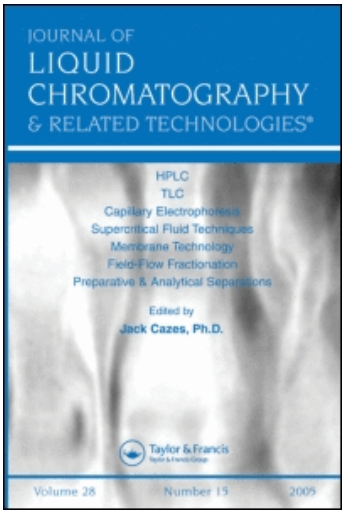

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Selective Precipitation of RNA, Supercoiled Plasmid DNA, and Open-Circular Plasmid DNA with Different Cationic Surfactants

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Abstract: Precipitation of RNA and plasmid DNA in clarified lysate produced from a bacterial culture was studied using three cationic surfactants with a different number of alkyl groups in the hydrophobic tail and cationic head. All the precipitation mixtures contained 0.5 M of NaCl. The results obtained indicated that selective precipitation was achieved when different types of cationic surfactants were used. In this study, trimethyltetradecylammonium bromide (TTAB) appeared to be a promising precipitant for precipitation of supercoiled plasmid DNA.

Keywords: Plasmid DNA precipitation, Supercoiled DNA, Cationic surfactants

INTRODUCTION

In the past decades, the administration of therapeutic genes to patients has become a reality in many clinical trials for preventing or treating various diseases. Non-viral vectors would be preferred in clinical applications to minimize the risk of viral infection. This increases the demand for highly purified plasmids for use in gene therapy and plasmid based vaccines. Supercoiled multimeric plasmid molecules are of particular interest for biopharmaceutical purposes because they contain multiple copies of a functional gene, and can thereby be more efficient in transfection experiments than the monomeric form.[1]

The majority of plasmid molecules isolated from prokaryotes, such as *E. coli*, are negatively supercoiled (SC) with a long, thin, and branched

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structure, which is better adapted to an active role in cell physiology. A fraction of the population of plasmid molecules also can exist in a relaxed or open circular (OC) form, with no coiling of the double helix. Other variants such as linear, denatured, or multimeric also can be present in the culture media. It is generally believed that the supercoiled form of plasmid DNA is the physiologically active conformation, and it is this form that is optimal for cell transfection.[2,3] Due to the coiled nature of the circular double helix, plasmid DNA is not necessarily a homogeneous product. Isolation from *E. coli* host cells usually comprises different forms of plasmid DNA because supercoiling is lost by a breakage of single or double strand, and this results in open circular or linear plasmid structures. The challenge in downstream processing is essentially aimed at eliminating cellular components of the host like bacterial protein, lipids, lipopolysaccharides, and nucleic acids. Chromosomal DNA fragments obtained after cell lysis, high molecular weight RNA (ribosomal and messenger RNA), and plasmid variants, which may be ineffective in transferring gene expression, are particularly difficult to separate from supercoiled plasmids due to the similarities in physical and chemical structure.

Precipitation has been recognized as an effective and perhaps selective initial recovery step in plasmid DNA preparation, and many precipitants have been used for this purpose, for example, polyethylene glycol,[4,5] spermine,[6] spermidine,[7] and cationic surfactant, such as cetyltrimethylammonium bromide (CTAB).[8–11] Lately, it was discovered that RNA and plasmid DNA can be selectively precipitated by adding inorganic salt, such as NaCl or NH_4Cl, into a mixture of CTAB and plasmid clarified lysate during the precipitation.[12] Addition of NaCl or NH_4Cl, which was thought to cause weaker interaction between the nucleic acid and the cationic surfactant, also led to the DNA pellet that was easier to be dissolved after precipitation, thus, the difficulty in solubilizing the DNA pellet, which was encountered in preceding research,[9] was overcome.

In the current study, precipitation of components in clarified plasmid lysate with 3 different cationic surfactants: cetyltrimethylammonium bromide (CTAB), trimethyltetradecylammonium bromide (TTAB), and ethylhexadecyldimethylammonium bromide (EDAB), was performed. Selectivity of these surfactants in precipitation of RNA and plasmid DNA of two different conformations, supercoiled and open-circular, was evaluated and compared. The result indicated that TTAB could be a promising precipitant in selective precipitation of the desirable supercoiled plasmid DNA from the open-circular one.

EXPERIMENTAL

Reagents

Cetyltrimethylammonium bromide (CTAB), trimethyltetradecyl-ammonium bromide (TTAB), ethylhexadecyldimethylammonium bromide

(EDAB), and sodium chloride (NaCl) were all of reagent grade (Sigma, MO, USA).

Plasmid Preparation

pSVβ plasmid (Promega, WI, USA) was transformed and propagated in *Escherichia coli* DH5α (Invitrogen Corp., CA, USA) using established procedures for plasmid DNA transformation.[13] After 14 hours of culturing, bacterial cells were harvested and plasmid lysate was prepared by the alkaline lysis method.[14]

Batch Precipitation of Plasmid Clarified Lysate with Cationic Surfactant

Clarified plasmid lysate was mixed with surfactant at various concentrations and with sodium chloride to the final concentration of 0.5 M. The mixtures were incubated overnight at 4°C before being centrifuged at 13000 g. The precipitates were resuspended in TE buffer and stored at −20°C for further analysis.

Analysis

A sample of 5 μL and resuspended precipitates were loaded into 0.8% agarose gel running with TAE buffer under 20 V for 3 hrs. The gel was stained with 0.5 μg/mL ethidium bromide. After UV visualization, the gels were scanned to estimate the band area of open-circular and supercoiled plasmid DNA using Scion Image Release beta 4.02 for Windows. The relative percentage of supercoiled (%SC) was determined as follows:

$$\%\,\mathrm{SC} = \frac{\text{Area of SC band}}{\text{Total area}}$$

where *Total area* is the summation of the peak area obtained from bands of supercoiled and open-circular plasmid DNA.

%SC and %OC (obtained from subtracting %SC from 100) were plotted in a bar chart against concentrations of surfactant. This chart will give a general view of relative quantity between supercoiled and open-circular plasmid, after precipitation with different surfactant concentrations.

RESULTS AND DISCUSSION

The molecular structures of these three surfactants are depicted in Figure 1. CTAB and EDAB have one C_{16}-alkyl group in their hydrophobic carbon

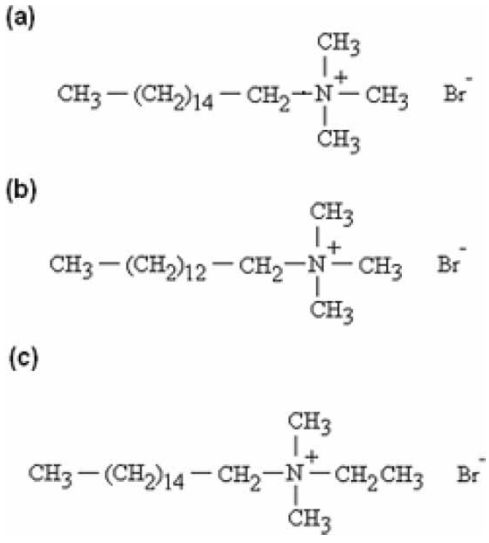

Figure 1. Molecular structures of (a) cetyltrimethylammonium bromide (CTAB), (b) trimethyltetradecylammonium bromide (TTAB), and (c) ethylhexadecyldimethylammonium bromide (EDAB).

chain compared with C_{14}-alkyl group in TTAB. The cationic heads of CTAB and TTAB similarly has three methyl groups, while that of EDAB has two methyl groups and one ethyl group. When used as precipitant in precipitation of components in clarified plasmid lysate, these three surfactant results in different contents of the precipitates.

When there was no NaCl in the mixture, CTAB could precipitate all the components, as shown in Figure 2. In Figure 3, which represents contents and

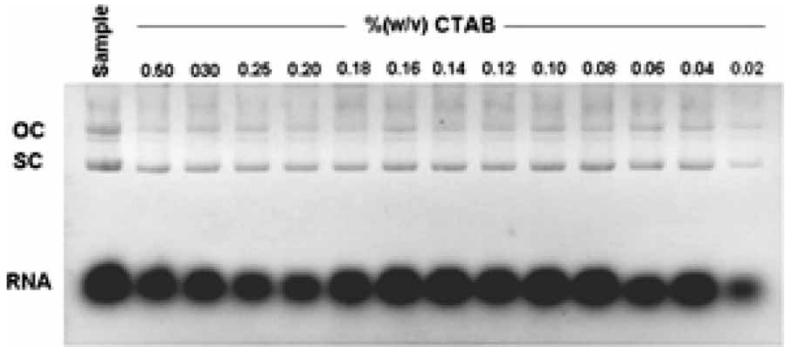

Figure 2. Agarose gel electrophoresis of the precipitates from clarified plasmid lysate using CTAB as precipitant with no NaCl addition.

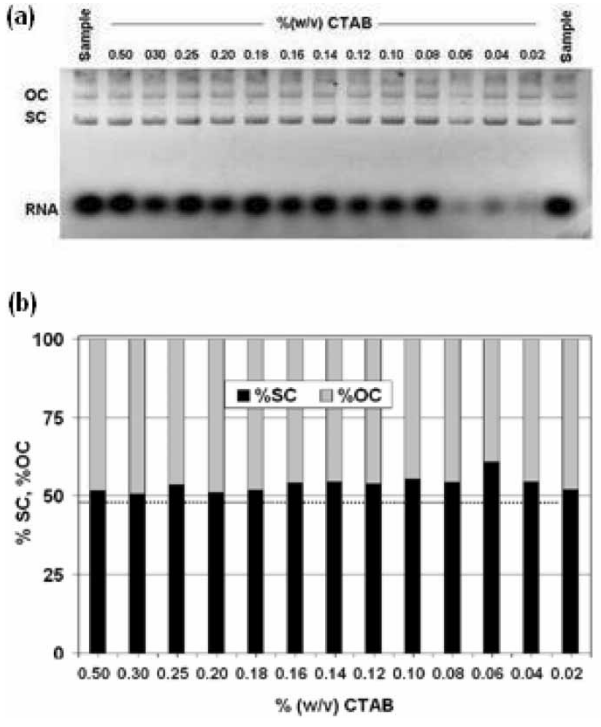

Figure 3. Precipitation of components in clarified plasmid lysate using CTAB containing 0.5 M NaCl. (a) agarose gel electrophoresis. (b) relative percentage of open-circular (OC) and supercoiled (SC) plasmid DNA in the precipitates.

relative percentages of supercoiled and open-circular in precipitates obtained after precipitation using CTAB containing 0.5 M NaCl, it is clearly shown that addition of NaCl created differential solubility between RNA and plasmid DNA. Agarose gel electrophoresis in Figure 3a shows that, in a solution of 0.08–0.50% (w/v) CTAB containing 0.5 M sodium chloride, RNA, open-circular (OC), and supercoiled plasmid DNA (SC) were all precipitated. Open-circular and supercoiled plasmid DNA were found to precipitate in a solution of very low concentration of this surfactant at 0.02, 0.04, and 0.06% (w/v), where only a slight amount of RNA was precipitated. As seen in Figure 3b, the relative percentage of open-circular and supercoiled plasmid DNA precipitated in each CTAB concentration was quite equal, and was not much different from that in clarified plasmid lysate, which contained 54% of supercoiled and 46% of open-circular plasmid DNA.

Precipitation of clarified plasmid lysate components using TTAB with no NaCl addition is as shown in Figure 4. Similar to CTAB, TTAB was capable to precipitate RNA, open-circular, and supercoiled plasmid DNA, even at concentrations as low as 0.04% (w/v). Figure 5 illustrates contents

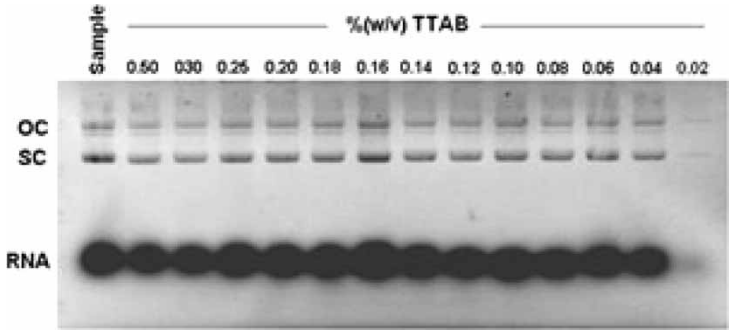

Figure 4. Agarose gel electrophoresis of the precipitates from clarified plasmid lysate using TTAB as precipitant with no NaCl addition.

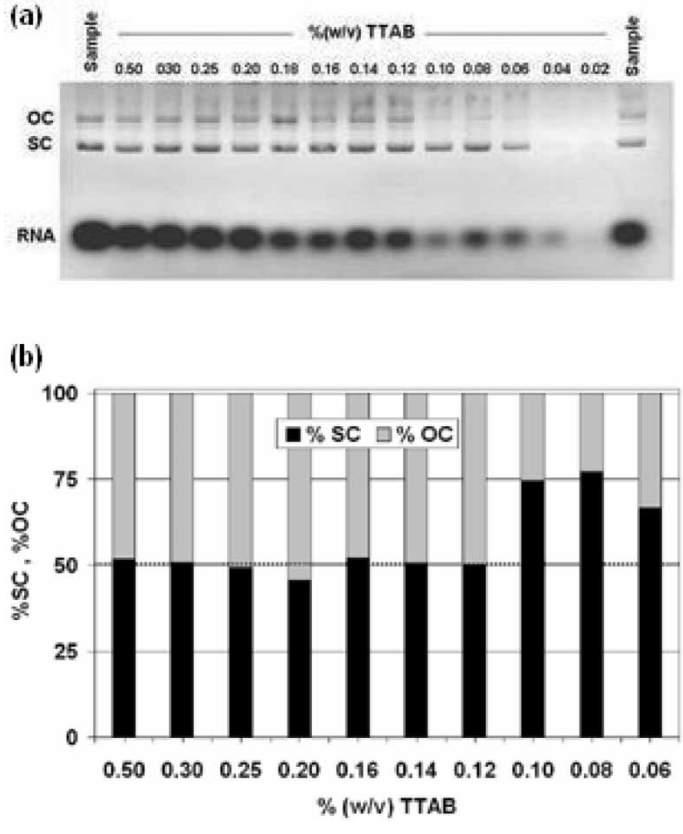

Figure 5. Precipitation of components in clarified plasmid lysate using TTAB containing 0.5 M NaCl. (a) agarose gel electrophoresis. (b) relative percentage of open-circular (OC) and supercoiled (SC) plasmid DNA in the precipitates.

and relative percentage of supercoiled and open-circular plasmid DNA from the precipitation with TTAB in the solution containing 0.5 M NaCl. In this system, RNA, open-circular plasmid DNA, and supercoiled plasmid DNA were all precipitated by TTAB at concentrations from 0.12% to 0.50% (w/v). At TTAB concentration of 0.06, 0.08, and 0.10% (w/v), reducing the amount of RNA was found in the precipitate. In addition to poor precipitation of RNA, open-circular plasmid DNA appeared not to be precipitated well at low TTAB concentrations, as indicated by a faint band of open-circular plasmid DNA in the agarose gel. The relative percentage of supercoiled plasmid DNA calculated for the precipitates obtained from precipitation using 0.06, 0.08, and 0.10% (w/v) TTAB were 75%, 77%, and 67%, respectively, which was approximately 25 to 40% higher than that in the clarified plasmid lysate. TTAB at a concentration of 0.04 slightly precipitates RNA from the clarified lysate, and both 0.04 and 0.02% (w/v) nearly did not cause precipitation of open circular and supercoiled plasmid DNA.

When NaCl was not added into the mixtures, EDAB was very efficient in precipitating RNA and plasmid DNA, as seen in Figure 6. Agarose gel electrophoresis in Figure 7a shows that with 0.5 M of NaCl in the solution, EDAB at concentrations from 0.02 to 0.16% (w/v) selectively precipitated plasmid DNA more than RNA. The relative percentage of supercoiled plasmid DNA, shown in Figure 7b, was observed to be about 64, 65, and 66% when 0.06, 0.08, and 0.12% (w/v) EDAB was used, respectively, which was approximately 20% higher than that in the clarified lysate sample. EDAB at very low concentration, 0.02% (w/v) EDAB, caused very slight precipitation of RNA, open-circular, and supercoiled plasmid DNA.

Complexes between DNA and cationic surfactant are formed through interaction of the negatively charged DNA phosphate groups with surfactant counterions, and stabilized by hydrophobic interaction of the hydrocarbon tails of the surfactant molecules.[15] For the surfactant that possesses the

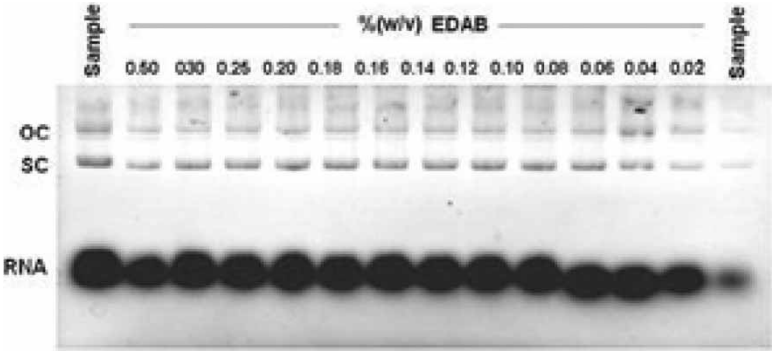

Figure 6. Agarose gel electrophoresis of the precipitates from clarified plasmid lysate using EDAB as precipitant with no NaCl addition.

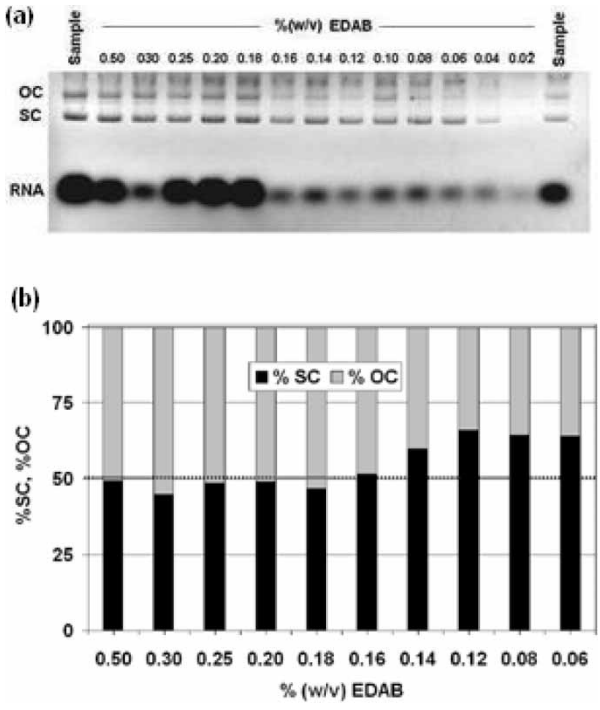

Figure 7. Precipitation of components in clarified plasmid lysate using EDAB containing 0.5 M NaCl. (a) agarose gel electrophoresis. (b) relative percentage of open-circular (OC) and supercoiled (SC) plasmid DNA in the precipitates.

same ionic head, the one that has a shorter hydrophobic tail is thought to interact less strongly and with less coordination, because the gain in energy due to the inclusion of the hydrophobic tails in a micelle like environment is lower.[16] The surfactant with the longer chain length binds more easily to DNA, leading to the formation of precipitate for smaller amounts of the DNA. In other words, a large amount of the shorter chain length surfactant is needed to induce the compaction of DNA molecules, and a smaller amount of the longer chain length surfactant is needed to promote the phase separation. This is an indication of the importance of the hydrophobic interaction between the surfactant molecules, and evidence of the known fact that complexes are formed with DNA and surfactant aggregates.[17] This reason could explain TTAB at low concentration was less efficient than CTAB in precipitating the components in plasmid lysate.

When comparing CTAB with EDAB, which have the same length of hydrophobic alkyl chain, EDAB appeared to be less efficient than CTAB in precipitation of the component in clarified plasmid lysate, especially at a concentration lower than 0.16% (w/v). This could be the result from the

difference in the size of the cationic head of these two surfactants. The cationic head of CTAB is made of 3 methyl groups, while that of EDAB is made of 2 methyl groups and an ethyl group. The ethyl group might obstruct the positive charge of the surfactant to interact with the negative charge of the phosphate group on the molecule of nucleic acid.

CONCLUSIONS

Results obtained from batch precipitation of clarified plasmid lysate using 3 cationic surfactants with different molecular structures show that the length of hydrophobic alkyl chain and the size of cationic head of the surfactant could have an impact on the components being precipitated. In addition, RNA, open-circular, and supercoiled-plasmid DNA can be selectively precipitated when an inorganic salt like sodium chloride was added into the surfactant solution. From this study, TTAB at concentrations of 0.06 to 0.10% (w/v) containing 0.5 M NaCl appeared to be a promising precipitant to precipitate supercoiled plasmid DNA, since this surfactant preferably precipitated supercoiled plasmid DNA more than the open-circular one.

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