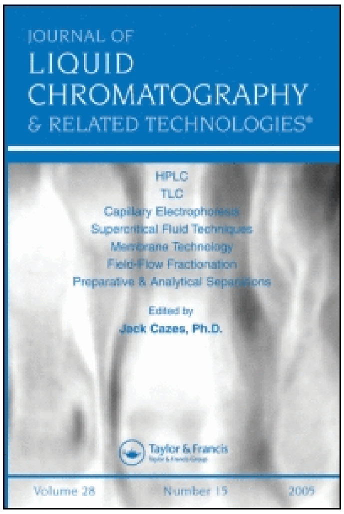

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Transition Between Supercoiled and Open Circular Plasmid DNA During Alcohol Precipitation

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ABSTRACT

Precipitation of a 6.9 kb pSVβ plasmid DNA using three different alcohols, i.e., methanol, ethanol, and 2-propanol, as precipitating agents was performed. Precipitate composition was analyzed, and it was found that the quantity of supercoiled plasmid DNA recovered varied according to type and concentration of alcohol. Precipitation with 2-propanol concentration greater than 70% (v/v) resulted in mostly open circular plasmid DNA, which could be converted back to supercoiled by diluting 2-propanol concentration in the process. It appeared in this study that 2-propanol at high concentration could affect supercoiling of the plasmid, and lead to the temporary supercoiled-open circular transition without covalent breaking of the strands. Supercoiled DNA quantity slightly

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decreased in precipitation with methanol at high concentration, but remained quite constant in precipitation using ethanol.

Key Words: Alcohol precipitation; Supercoiled DNA; Plasmid DNA conformational transition.

INTRODUCTION

Recently, a novel separation system, centrifugal precipitation chromatography (CPC), has been developed. Conceptually, a moving concentration gradient of precipitating agent is generated through a long separation channel under a centrifugal force field. Biomolecules of interest undergo a repetitive process of precipitation–dissolution and, thus, separation according to their solubility in precipitating agents with different concentrations. This system eliminates various complications, such as adsorptive sample loss and deactivation caused by solid support. It is also quite flexible to manipulate and scale-up. CPC has been used to successfully fractionate protein mixtures, purify monoclonal antibody from cell culture supernatant,[1] and separate recombinant ketosteroid isomerase from a crude *Escherichia coli* lysate.[2]

In an attempt to apply CPC to the separation of supercoiled plasmid DNA from a DNA mixture, selection of the most suitable precipitating agent is crucial. Alcohol, such as ethanol or 2-propanol are commonly used to precipitate the plasmid during a plasmid production process. The study of alcohol precipitation of supercoiled and open circular plasmid DNA will, thus, provide information for selecting the alcohol precipitating agent for supercoiled DNA isolation in CPC. In this study, batch precipitation of a 6.9 kb pSVβ plasmid DNA showed that methanol, ethanol, and 2-propanol, with concentrations between 40–90% (v/v), resulted in different quantities of supercoiled pSVβ plasmid DNA after precipitation.

EXPERIMENTAL

Plasmid Preparation

pSVβ plasmid (Promega, WI) was transformed and propagated in *E. coli* DH5α (Invitrogen Corp., CA) using established procedures for plasmid DNA transformation.[3] After 14 hr of culturing, bacterial cells were harvested and plasmid lysate was prepared by the alkaline lysis method.[4]

Alcohol Precipitation of pSVβ Plasmid DNA

Plasmid lysate was mixed with alcohol until desired final alcohol concentrations were reached. Salts, such as ammonium acetate, were not added in the mixtures in order to eliminate a possible effect of salt on plasmid DNA structure. The mixtures were then centrifuged and the precipitates and supernatants were separately collected for further analysis.

Analysis

Absorbance at 260 (A_{260}) and 280 (A_{280}) nm of precipitates and supernatants was measured using a spectrophotometer (Shimatzu, UV-160). Concentration of total DNA was calculated from the absorbance at 260 nm: an A_{260} of 1.0 corresponds to a 50 μg/mL double stranded DNA solution.

Precipitates and supernatants obtained from alcohol precipitation of pSVβ plasmid DNA were subjected to 0.8% agarose gel electrophoresis in wide mini-Sub Cell® GT system (Biorad 170-4-420) at 75 V for 80 min. Gel that was stained with EtBr was visualized, and band intensities on the gels were converted into peak areas using Scion Image Release Beta 4.0.2 for Window (Scion Corporation, MD). A percentage of supercoiled or open-circular was determined by dividing their peak areas with that of total DNA, which was the summation of the two.

RESULTS AND DISCUSSION

Percent precipitation of pSVβ plasmid DNA was calculated as the ratio of A_{260} of precipitates to A_{260} of the starting sample. As seen from Fig. 1, only 50% (v/v) of 2-propanol was used to precipitate more than 80% of the pSVβ plasmid from the sample solution, while 60% and 70% of ethanol and methanol, respectively, were required in order to achieve the equal percent precipitation. This result is expected as addition of alcohols lowers the dielectric constant of a solution and, hence, reduces the solvating power of the solvent (water, in this case) causing a precipitate to form.[5] The lower the dielectric constant of the alcohol molecule, the higher its ability to pull down the dielectric constant of the solvent. Therefore, less 2-propanol ($\varepsilon_{\text{2-propanol}} = 18.3$, at 25°C) was needed to precipitate the plasmid than that of methanol or ethanol ($\varepsilon_{\text{methanol}} = 32.6$ and $\varepsilon_{\text{ethanol}} = 24.3$ at 25°C).

Figures 2(a)–(c) depict agarose gel electrophoresis of pSVβ precipitates obtained from precipitation using methanol, ethanol, and 2-propanol, respectively. Faint bands in lane 1 in Fig. 2(a) indicate a small quantity of plasmid

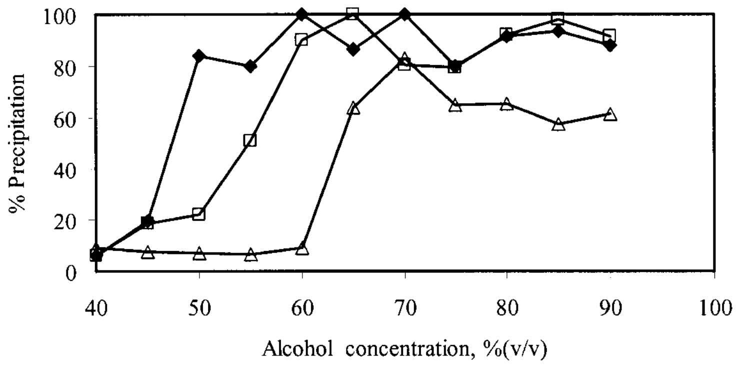

Figure 1. Percent precipitation of pSVβ plasmid DNA when precipitated with methanol (△), ethanol (□), and 2-propanol (◆).

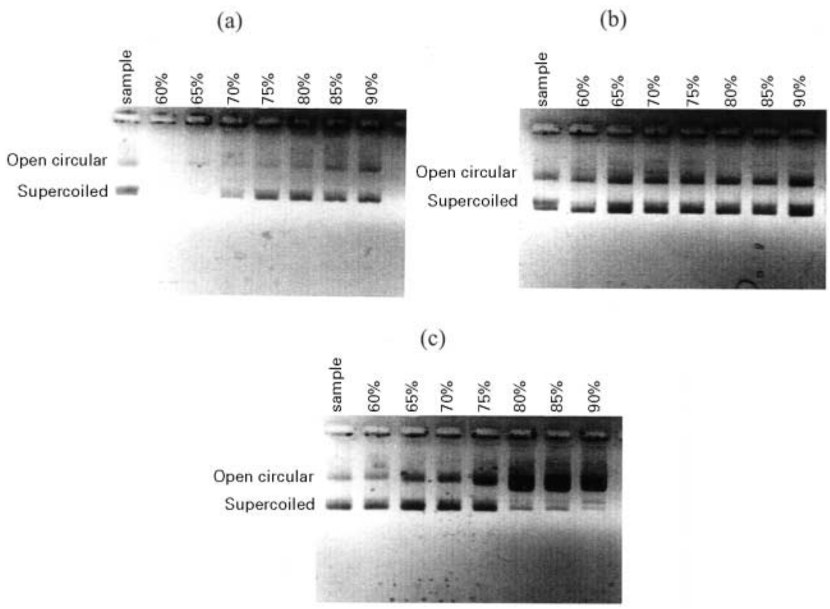

Figure 2. Agarose gel electrophoresis of pSVβ after precipitation with (a) methanol, (b) ethanol, and (c) 2-propanol. Lane marked "sample" is pSVβ before precipitation, and percentages above other lanes indicate percent by volume of alcohol used for precipitation.

DNA, which agrees with the low ability of 60% (v/v) methanol in precipitating plasmid DNA. For precipitates obtained from 2-propanol precipitation, the intensity of the bands representing open circular pSVβ plasmid DNA increased when the concentration of 2-propanol increased, while those of supercoils continuously decreased until nearly disappearing at 2-propanol concentration of 90%. It appeared as if the transition from supercoiled to open circular forms of pSVβ plasmid occurred when concentrations of 2-propanol were increased. Quantities and percentage of supercoiled pSVβ plasmid DNA were determined and are shown in Figs. 3 and 4, respectively. We found that at concentrations of 2-propanol higher than 70% (v/v), percentages and quantities of supercoiled pSVβ in the precipitates drastically decreased. At 2-propanol concentrations higher than 80% (v/v), less than 10 mg/L of supercoiled pSVβ was recovered. Precipitates resulting from high concentrations of methanol showed slightly decreased yields of supercoiled pSVβ as well. However, yield and percentages of supercoils obtained from precipitation using ethanol remained fairly constant.

Previous studies have reported that water appears to stabilize the DNA double helix and removal of water by alcohol molecules could cause destabilization of the DNA double helix,[6,7] or could be a reason for the presence of a particular DNA structure found in alcohol solution.[8] It was, also, found that hydrophobic interactions between alcohols and nucleic acid bases were relatively important in the process of DNA denaturation.[7] Therefore, we postulated here, that water might have been displaced from the plasmid molecules and 2-propanol could have interacted with the base component of the plasmid DNA, causing dramatic conformational transition from supercoiled to open circular as observed. Since solubility parameters of nucleic acid bases are

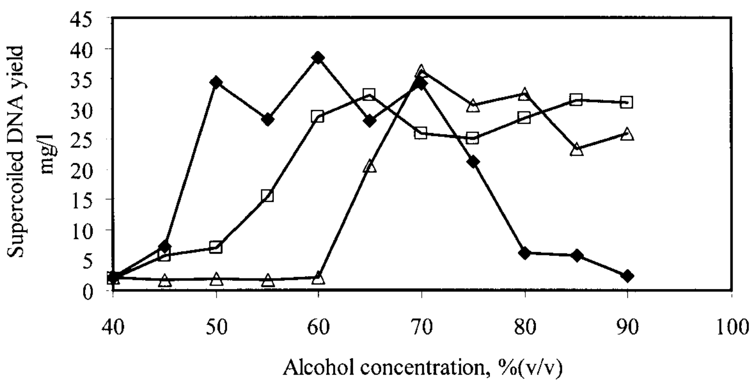

Figure 3. Yield of supercoiled pSVβ plasmid DNA (mg of supercoiled per liter of culture broth) after precipitation with methanol (△), ethanol (□), and 2-propanol (◆).

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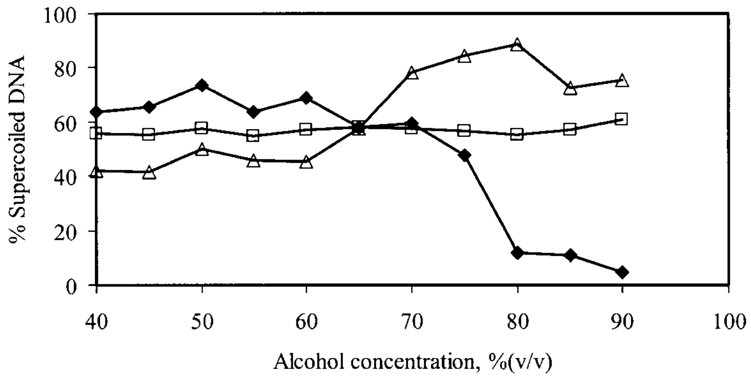

Figure 4. Percentages of supercoiled pSVβ plasmid DNA in the precipitates from methanol (△), ethanol (□), and 2-propanol (◆) precipitation.

varied in each alcohol solution,[7] different results were obtained in precipitation using methanol and ethanol.

We conducted experiments to examine if the conformation of DNA as open circular after precipitation with high concentration of 2-propanol was permanent. In this particular experiment, 2-propanol was added into the plasmid DNA solution until the final concentration of 90% (v/v) was reached. The mixture was then incubated at 4°C for 30 min before it was divided into three parts with equal volume. The first part was centrifuged at 12000 *g* in the microcentrifuge for 20 min; the precipitate and supernatant were collected for further analysis. TE buffer was added to the second and the third part of the mixture until the final concentration of 2-propanol was decreased to 75% and 60% (v/v), respectively. The diluted mixtures were incubated for 10 min at 4°C before they were centrifuged. Precipitates and supernatants were collected for the analysis as described previously. The experiment was repeated with different incubation times of the diluted mixtures.

As shown in Fig. 5, pSVβ-plasmid DNA was mostly in open circular form when it was mixed with 2-propanol to the final alcohol concentration of 90% (v/v) (at time = 0). At this point, only about 5% of plasmid DNA was supercoiled. Dilution of 2-propanol concentration before precipitation resulted in precipitates with higher percentages of supercoiled pSVβ. When the diluted mixtures were incubated longer before they were centrifuged, the percentage of supercoiled DNA was further increased and approaching the values showed in Fig. 3 for corresponding 2-propanol precipitation. Dilution of 2-propanol from 90% (v/v) to 60% (v/v) gave slightly higher supercoils percentages

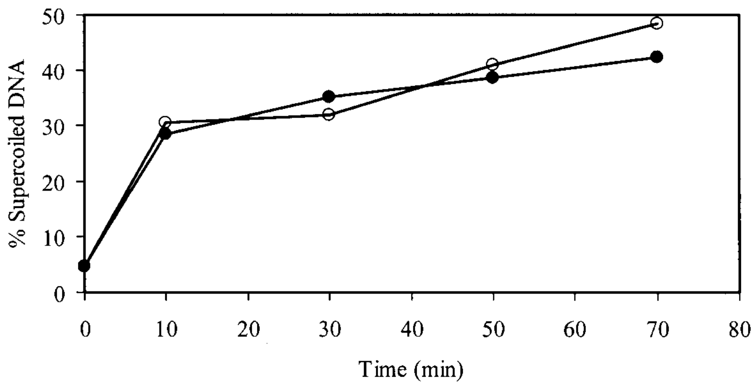

Figure 5. Percentages of supercoiled DNA after 2-propanol concentration was diluted from 90% to 75% [●] and 60% [○].

than dilution to 75% (v/v), which could have resulted from higher differences in 2-propanol concentrations, in which the plasmid was consecutively dissolved.

Observation of the temporary transition in conformation of pSVβ plasmid DNA due to the change in 2-propanol concentration, agrees with the conclusion that the denaturing effect of the alcohols on DNA is reversible.[9] 2-Propanol at high concentration might have affected supercoiling of pSVβ plasmid, and led to open circular conformation without covalent breaking of the nucleic acids strands, as no linear DNA could be observed.

CONCLUSION

Quantities of supercoiled pSVβ plasmid DNA remained in precipitates after alcohol precipitation with varied types and concentrations of alcohol molecules. The ability of methanol, ethanol, and 2-propanol to remove water from molecules of pSVβ plasmid DNA, as well as interaction between alcohol and nucleic acid bases, may be the cause of supercoiled-open circular transition occurring during alcohols precipitation of pSVβ plasmid.

In this study, 2-propanol was the most efficient precipitating agent, as yields of 36, 32, and 38 mg supercoiled pSVβ per liter of culture broth were obtained from precipitation using 70% (v/v) methanol, 65% (v/v) ethanol, and 60% (v/v) 2-propanol, respectively. However, 2-propanol appeared to strongly induce transition from supercoiled to open circular conformation during precipitation. Since supercoiled plasmid DNA is thought to be more effective at transferring gene expression than open-circular, linear, multimetric, or partially denatured DNA,[10] results of this study could be useful in selecting the appropriate precipitating agent for plasmid DNA precipitation, especially if a high content of supercoiled plasmid is a critical goal.

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