Laser Trapping of an Individual DNA Molecule Folded Using Various Condensing Agents

Yukiko Matsuzawa,*,† Ken Hirano,† Kohei Mori,†
Shinji Katsura,† Kenichi Yoshikawa,‡ and Akira Mizuno†

Department of Ecological Engineering
Toyohashi University of Technology
Toyohashi, Aichi, 441-8580, Japan
Department of Physics, Kyoto University
Kyoto, 606-8502, Japan

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Conformational change of DNA from a coiled state into a globular state is induced by various condensing agents, such as PEG (poly(ethylene glycol)) in the presence of low-molecular weight cation, inorganic multivalent cation, polyamine, cationic surfactant, etc.[1] The DNA strand is formed into a hexagonal arrangement [2−4] and shaped into a toroidal or a rod structure by condensing agents,[5,6] so that the effective volume of the DNA decreases on the order of $\sim 10^{-4}$. Thus far, most studies concerning laser trapping of a biopolymer have been carried out by anchoring the polymer chain to a micrometer-sized latex bead.[7] Recently, however, effective laser trapping without the use of a latex bead has been shown to be possible for compacted DNA.[8,9] Even though various mechanisms by which different chemical agents induce DNA condensation have been examined, the optimum conditions for noninvasive trapping of macromolecules remain unclear. In the present contribution, we report the difference in effectiveness of trapping or manipulating globular DNA for three different condensing agents: PEG6000 (poly(ethylene glycol) 6000, from Nihon Oils and Fats Co Ltd.)-$MgCl_2$, spermidine (Nacalai Tesque Inc.), and CTAB (cetyltrimethylammonium bromide, Tokyo Kasei Kogyo Co., Ltd.).

T4 phage DNA (166 kbps, NipponGene) was used in the present study. The DNA was mixed with the condensing agents, DAPI (4′,6-diamidino-2-phenylindol), as a fluorescent dye, and 2-ME (2-mercaptoethanol), as an anti-oxidant for suppression of photobleaching, in a 20 mM MOPS (3-(*N*-morpholino)propanesulfonic acid) buffer (pH 7.2). The final concentrations were as follows: 20 mM MOPS buffer, 0.3 μM DNA in nucleotide, 0.6 μM DAPI, 2%(v/v) 2-ME. The samples were maintained for 2 h at room temperature. The critical concentrations necessary to induce DNA condensation completely under each condition were measured by changing the concentration of condensing agents [10] and were found to be [PEG] = 60 mg/mL, [$MgCl_2$] = 30 mM; [spermidine] = 159 μM; [CTAB] = 50 μM.

Figure 1 exemplifies trapping on globular DNA using a Nd:YAG laser (1064 nm). In response to the mechanical motion of the microscope stage, the trapped DNA (depicted by the closed arrow) remained in the same position, showing no noticeable change in conformation, whereas the free DNA (depicted by the opened arrow) moved from right to left (Figure 1a−c). When the rate of stage motion exceeded a critical value, the trapped DNA was released from the laser focal point (Figure 1d).[11]

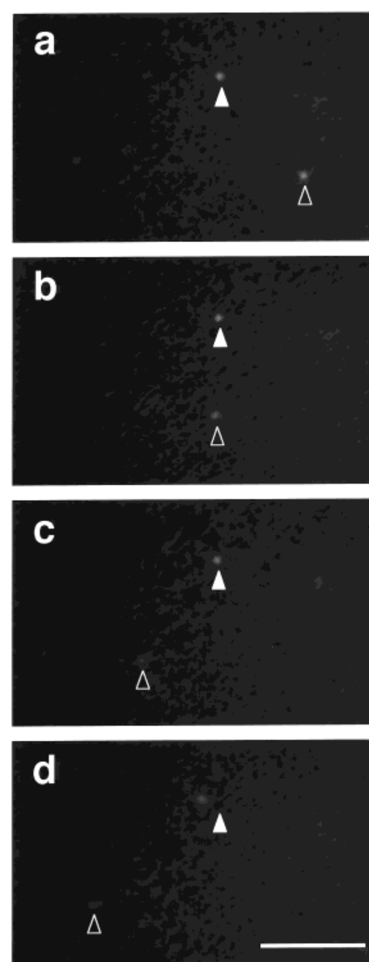

Figure 1. Trapping of globular DNA using a Nd:YAG laser. Scale bar indicates 15 μm. The trapped DNA molecule is indicated by the closed arrows, and the free DNA molecule is indicated by the open arrows. (a−c) The microscope stage was moved from right to left, and as a result the free DNA moved along with the stage. (d) The trapped DNA was released from the laser focus point when the critical rate of stage motion was exceeded.

Optically trapped DNA can be manipulated in any horizontal direction (as shown in Figure 1). Horizontal force (F) can be estimated by Stokes' law as follows:

$$F = 6\pi\eta R v$$

where η, R, and v are the viscosity of the solvent, the hydrodynamic radius of the globular DNA, and the critical velocity, respectively. The viscosity of the solvent was measured using a TOKIMEC Visconic ELD viscometer at 22 ± 0.2 °C, and the values obtained in PEG-$MgCl_2$, spermidine, and CTAB are 2.77, 1.06, and 0.95 mPas, respectively. The size of the globular DNA as judged from the fluorescence image is overestimated due to a blurring effect in the fluorescence image. From the measurement of the Brownian motion on the individual DNA molecules using fluorescence microscopy, the time-dependent translational dis-

* Author to whom correspondence should be addressed.
† Toyohashi University of Technology.
‡ Kyoto University.

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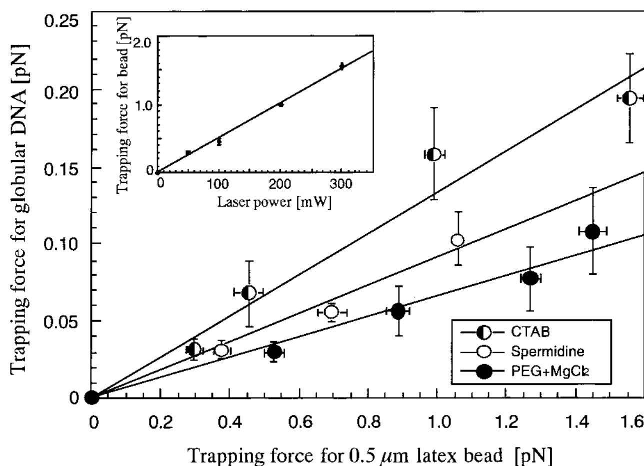

Figure 2. Change in trapping force for globular DNA with respect to the trapping force for 0.5 μm latex beads. Laser trapping was performed at 50, 100, 150, 200 mW. (insert) Relationship between the trapping force for 0.5 μm latex bead and laser power.

placement of individual globular DNA was measured in a quantitative manner to estimate the diffusion coefficient. The hydrodynamic radii were thus evaluated from the diffusion coefficient of the DNA molecules based on the Stokes−Einstein relationship:[12] 58 ± 5 nm, 51 ± 6 nm, and 95 ± 19 nm for the DNA with PEG-$MgCl_2$, spermidine, and CTAB, respectively.

The trapping force of the laser depends not only on the laser power but also on the optical conditions. Therefore, rather than varying laser power, we used the trapping force for 0.5 μm latex beads, which is proportional to laser power (see insert figure in Figure 2).[13] At a fixed laser power, we measured the critical velocity for the latex bead and each globular DNA. The critical velocities of the globular DNAs enabled by CTAB and spermidine were several times faster than those enabled by PEG-$MgCl_2$. Figure 2 shows the relationship between the trapping force for globular DNAs with the reference to that for the 0.5 μm latex bead. This enables comparison of the trapping force for different optical conditions. Spermidine causes DNA to condense in an ion-exchange reaction between counterions on DNA and spermidine,[14] and PEG is thermodynamically unfavorable to DNA. PEG causes the compaction of DNA with crowding effect, i.e., the incompatibility between flexible and stiff polymers.[10] In addition, the negative charge screening with magnesium cation causes DNA condensation.[8] CTAB causes DNA condensation for a cationic micelle-like aggregation, even below the critical micelle concentration (CMC).[4] All of the systems are known to induce the conformational change of an elongated and coiled DNA into a globular state in a discrete transition.

In general, the trapping force toward the center of the laser beam (F) for an object with permittivity (ϵ_D) and volume (V) in an electric field (E) within the environment having permittivity (ϵ_S) is represented as follows:[15,16]

$$F \propto (\epsilon_D - \epsilon_S) \cdot V \cdot grad\, E^2$$

Compared with the volume of latex beads, the volume of the globular DNA with CTAB is one order smaller, corresponding to the large decrease in trapping force compared to that of the 0.5 μm latex beads. As for the difference in trapping force between PEG and spermidine, PEG around the globular DNA is expected to increase the permittivity due to the large concentration of PEG (the ϵ_s of the PEG and spermidine solutions under this condition was found to be 1.345 and 1.338, respectively, based on measurement of refraction). Accordingly, the difference in permittivity between globular DNA and the PEG solution is lower than that between globular DNA and the spermidine solution.

In conclusion, the effectiveness of Nd:YAG laser trapping was found to be the highest for the globular DNA that was assembled with surfactant molecules. This result was due in part to the relatively large permittivity of the folded product. Selection of appropriate folding conditions is essential in achieving effective DNA trapping on single macromolecules.

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