APG mixtures as well as with CTAB alone was studied with the emphasis on revealing the influence of APG of different chain lengths on the phase behavior. Since the occurrence of turbidity can be seen as an indicator of strong interaction within the system [23], we determined the turbidity boundary spectroscopically. One example is

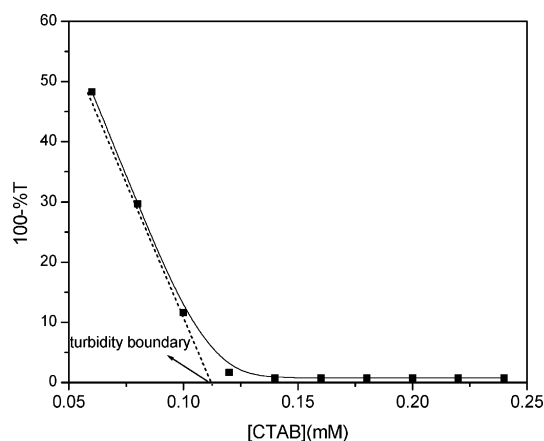


Fig. 2 Determination of the turbidity boundary. [DNA] = 0.07 mM, molar ratio CTAB/APG = 1, $T = 25$ °C, $\lambda = 350$ nm

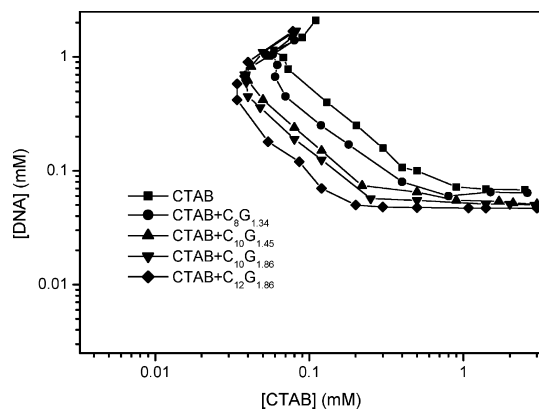


Fig. 3 Phase diagram for DNA/CTAB in the absence and presence of various APG samples. Molar ratio CTAB/APG = 1, $T = 25$ °C

shown in Fig. 2. We measured the turbidity boundary of the system at two wavelengths, 350 and 400 nm, and averaged the two values. The obtained phase map is shown in Fig. 3. The molar ratio of CTAB to APG was 1 for all points in the figure. As turbidity occurred at very low concentration, a logarithmic scale was employed for better comparison.

All of the DNA/surfactant systems, whether containing APG or not, display large turbid regions (Fig. 3). As for CTAB alone, its interaction with DNA leads to a phase separation into a diluted phase and a phase with high DNA and surfactant concentration. This is expected because several systems of polyelectrolyte and oppositely-charged surfactant show such phenomena [24, 25, 26, 27]. It is assumed that the driving force for the precipitation is the strong electrostatic interaction between polyelectrolyte and the surfactant counterion, leading to neutralization of the polyelectrolyte chain and hence precipitation. On the other hand, for APG-containing systems, the turbid regions expand with increasing chain length of APG (Fig. 3), indicating that the hydrophobic chain of APG plays an important role in the phase separation. The turbid regime for $C_{10}G_{1.86}$ has a slight but evident increase when compared to $C_{10}G_{1.45}$ that has less glucose units in the head group. The difference in the two-phase region for two C_{10} APG samples implies that the contribution of the head group of APG to the phase behavior is also evident, although the contribution is not larger than that of the alkyl chain.

Interestingly, the trend that two-phase regions increase with the alkyl chain of APG is more evident for dilute DNA concentrations. In the region of higher DNA concentrations (Fig. 3), the turbidity curves for APG-containing and APG-free systems overlap or cross over each other, showing no significant increase of the two-phase region. To further demonstrate this, three DNA concentrations, 0.2, 0.4 and 4 mM were chosen to show this difference. The minimum molar concentration of CTAB needed for the occurrence of turbidity in the presence and absence of various APG samples were determined. The CTAB concentration needed to make the DNA sample turbid in the absence of APG was normalized to unity for easy comparison.

As clearly shown in Fig. 4, APG's influence on the phase behavior of DNA/CTAB is more evident at low DNA concentrations. As the DNA concentration increases, the influence of APG decreases. At low DNA concentration, with the increase of alkyl chain length of APG, the turbidity boundary moves to lower CTAB concentrations. At high DNA concentration, however, APG has little, if any, influence on the turbid region, at least within the range examined. It seems that two mechanisms of the precipitation process exist at low and high DNA concentrations. Indeed, this kind of asymmetry in phase behavior has also been observed for

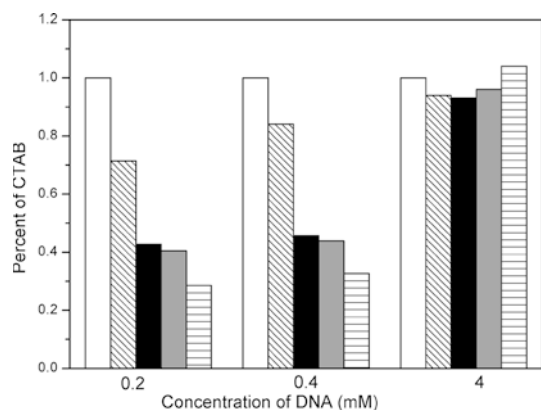


Fig. 4 Relative fraction of CTAB needed for phase separation in the presence of various APG. The empty bars correspond to CTAB alone; diagonally slashed bars correspond to CTAB + C₈G_{1.34}; black bars correspond to CTAB + C₁₀G_{1.45}; gray bars correspond to CTAB + C₁₀G_{1.86}; horizontally-lined bars correspond to CTAB + C₁₂G_{1.42}. Molar ratio CTAB/APG = 1. $T = 25^\circ\text{C}$

DNA/CTAB in the presence of salt. However, the author did not explain this phenomenon [19]. Therefore, we found it necessary to perform further studies of the salt's effect on the phase behavior.

Salt effects

The results are shown in Fig. 5 with the corresponding salt-free system as a comparison (for simplification only one sample, C₁₀G_{1.45}, was used). It is generally accepted that addition of salt will, due to its screening effect, weaken the interaction between polyelectrolyte and the oppositely-charged surfactant [28, 29, 30]. As mentioned above, polyelectrolyte complexes with oppositely-charged surfactants release counterions into the bulk, which compensates for the entropy loss of the complex formation. Addition of salts suppresses this compensation effect, leading to delay of the precipitation [31]. Therefore, the addition of salt should also weaken the interaction between DNA and CTAB or the CTAB/APG mixture. However, whether for the APG-containing or the APG-free system, the two-phase region in the presence of salt is slightly expanded (Fig. 5). This is even the case if viewed at lower DNA concentration (lower than 1 mM). It now becomes clear that salt and APG can both result in an asymmetry in the phase diagram, indicating that their influence on the phase behavior may be of the same origin.

Phase behavior at low DNA concentrations

Turbidity can be approximated as a critical aggregation concentration (c_{ac}) between polymer and surfactant of opposite charge [32], so that at low DNA concentra-

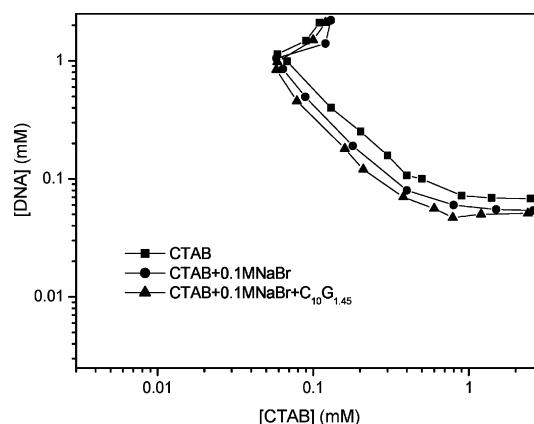


Fig. 5 Phase diagram of DNA/CTAB and DNA/CTAB/C₁₀G_{1.45} system in the presence and absence of NaBr. Molar ratio CTAB/APG = 1. $T = 25^\circ\text{C}$

tions, both the addition of APG or salt can induce earlier formation of a mixed micelle-like aggregate. However, one may argue that the added APG may only bridge the individual CTAB/DNA complexes, causing earlier turbidity. In this case, an increased amount of APG should cause earlier turbidity. As shown in Fig. 6, the mixing ratio of APG and CTAB has a remarkable influence on the starting point of turbidity. All samples with C8, C10 or C12APG have an optimal mixing ratio where turbidity occurs earliest. The CTAB concentrations needed for the occurrence of turbidity are least for C8 and C10APG with $\alpha_c = 0.8$ ($\alpha_c = [\text{CTAB}]/([\text{CTAB}] + [\text{APG}])$), and for C12APG with $\alpha_c = 0.4$. These results clearly exclude the assumption that the earlier turbidity is only due to the bridging effect of APG. For C12APG, relatively more APG is needed for the turbidity to occur. This may be attributed to the very small cmc for C12APG. As a comparison, the cmc's for various APG/CTAB mixtures in DNA-free aqueous solution were measured (Fig. 7). The minimum in cmc for all four APG samples indicates the synergism between CTAB and APG.

Combination of Fig. 6 and Fig. 7 reveals that for all four APG samples, the mixing ratio of CTAB/APG at the earliest occurrence of the turbidity roughly corresponds to the cmc minimum of the mixed surfactant system. Therefore, the addition of APG may facilitate the formation of mixed aggregate between surfactant and DNA owing to the incorporation of APG into the mixed micelles. The mixed micelles then coat the DNA, leading to charge neutralization and turbid solutions.

Meľnikova et al. [33] reported that the addition of dioleoyl phosphatidyl ethanolamine (DOPE) to the dodecyltrimethylamine oxide (DDAO)/DNA system caused the formation of "vesicular" DDAO instead of "micellar" DDAO, which facilitated the interaction between DDAO and DNA. In the present study, we

Fig. 6 Phase behavior of DNA/CTAB/APG as a function of the molar fraction of CTAB in the CTAB/APG mixtures. Thick black bars correspond to precipitation. [DNA]=0.2 mM. **a** for $C_8G_{1.34}$, **b** for $C_{10}G_{1.45}$, **c** for $C_{10}G_{1.86}$ and **d** for $C_{12}G_{1.42}$. $T=25\text{ }^{\circ}\text{C}$

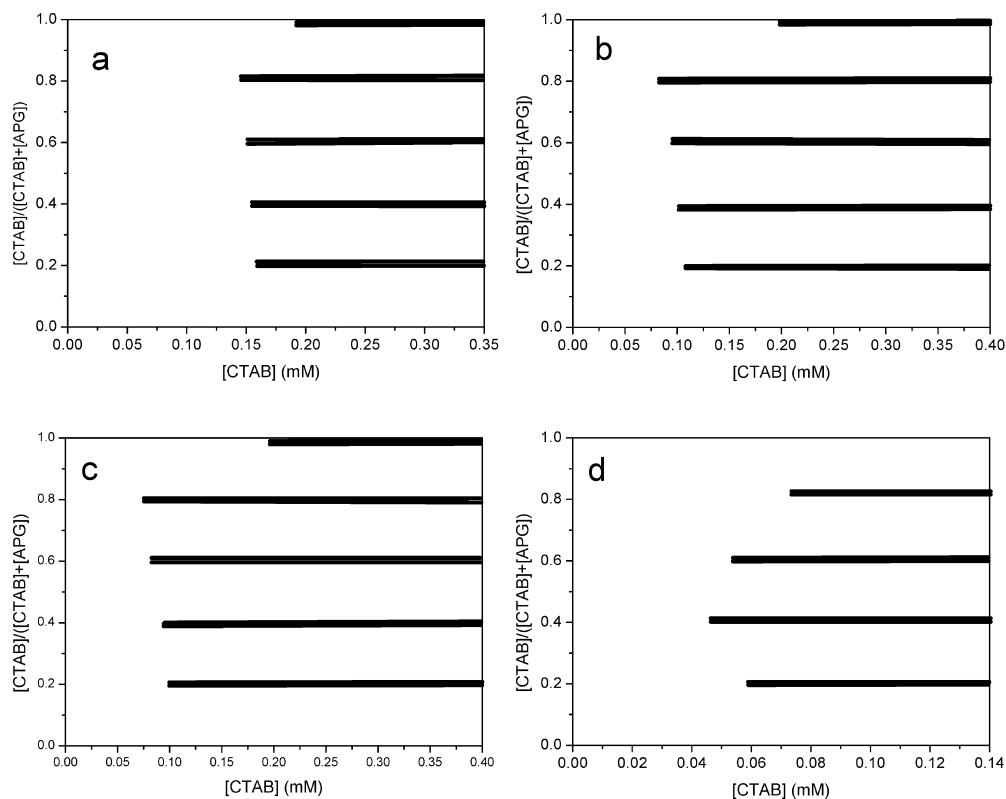
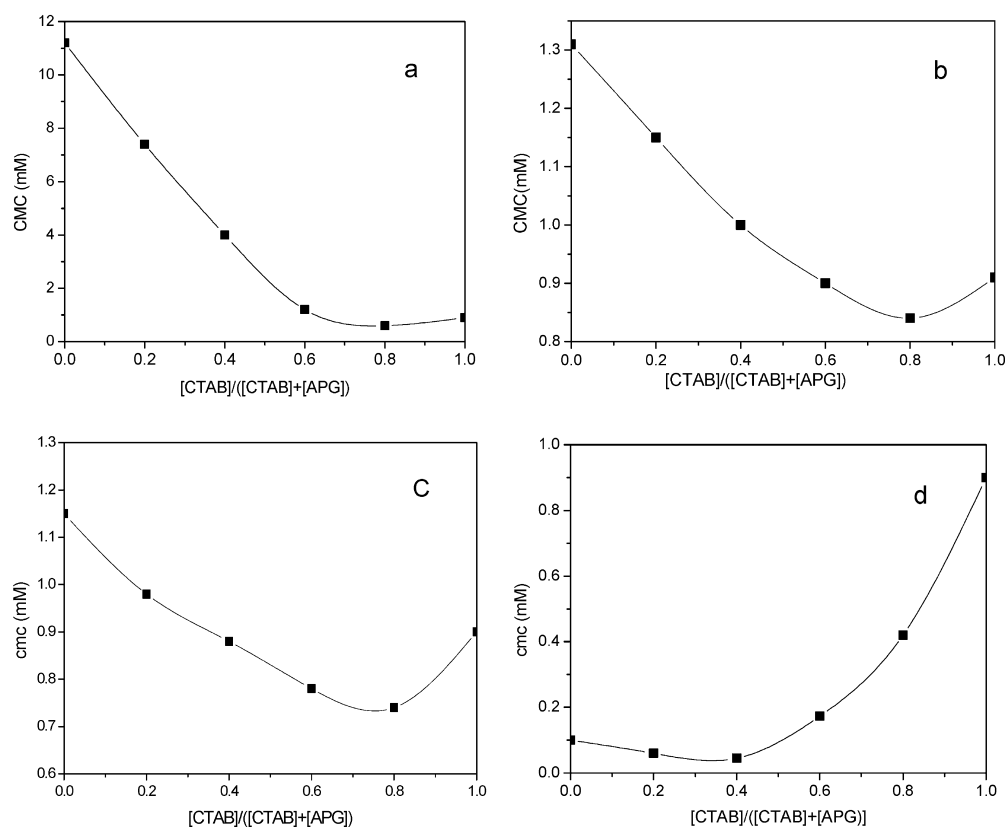


Fig. 7 Cmc of CTAB/APG mixtures vs. molar fraction of CTAB in CTAB/APG mixtures. **a** $C_8G_{1.34}$, **b** $C_{10}G_{1.45}$, **c** $C_{10}G_{1.86}$ and **d** $C_{12}G_{1.86}$. $T=25\text{ }^{\circ}\text{C}$



assume APG as a carrier for CTAB, and that they form mixed assemblies with optimal packing manner.

Mechanisms for DNA precipitation

We assume that there are two different precipitating mechanisms at low and high DNA concentrations. In the case of low DNA concentrations, cationic surfactants bind to DNA in a cooperative manner [34]. Only after complete saturation of one DNA chain do the surfactants begin to bind to another chain, which is comparable to the formation of surfactant micelles. The DNA/CTAB/APG complex may also be seen as a special cationic/nonionic surfactant mixture. The only difference to common surfactant mixtures is that the counterion for cationic surfactant is more or less "rigid". That is, the cationic surfactants should not only overcome the electrostatic repulsion between their polar head groups, but the force exerted by the DNA chain. Since at low DNA concentrations the surfactant concentration is much higher than the DNA concentration, it is reasonable to assume that the process is hydrophobicity-dominated. The addition of APG seems to facilitate this process, considering that incorporation of APG stabilizes mixed micelles and promotes aggregation of CTAB with DNA; in addition, APG has many hydroxyl groups and may adsorb onto the DNA via hydrogen bonding. These two effects may greatly strengthen the interaction between CTAB and DNA. This may further be proved by our observation that nonionic surfactant of polyoxyethylene type has no significant effect on the CTAB/DNA phase behavior. On the other hand, incorporation of APG in CTAB micelles may be energetically favorable, considering that APG can stabilize the micelles by screening the repulsion between the ionic head groups of the CTAB cations. As a result, the association of CTAB with DNA is facilitated by the addition of APG.

When salt is added, the association between CTAB and DNA is strengthened due to the increase of the surfactant's hydrophobicity by the "salting out" effect

[35]. This corresponds to the decrease of the cmc of the ionic surfactants by salt addition. In addition, the added salt screens the charges of DNA, making its conformation less extended, and so facilitates the cooperative binding of surfactants. In this case, the resulting complex may contain only one DNA chain (this has been demonstrated by Mel'nikov et al. [11]), and it is these complexes that aggregate into visible precipitation.

At high DNA concentrations, it is unlikely that surfactants bind to DNA in a cooperative manner due to the influence of neighboring DNA chains. In this case, the surfactants may bind to DNA at random sites. The partially neutralized DNA chains may form initial inter-chain aggregates when binding on one chain is not saturated. As a result, the resulting complex may contain many DNA chains with part of DNA still charged. In this case, the low surfactant concentration compared to DNA makes it difficult for strong hydrophobic association to occur. The key step for binding may be random electrostatic interactions between surfactant head groups and phosphate groups of DNA. This may be called an electrostatic-dominated process. In this case, the addition of salt weakens DNA/CTAB interaction due to the electrostatic screening effect. As for APG, however, due to its nonionic nature, it can hardly exert any influence on the interaction between DNA and CTAB.

Our above assumption is based on the asymmetry of the phase map at low and high DNA concentrations when salt or APG is added. However, as observed for the polyacrylic acid/cationic system, the added salt weakens the interaction between the polyelectrolyte and surfactant at all polymer concentrations [36, 37, 38]. So it can be inferred that the inherent tendency of the DNA double helix chain to be compact [39] plays an important role in the hydrophobic-driven association process.

Zeta potential and particle size

The above assumption was further proved by zeta potential measurements (Fig. 8). At 0.2 mM solution

Fig. 8 Zeta potential of DNA/CTAB system and DNA/CTAB/ $C_{10}G_{1.45}$ system. Short dashed lines indicate turbidity occurrence. **a** 0.2 mM DNA, **b** 2 mM DNA. Molar ratio CTAB/ $C_{10}G_{1.45}$ = 1. $T = 25^\circ\text{C}$

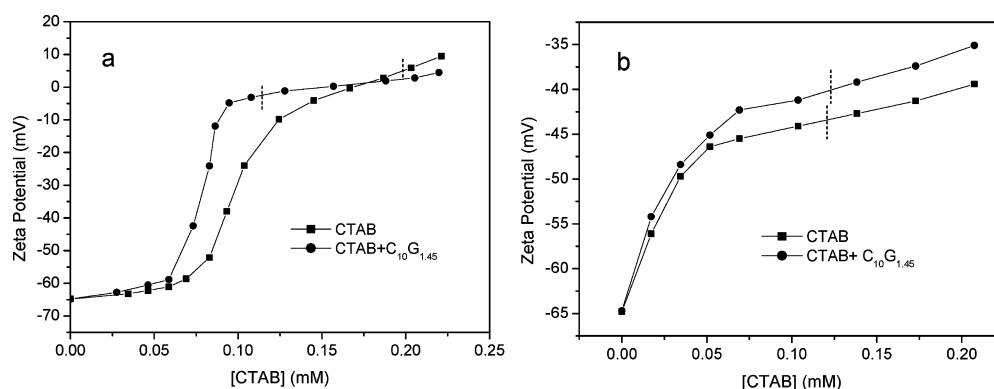
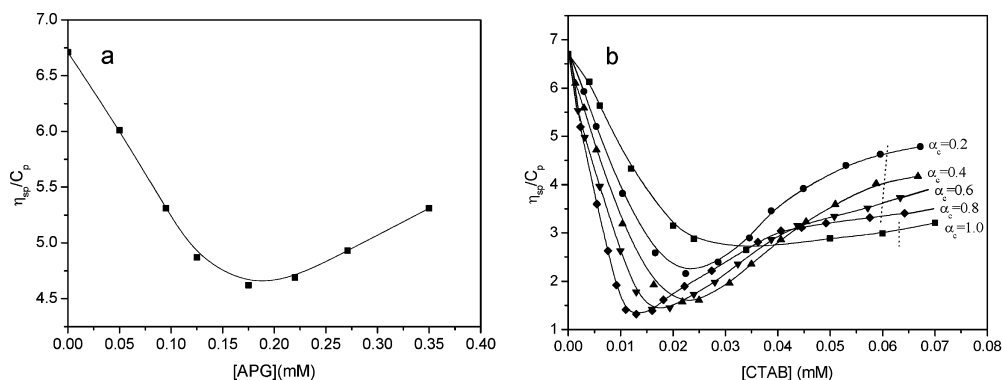


Fig. 9 Reduced viscosity of 0.1 mM DNA solutions in the presence of **a** $C_{10}G_{1.45}$ and **b** CTAB/ $C_{10}G_{1.45}$ of various ratios vs. CTAB concentration. α_c is the molar fraction of CTAB in the surfactant mixture. Concentration of DNA = 0.2 mM. Dotted line corresponds to the onset of turbidity. Ionic strength was 0.1 by adding NaCl. $T = 25^\circ\text{C}$



DNA, the zeta potential of DNA as a function of CTAB concentration gives a sigmoidal curve and approaches zero when precipitation occurs, showing that before precipitation occurs, most complexes have already been neutralized. The addition of $C_{10}G_{1.45}$ leads to a similar result. Before the zero point of charge is reached, the zeta potential for the APG-containing system is always larger than that of the APG-free system probably due to the presence of APG molecules that drive the counterions nearer to the DNA with a similar result to electrostatic screening. In the case of more concentrated DNA solutions (2 mM), the complex assumes a high negative zeta potential until and even after the precipitation occurs. The zeta potential for the APG-containing system is larger than CTAB alone, too.

We chose two supernatant samples of DNA/CTAB/APG exactly where visible precipitation occurred (at 0.2 mM and 2 mM of DNA), and found that the average hydrodynamic radius for the 2 mM solution was one order of magnitude higher than for the 0.2 mM solution. This, combined with the zeta potential results, again supports the notion that the complexation of DNA/CTAB/APG at low DNA concentrations may resemble the formation of micelles in pure surfactant solutions: a hydrophobicity-driven cooperative process. At high DNA concentrations, the binding is dominated by electrostatic interaction and the resultant complex is still negatively charged until turbidity occurred.

Viscosity measurement results

Calf thymus DNA was used because it has longer chains, making it easier to detect the conformational changes. In addition, 0.1 M NaCl was added to avoid possible denaturation of DNA during the measurements that may lead to constant decrease of viscosity even without adding surfactant. Except for $\alpha_c = 1$, all viscosity curves go through a minimum (Fig. 9), indicating that the conformation DNA/surfactant aggregates is most compact at these surfactant concentrations. After the mini-

mum, larger assemblies formed which increases viscosity. The addition of APG not only shifts the minimum to smaller CTAB concentrations but decreases significantly the size of the formed complexes as indicated by a smaller viscosity. Note that also at $\alpha_c = 0.8$ the aggregate in the solution seems mostly compact (the strongest synergism for CTAB/ $C_{10}G_{1.45}$ also occurs at $\alpha_c = 0.8$, Figs. 6 and 7). This again supports the assumption that at low DNA concentration, complexation proceeds in a process similar to formation of mixed micelle, and the optimal packing of surfactants leads to smaller complexes.

Conclusions

The most important observations previously described are summarized as follows:

1. The addition of APG to CTAB/DNA has a significant influence on their phase behavior. At low DNA concentration, APG addition shifts the turbidity to lower CTAB concentrations. At high DNA concentration, however, APG exerts little influence on the phase behavior of CTAB/DNA. This leads to asymmetry of the phase map. The addition of salt can also lead to such phase map asymmetry.
2. Two different precipitating processes are described for low and high DNA concentrations: hydrophobicity- and electrostatics-driven processes. At low DNA concentrations, the precipitating process proceeds like the aggregation of common micelles. This process can be facilitated by either the addition of salt or APG. At high DNA concentrations, CTAB interacts with DNA through random electrostatic binding that is weakened by the addition of salt and is not influenced significantly by APG.
3. Light scattering and zeta potential measurements further support the above assumptions. Viscosity measurements at low DNA concentration showed that the addition of APG leads to the formation of

more highly-compacted aggregates. This may result from the optimal packing of the surfactant mixture, provided that at low DNA concentration the DNA-surfactant interaction is a hydrophobicity-driven process.

Acknowledgements The Chinese Natural Science Research Fund has supported this work (Grant No. 20233010). Dr. Jianjun Lu is acknowledged in particular for assistance with light scattering measurements and helpful discussion.

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