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Single Molecule Fluorescence Imaging and Its Application to the Study of DNA Condensation

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Single molecule fluorescence imaging incorporated with optical tweezers and a laminar flow cell has been used to monitor the kinetic process of DNA condensation induced by spermidine. It was found that at least two steps were involved in the condensation process of the hydrodynamically-stretched linear DNA; a lag period followed by a rapid collapse of DNA. The lag time increased with the flow speed and the collapse time remained short within the range of the flow speed studied. The effect of salt concentration on the condensation process was examined, and the results suggest that the longer lag time observed in the higher salt buffer probably results from the displacement of bound cations and rearrangement of spermidine on the DNA. The flow-speed dependence of the lag time suggests that a nucleation event at the free end of the DNA, i.e. formation of a loop, may play a vital role in the kinetic process of condensation.

KEY WORDS: Single molecule fluorescence imaging; optical tweezers; spermidine; DNA condensation.

INTRODUCTION

Single molecule fluorescence imaging has recently attracted great attention in the field of biological science [1,2]. It enables detection of the reactions of single biomolecules such as DNA which are not visible using conventional optical microscopes because of their small size. This is achieved by tagging biomolecules with fluorophores and visualising them using the fluorescence microscope. One of the difficulties of the kinetic study of bio-reactions is that the reactions of the biomolecules cannot be synchronised even if they are initiated at the same time. Therefore, ambiguity in the determination of the dynamic parameters of bio-reactions is inevitable because the real values are usually swamped by ensemble-averaging. Single molecule fluorescence imaging is able to avoid the problem caused by the non-synchronised behaviour of biomolecules because all the data are measured from individual biomolecules.

Single molecule fluorescence imaging combined with optical tweezers offers further opportunities for the study of bio-reactions, especially DNA-related interactions. It allows not only detection of the behaviour of single DNA but also its manipulation. By tethering a single DNA labelled with fluorescent dye molecules (YOYO-1) to a bead, trapping the bead with the optical tweezers and then stretching the DNA with a liquid flow, a linear single DNA molecule may be visualised using a fluorescence microscope and positioned by moving the trapped bead. This novel technique has been applied to studies of mechanical properties of protein motors [3,4], physical properties of DNA [5], protein-DNA interactions [6] and DNA condensation [7].

DNA condensation is defined as a decrease of the volume occupied by a DNA molecule from the large space loosely occupied by a worm-like chain to an orderly compact state[8]. This biological mechanism is used by organisms to pack their genomic DNA in a confined space. The compression of volume can reach as high as 7000-fold for T4 phage DNA packed inside the virus capsid [9].

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Condensation of DNA may be provoked by condensing agents such as polyamines and basic proteins. It is also significantly affected by other factors such as macromolecule concentration and the volume exclusion mechanism, salt concentration, pH and solvents [8,10]. This biological mechanism is therefore a complicated process involving interactions between DNA, condensing agents and all other components in the surrounding environment.

DNA condensation has been an interesting topic in the fields of biophysics, biochemistry and molecular biology not only because of its biological significance in storage and protection of genetic information, but also because of the complex interactions involved. It has been suggested that condensation involves displacement of bound counterions, rearrangement of condensing agents on DNA, charge neutralisation, hydration and configuration change of DNA [8,9]. Many measurements have been done to determine the dimensions and shapes of condensed DNA [11–13] and the critical concentrations of different condensing agents under different buffer conditions [14,15]. However, few studies have been done to obtain kinetic information on the highly dynamic process. Single molecule fluorescence imaging combined with optical tweezers and/or a flow cell has recently emerged as a promising technique for the kinetic study of DNA condensation. This combined technique is able to avoid the problem of low sensitivity often encountered by using conventional techniques. That is the difficulty to distinguish between condensation, aggregation and precipitation of DNA. A study of chromatin assembly using this single molecule technique has revealed that the assembly process involves at least three steps of similar time scale [16]. In addition, a combination of the imaging technique, optical tweezers and a flow cell has been used to monitor, in real time, the protamine-induced collapse of single DNA, and to obtain the binding and dissociation rates for protamine [7].

In this paper, we present a systematic study of the condensation of a 24,000 base pair fragment of phage lambda DNA induced by the condensing agent, spermidine $(NH_3C_3H_6NH_2C_4H_8NH_3)^{3+}$. The effects of flow speed and salt concentration on the condensation have been examined. The aim of the study is to provide experimental information to aid the development of a kinetic model for condensation of DNA.

MATERIALS AND METHODS

Preparation of the 24,000 Base Pair Fragment of Lambda DNA

The 24,000 base pair fragment of lambda DNA was obtained by mixing phage lambda DNA (New England Biolabs) with endonuclease Xba I (Sigma) in the buffer of 50 mM Tris-HCl, pH 7.5, 10 mM $MgCl_2$, 100 mM NaCl and 1 mM dithioerythritol and incubating for 1 hr at 37°C. Xba I cleaves phage lambda DNA at position 24,508 and thus generates a fragment of DNA about 24,000 bp in length which gives a contour length of 8.2 μm.

Preparation of Fluorescently Labelled DNA-Bead

The preparation method used here was adapted from Bianco *et al.* [6]. The fragment of lambda DNA was firstly tagged with a biotin molecule at one of its cohesive ends, by annealing and ligating a complementary 12-mer oligonucleotide with a biotin molecule attached at the 3′-end of the oligo (Oswel). The biotin-tagged lambda DNA (0.9 μM) was tethered onto 1 μm streptavidin-coated polystyrene beads (Bangs Laboratories) through the biotin-streptavidin reaction in 20 mM Tris-HCl, pH 8 for 1 hr at 37°C. The bead-conjugated DNA (5 pM) was fluorescently labelled by intercalating dye molecules (YOYO-1, Molecular Probes) in a ratio of 4 bp per YOYO-1, by incubation for 90 minutes at 37°C. The fluorescently labelled DNA-bead solution was then diluted to 0.2 pM with the diluting buffer including 20 mM Tris-HCl (pH 8), 2% 2-mercaptoethanol and an anti-fading agent [17] (a mixture of 2.3 mg/mL D-(+)-glucose, 0.1 mg/mL glucose oxidase, 18 μg/mL catalase and 20 mM 2-mercaptoethanol) before use.

Experimental Setup

The optical tweezers was generated by focusing the laser beam from a circularised Diode laser (wavelength = 785 nm, maximum power = 50 mW; Circulase) through an oil-immersion objective (100×, 1.4 numerical aperture; Nikon). An inverted epi-fluorescence microscope (Eclipse TE2000-U, Nikon) was used to combine the optical tweezers with fluorescence imaging detection. The fluorescently labelled DNA was excited with a high-pressure mercury lamp (HB-10104AF, Nikon) using the Nikon B-2A filter set. Successions of fluorescence images were captured and recorded by a cooled CCD camera (Imager 3, LaVision).

All the condensation experiments were performed in a Y-shaped flow cell [6,7]. Figure 1 shows the schematic diagram of the flow cell with a glass window on the bottom side of it. The two separate channels, 2 mm wide and ≈40 μm deep, were connected to two 1-mL syringes (the sample and reaction syringes). They were then joined to a 4 mm-wide channel of the same depth. The solutions in the computer-controlled individual syringes were introduced into the channels with the same speed. The bead

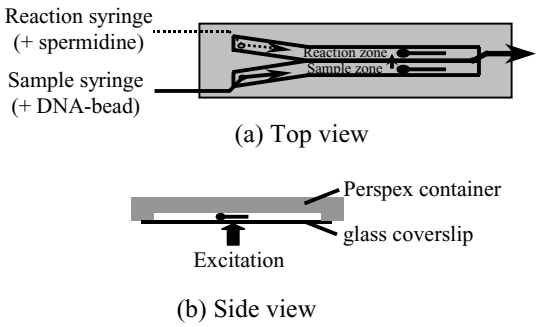

Fig. 1. A schematic diagram of the laminar flow cell used for the study. (a) The top view of the flow cell. The DNA-bead was pumped from the sample syringe and the spermidine from the reaction syringe into the channels with the same speed, producing a laminar flow with sample and reaction zones. By moving the optically trapped bead, the attached linear DNA was moved from the sample zone into the reaction zone where the condensation occurred. (b) The side view of the flow cell. The cell had a glass window on the bottom allowing excitation of fluorescent dye molecules intercalated in the DNA.

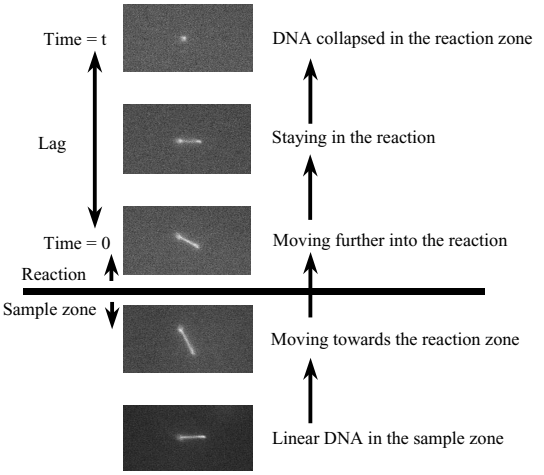

Fig. 2. Snapshot images of a typical example of the spermidine-induced DNA condensation process. The flow speed here was 175 μm/s and the stretched length of DNA was $\approx$6 μm. The lag time is the time needed for the start of collapse of hydrodynamically stretched DNA after the DNA entered the reaction zone.

was trapped and immobilised by the optical tweezers at about 10 μm above the glass window, and the single DNA molecule tethered on the bead was then stretched by the surrounding liquid flow.

Condensation Experiments

For the condensation measurements done in the buffer without added NaCl, the sample syringe contained 0.2 pM fluorescently labelled DNA-bead, and the reaction syringe 2 mM spermidine in the diluting buffer. For the measurements done in the high salt buffer, 5 mM NaCl was included in the diluting buffer for both the sample and reaction solutions. Both the sample and reaction solutions were pumped into the cell with the same speed, generating a laminar flow (the sample and reaction zones). The experiment was performed by firstly trapping a bead with a single attached DNA linearly stretched by the liquid flow, moving the DNA into the reaction zone and then observing the process of condensation. A range of flow speeds from 40 to 210 μm/s and buffers with/without 5 mM NaCl were examined here. All condensation experiments were carried out at room temperature.

RESULTS AND DISCUSSION

Condensation by spermidine of hydrodynamically-stretched linear DNA has been observed in buffers of different NaCl concentrations over a flow range of 40 to 210 μm/s. The images shown in Fig. 2 are snapshots of the spermidine-induced condensation process. Once the linearly stretched DNA was moved into the reaction zone containing 2 mM spermidine, two consecutive steps were observed in the condensation process regardless of the experimental conditions, namely a lag period followed by a rapid collapse of the DNA. The lag period appeared to be the dominant step in the whole condensation process because it lasted for a few seconds while the collapse step took only milliseconds. The effect of NaCl concentration on the lag time was examined to find out the cause of the lag period. As shown in Fig. 3, a longer lag time was observed for the condensation of DNA in the higher salt buffer. The work done by Wilson *et al.* [18] suggests that approximately 90% of the DNA phosphate charge must be neutralised for collapse to occur. Therefore, the lag period probably results from at least two factors; displacement of bound Na^+ ions and rearrangement of spermidine on the DNA so that a critical binding fraction may be reached before collapse occurs. Higher NaCl concentration affects the competition of bound Na^+ and spermidine such that the lag period is prolonged as observed in the study here.

The effect of flow speed on the lag time was also studied and the results are shown in Fig. 3. It was found that the lag time increased with increasing flow speed over the range from 40 to 210 μm/s. A simulation study performed by Ostrovsky *et al.* [19] suggests the importance of the motion of the free ends of DNA, i.e. nucleation, in the kinetic model of DNA condensation. A condensation study of bacterial chromosomes using the fluorescence imaging technique showed that collapse was initiated at

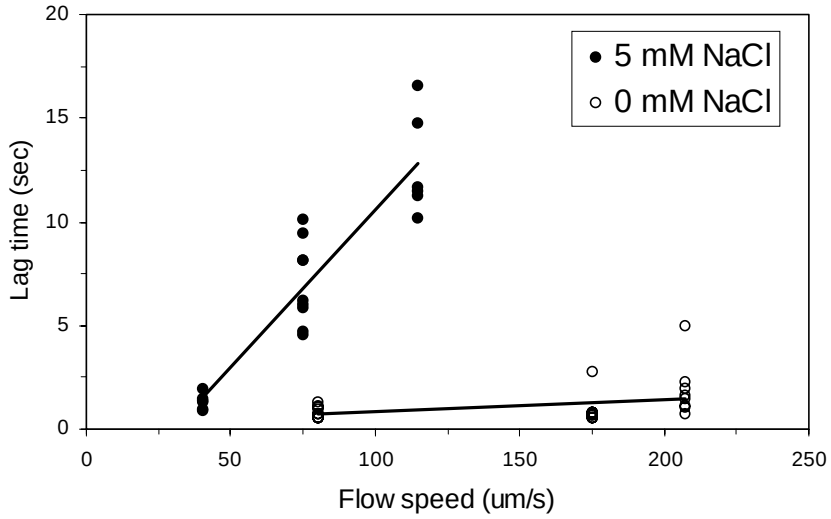

Fig. 3. A diagram of the lag time as a function of the flow speed applied on the single DNA in the buffer with (•) and without (○) 5 mM NaCl.

the free end by forming a small loop [20]. Therefore, the flow-speed dependence of the lag time may be caused by the nucleation event at the free end of the DNA, i.e. formation of a loop. At lower flow speed, the diffusion of the free end of the DNA and thus the probability of the formation of a loop is higher so the collapse of DNA may be triggered earlier, resulting in a shorter lag time. On the other hand, higher flow speed lowers the frequency of nucleation, resulting in a longer lag time.

The collapse rate of the DNA observed here was fast and at the limit of our experimental system. The spermidine-induced collapse, which was in the time scale of milliseconds, occurred more rapidly than with other condensing agents such as protamine [7] and histones [16], which occurred on the time scale of seconds. The observation of different collapse time scales may suggest that, although both small polyamines and proteins condense DNA, the interactions between DNA and the condensing agents are different and therefore result in different kinetic mechanisms for collapse of DNA.

CONCLUSIONS

The technique of single molecule fluorescence imaging combined with optical tweezers and a laminar flow cell has demonstrated here its unique ability for revealing kinetic information on DNA condensation. It was found that the spermidine-induced condensation of hydrodynamically-stretched linear DNA involves at least two steps, a lag period followed by a collapse of DNA. The lag time increased approximately linearly with the flow speed over the range studied. The collapse time after the lag period remained very short for all flow speeds. The longer lag period occurring in higher ionic strength indicates that displacement of bound counterions and rearrangement of sperimidine on the DNA are involved in determining the lag time. The flow-speed dependence of the lag time suggests that the condensation process is dominated by a nucleation event at the free end of the DNA which becomes less frequent at high flow speeds.

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