Direct laser trapping of single DNA molecules in the globular state

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ABSTRACT

A sharply focused laser is able to trap small particles at the laser focal point due to the difference in refractive index of the particles and that of the surrounding medium. This technique, called laser trapping, can be used to manipulate animal or bacterial cells without any contact and has been widely applied in biological research. However, it has been difficult to trap biological macromolecules such as DNA molecules, because these molecules give a low difference in refractive index and cannot overcome Brownian motion. DNA molecules can be transformed to a condensed globular state. This transformation results in a higher refractive index of DNA due to its increased density. We demonstrate in this paper that a single DNA molecule can be optically trapped using a Nd:YAG laser (1064 nm wavelength) upon transformation from the coiled state to the globular state.

INTRODUCTION

Manipulation of single DNA molecules has been recognized as an important technique for biochemistry and molecular biology. Recently, several different methods have been developed to manipulate a single DNA molecule: (i) laser trapping of a DNA molecule anchored to a polystyrene bead (1–3), which affords us one of the most important techniques to transport and stretch individual DNA molecules; (ii) attaching and stretching DNA molecules on a coverslip modified with chemicals (4–5) or using electrical force (6), applied to mapping of restriction enzyme sites on DNA molecules; (iii) attaching and removing with a STM tip (7). In spite of these developments, it has been difficult to trap a single DNA molecule optically without first attaching it to a bead. Optically trapped objects require higher refractive indices than that of the surrounding medium, however, DNA molecules in the coiled state do not have a great enough difference in refractive index.

Visualization of DNA molecules

To determine the dependence of the DNA structure on the concentration of PEG and MgCl₂ under our experimental conditions, we observed the fluorescent image under the microscope. The samples were prepared as follows. The PEG (average molecular weight 6000; Nihon Oils and Fats Co. Ltd) was dissolved in a sodium phosphate buffer (pH 7.2) and mixed with T4 phage DNA (166 kb), 4′,6-diamidino-2-phenylindole (DAPI), a fluorescent DNA groove-binding dye, and 2-mercaptoethanol (2-ME), an antioxidant to suppress photobleaching. The final concentrations were as follows: 20 mM sodium phosphate buffer, 0.6 μM DNA (nucleotide concentration), 0.6 μM DAPI and 4% (v/v) 2-ME. The concentrations of PEG and MgCl₂ were chosen depending on the experimental conditions.

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Figure 1. Schematic diagram of the laser trapping system. The optical trap is placed in the object plane of the objective by adjustment of the collimation of the expanded Nd:YAG laser beam. A globular DNA molecule can be trapped at the focus of the laser and be positioned in the object plane by movement of the x–y stage. DM1 and DM2, dichroic mirrors; ND, neutral density filter; RM, reflective mirror; BF1–BF3, barrier filters. BF1 lets through only the excitation light and BF2 only the emission light. BF3 eliminates scattered light of the Nd:YAG laser.

RESULTS

DNA phase transition from the coiled state to the globular state

Figure 2 shows the dependence of the state of DNA molecules on the concentrations of PEG and MgCl$_2$. The state of the DNA was determined by observing its fluorescence image. We observed three distinct regions denoted here as: A, coiled; B, coexistent coiled/globular; C, globular. In region B, the transition between the coiled and the globular DNA could be plainly distinguished by observing the DNA molecules. This behavior of the transition is similar to that in PEG/NaCl systems (15), in which an individual coiled DNA molecule undergoes a first order phase transition to the globular state (16). Magnesium cations in PEG solution more readily induce DNA condensation compared with sodium cations. For instance, in 60 mg/ml PEG solution, a NaCl concentration >450 mM is necessary to change DNA completely into the globular state; no coiled DNA was observed. Figure 3 shows laser trapping of a globular DNA molecule. Free DNA molecules were translated by moving the microscope stage. The globular DNA molecule indicated with white arrows was trapped at the laser focal point as a free DNA molecule moved leftward (Fig. 3a–c) or upward (Fig. 3c–e). At this laser power, it was possible to trap globular DNA, however, coiled DNA could not be trapped. When a coiled DNA molecule arrived at the laser focal point on moving the stage, it was pushed downward by the optical pressure of the laser beam and escaped the trap (data not shown).

Laser trap of globular DNA

Globular DNA induced by PEG/MgCl$_2$ was optically trapped using 180 mW of 1064 nm light from a Nd:YAG laser. The infrared laser induces less damage to biological molecules such as DNA compared with visible light from krypton ion or argon ion lasers (17). Under the conditions [PEG] = 60 mg/ml and [MgCl$_2$] = 50 mM, all of the DNA existed in the globular state; no coiled DNA was observed. Figure 3 shows laser trapping of a globular DNA molecule. Free DNA molecules were translated by moving the microscope stage. The globular DNA molecule indicated with white arrows was trapped at the laser focal point as a free DNA molecule moved leftward (Fig. 3a–c) or upward (Fig. 3c–e). At this laser power, it was possible to trap globular DNA, however, coiled DNA could not be trapped. When a coiled DNA molecule arrived at the laser focal point on moving the stage, it was pushed downward by the optical pressure of the laser beam and escaped the trap (data not shown).

DISCUSSION

We have also succeeded in trapping globular DNA condensed by other agents, such as spermidine and PEG/NaCl (data not shown). These results strongly suggest that condensation of DNA into the globular structure, which may induce a higher refractive index and lower viscosity drag force due to its decreased size, is essential for laser trapping of DNA molecules. Laser trapping of a single supercoiled $\lambda$ DNA molecule using a krypton ion laser operating at 647 nm was reported by Chiu et al. (18). In their experiment, $\lambda$ DNA molecules were stained with YOYO dye. We have observed that YOYO dye induces DNA condensation to the globular state within ~30 min in the absence of any condensing agents. This suggests that the mechanism of trapping observed by Chiu et al. might be attributed to YOYO-induced condensation, but the DNA structure is as yet unknown. The globular transformation induced by condensing agents has been extensively studied by means of spectroscopy and microscopy, so that the...
morphology of and experimental conditions for globular DNA have already been determined (19–21). Laser trapping of a single DNA molecule can possibly be used for mapping. Reversibility of the transformation between the coiled and the globular states is required for this application. The globular DNA in our system could easily be reverted to the coiled state by reducing the PEG or MgCl₂ concentration. However, it might be difficult to transform DNA condensed by YOYO reversibly, because YOYO dye binds to DNA molecules with high affinity.

Handling large chromosome size DNA molecules (more than several hundred thousand base pairs) in solution is difficult, and the optical trapped DNA molecule remained stationary. (f) The trajectories of the free and the trapped DNA molecules are illustrated schematically.

to the development of an in situ hybridization technique for chromosomal DNA and successive preparation of DNA fragments from the terminus. In conclusion, we propose that laser trapping of globular DNA, reversible to the coiled state, will contribute to various fields of biological studies based on single molecules.

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