

Highly Concentrated Ethanol Solutions: Good Solvents for DNA as Revealed by Single-Molecule Observation

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We observed single DNA molecules at different ethanol concentrations by using fluorescence microscopy. Large single DNA molecules undergo reentrant conformational transitions from elongated coil into folded globule and then into elongated coil state, accompanied by the increase of the concentration of ethanol in a low-salt aqueous environment. The second transition from globule into the coil state occurs at around 70% (v/v) ethanol. From circular dichroism (CD) measurements, it is confirmed that the reentrant transition of the higher order structure proceeds together with the transitions of the secondary structure from B to C and, then, from C to A in a cooperative manner. The determined mechanism of the reentrant transition is discussed in relation to the unique characteristics of solutions with higher ethanol content, for which clathrate-like nanostructures of alcohol molecules are generated in the surrounding water.

Ethanol precipitation is often used to obtain DNA molecules extruded from cells. An ethanol solution of around 70% (v/v) is commonly used to induce DNA precipitation.^[1] A decrease in ionic dissociation at lower dielectric constants in the presence of ethanol is considered to be the mechanism that underlies DNA precipitation. The dielectric constant of ethanol is approximately one third that of water, which could lead to the expectation that a higher concentration of ethanol would be more favorable for obtaining precipitates of DNA. However, protocols do not recommend the use of an ethanol concentration above 70% (v/v) for DNA precipitation.^[1] Although it is not clear why currently available experimental protocols avoid higher concentrations there seems to be an expectation that

70% (v/v) ethanol is suitable for avoiding contamination from cell extracts.

It has been a long-standing puzzle why the entropy of an ethanol solution is much lower than that expected theoretically, based on the randomly mixed state.^[2] This phenomenon has been attributed to the formation of icelike or clathrate-like nanostructures with alcohol molecules in the surrounding water.^[3] Based on neutron-diffraction measurements in a methanol–water mixture with a molar ratio of 7:3, it has been reported that most of the water molecules exist as small hydrogen-bonded strings and clusters surrounded by close-packed methyl groups.^[4] Since that report, several experimental and theoretical studies have suggested the formation of clusters of alcohol molecules.^[5] In spite of these recent studies stimulated by the results of neutron diffraction, as far as we are aware, there seems to be no studies on specific chemical and/or biochemical properties associated with the formation of such nanosized molecular clusters, including abnormality of the solvability of biomacromolecules and/or polyelectrolytes.

In the present study, we performed single-molecule observations of large genomic DNA molecules in ethanol–water solutions under low-salt conditions. We found that individual DNA molecules exhibit a folding transition from an elongated coil to a compact state upon an increase of the ethanol concentration to above 50% (v/v). Interestingly, large genomic DNA molecules undergo an unfolding transition from a compact to an elongated coil state upon a further increase in the alcohol concentration up to 80% (v/v) in an ethanol–water solution under low-salt conditions. We discuss the unexpected phenomenon of decondensation of DNA in relation to the nanoscaled segregation of the ethanol–water solution.

Figure 1 (left) shows examples of fluorescence microscopic images of single λ -DNA molecules at different ethanol concentrations, where the DNA molecules are undergoing translational and intrachain Brownian motion.^[6] The histograms in Figure 1 (right) shows the long-axis length, L , where L is defined schematically at the top of the histograms. At a low concentration of ethanol, that is, at 0–40% (v/v), DNA molecules assume an elongated coil conformation that exhibits significant intrachain Brownian motion. At 50% (v/v), around three quarters of the DNA molecules display a coil conformation, and the other quarter exhibits a folded compact conformation, as shown in the histograms. At 60% (v/v) ethanol, more than half of the DNA molecules exhibit a folded compact state, which is characterized as a bright optical dot with relatively large translational Brownian motion. Upon a further increase in the ethanol concentration up to 70% (v/v), almost all of the

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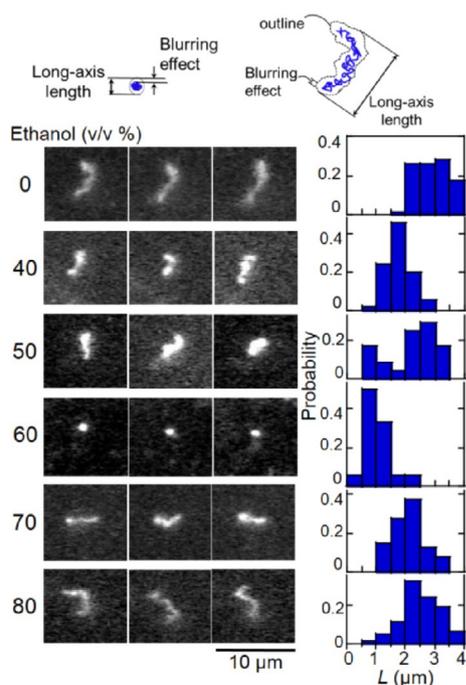


Figure 1. Observation of single DNA molecules at different ethanol concentrations. Left: Fluorescence microscopy images of single λ -DNA molecules undergoing intra- and intermolecular fluctuation in solutions with different ethanol concentrations. The time interval is 0.5 s. Right: Probability distribution of long-axis length, L (see the top scheme for a definition) of DNA in solution (λ -DNA, 30 μm in nucleotide units).

DNA molecules assume an elongated coil conformation, thus indicating a reentrant transition. At 80% (v/v), L reverts to the level seen at low ethanol concentrations. Thus, it becomes clear that the essential features of the DNA conformations are the same at low concentrations, 0–40% (v/v), and at a high concentration, 80% (v/v).^[7]

To study the change in the secondary structure of DNA depending on the ethanol concentration, we measured the circular dichroism (CD) spectra of λ -DNA. As shown in Figure 2, for the spectra obtained at 0 and 40% (v/v), the positive band at around 278 nm and the negative band at around 248 nm indi-

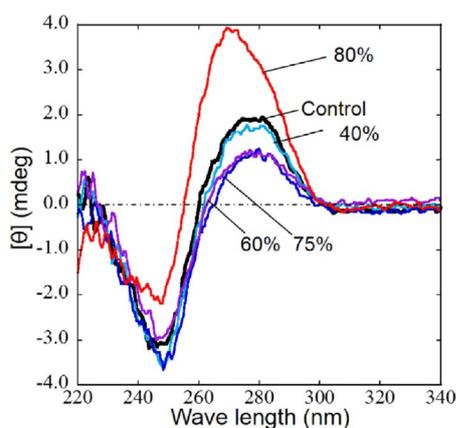


Figure 2. CD spectra of DNA (λ -DNA, 30 μm in nucleotide units) at different ethanol concentrations.

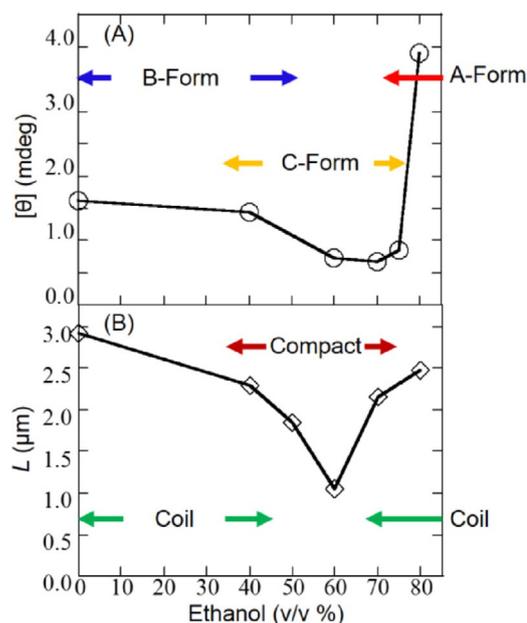


Figure 3. Comparison of the effect of the ethanol concentration between secondary (A) and higher-order structures (B) of λ -DNA, as evaluated by CD spectra and fluorescence microscopy, respectively. In (A), ellipticity, $[\theta]$, at 270 nm is given for the same conditions as in Figure 2.

cate that the secondary structure is in the B form. A significant decrease in the positive Cotton effect around 280 nm was observed at 60 and 70% (v/v) ethanol, which implies a transition from the B form to a C-like form in the secondary structure of DNA. A further increase in the ethanol concentration causes a change to an A-like conformation, as revealed by the significant increase in the positive Cotton band at around 270 nm.^[8]

The CD measurements indicate that the secondary structure of DNA exhibits a successive change, B \rightarrow C \rightarrow A, accompanied by a reentrant transition of the higher order structure, coil \rightarrow compact \rightarrow coil conformation (Figure 3).^[9] These experimental observations suggest that a large DNA molecule undergoes an unfolding transition from a compact to an elongated coil state upon an increase in the ethanol concentration from 60 to 80% (v/v).

Interestingly, a previous study involving a neutron-scattering experiment suggested the formation of nanoclusters of water molecules at high alcohol concentrations.^[4] Thus, the unfolding transition of compact DNA at higher ethanol concentrations is attributable to the preferred association of such nanoclusters of water with double-stranded DNA. Through this hydration mechanism, the phosphate groups dissociate to a negatively charged state, accompanied by the release of counter cations to the neighboring water-rich environment. As a result, as highly charged polyelectrolytes, solutions with a high ethanol concentration become good solvents for DNA.

Finally, we addressed the effect of the reentrant transition at a macroscopic level. Figure 4 shows visual images of an ethanol solution of 1.0 mM calf thymus DNA. In the absence of NaCl, a precipitate is generated at 60% (v/v) ethanol, whereas DNA is fully solvable at 80% (v/v) ethanol, as indicated by the clear solution. With the addition of 200 mM NaCl, precipitates

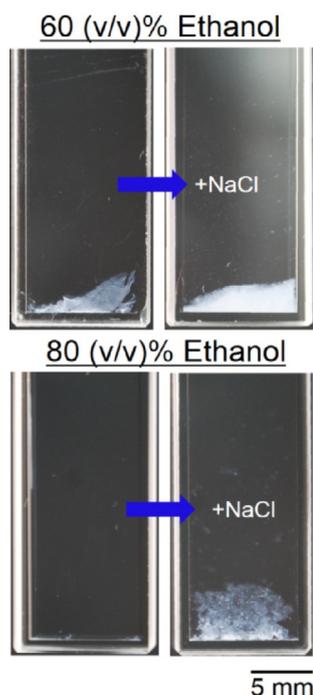


Figure 4. Visual inspection on the solvable state of DNA. For comparison, photographs are shown for 60 and 80% (v/v) ethanol solutions of calf thymus DNA (1.0 mm in nucleotide units) in the absence and presence of 200 mM NaCl.

of DNA are seen for both the 60 and 80% (v/v) solutions, which corresponds to the well-known phenomenon of 'ethanol precipitation'.

Nowadays, ethanol precipitation is routinely used in molecular biology, as a result of many previous efforts to improve the efficiency of the isolation and purification of natural DNA molecules. However, less attention has been given to physicochemical approaches in the great efforts to establish the protocol of ethanol precipitation. The interesting property of highly concentrated ethanol solutions that was reported in the present study may lead to further findings with regards to the stability of a rich variety of macromolecules, including proteins and synthetic polyelectrolytes. Future research in this area could involve physicochemical studies concerning the state of water molecules adjacent to DNA molecules and/or charged chemical species, where the application of neutron diffraction would provide information on the unique property of highly concentrated ethanol solutions.

Experimental Section

λ -DNA (48 kbp) was purchased from Nippon Gene (Toyama, Japan) and calf thymus DNA was obtained from Wako (Osaka, Japan). The fluorescent cyanine dye quinolinium [(1,1'-[1,3-propanediyl-bis[(dimethyliminio)-3,1-propanediyl]]bis[4-[(3-methyl-2(3H)-benzoxazolylidene)-methyl]]]tetraiodide) was purchased from Molecular Probes Inc. (Eugene, OR, USA). 2-Mercaptoethanol (2-ME) and other chemicals were obtained from Wako Pure Chemical Industries (Osaka, Japan). The cyanine dye YOYO-1 at a concentration of 0.2 μ M was

added to the DNA solution, together with the antioxidant 2-ME [4% (v/v)], to visualize individual DNA molecules by fluorescence microscopy. Details of the experimental conditions for the observation of single DNA molecules was essentially the same as in our previous study.^[10] Observations were carried out at around 24 °C with a DNA concentration of 30 μ M in nucleotide units. Fluorescence images of DNA molecules were observed using a microscope (Axiovert 135 TV, Carl Zeiss, Germany) equipped with an oil-immersed 100 \times objective lens, and recorded on DVDs by using a highly sensitive electron-bombarded charge-coupled device (EBCCD) (Hamamatsu Photonics, Hamamatsu, Japan). The recorded video images were analyzed with the freely available image-processing software ImageJ (National Institute of Mental Health, MD, USA). CD spectra of λ -DNA (30 μ M in nucleotide units) dissolved in ethanol–water solutions were measured at 25 °C with a CD spectrometer (J-720w, JASCO, Japan). The cell path length was 1 cm. Measurements were performed at a scan rate of 100 nm min⁻¹ and CD spectra were obtained as the accumulation of three scans.

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