ABSTRACT

We combined molecular tagging with microfluidics to study kinetics in a single DNA molecular digestion. Moving tags of both DNA ends revealed initial breakup of a single DNA molecule into fragments induced by limited enzymatic digestion and subsequent rapid formation of transient structure in encounter complex. The method enables us to analyze rapid dissociation of weakly bound complexes derived from intramolecular transfer.

KEYWORDS: Single-Molecule Detection, Enzymatic Digestion, Kinematics, Molecular Tagging

INTRODUCTION

DNA digestion is a notable biological process in which proteins search for a favorable interaction with DNA. For example, when a restriction enzyme binds to a target sequence, DNA undergoes a large conformational change [1]. The mechanism is poorly understood, but it has been hypothesized on theoretical grounds that the binding kinetics may be enhanced by a DNA-sliding effect [1, 2], where the DNA binds weakly and non-specifically to its target enzyme prior to the DNA break. Here we show our method for visualizing the event in real time.

EXPERIMENTAL

The temporal molecular action was monitored based on motion capture (MoCap) experiments (Figure 1). The MoCap reads biomechanical motion signals from attached markers [3]. By using a molecular tagging with quantum dot (QD), motion tracking and position sensing of single DNA molecule gaining access to restriction enzyme is possible on a microchannel surface. Our previous method could measure the duration with single molecular marker [4], but could not capture the moment of digestion. Recently the performance improved with dual tagging technique. The recorded geometric data gives an advanced interpretation of the movements without imaging whole visual appearance of DNA molecule. This is innovative from a methodological point of view, since conventional intercalating dyes for DNA imaging cause an inhibitory effect on DNA digestion [5].
RESULTS AND DISCUSSION

Figure 2A shows the actual moment of single DNA digestion under flow. The flowing DNA molecule formed a transient encounter complex on binding to the restriction enzyme (Apa I) on the microchannel surface. The encounter complex evolved by way of this intermediate to the next state with dissociation of a fragment of digested DNA molecule. These steps were completely traced by tagged QDs (Figure 2B).

To obtain further insights into the stepwise mechanism of the coupled DNA binding and moving, we analyzed trapping times (t) of both QDs, where t represents velocity decay and relaxation of the DNA molecule in fluid. Two-step process in Figure 3A corresponds to the digestion of one restriction site by Apa I. On the other hand, when another restriction enzyme (Spe I) is used as a control, no restriction enzyme cutting site exists in the DNA molecule (Figure 3B). At this time, the tagged QDs are detected as a mixture throughout the period of observation, and the process is one-step. These provide understanding of a molecular mechanism by which the encounter complex is maintained after the DNA break.

Figure 2: Video monitoring of enzyme area. (A) Recorded fluorescent images after conversion into individual sequential frames (interval: 0.1 s, scale bar: 10 μm). CCD camera was set at the edge of the enzyme area to capture images of fresh DNA. The flow kept movements of QDs-tagged DNA molecules constant, but the enzyme changed their movements; progressive slowing, stopping, and accelerating of the QD images upon arrest were seen. (B) Suggested scheme of the monitoring. QD1 and QD2 were named as shown.

Figure 3: Analysis of geometric data. (A, B) Trajectories of QD1 and QD2 obtained from the video monitoring. Displacements of QDs were measured, and their velocities were calculated. Marked red areas show the time course of QD when it became almost stationary due to the interaction between DNA and restriction enzyme. The time lengths were defined as t1 and t2 for QD1 and QD2, respectively, to evaluate lifetime of related interactions. Restriction maps of λDNA for Apa I (one restriction site) and Spe I (no restriction site) are also shown. (C, D) Scatter plots of t1. Insets are explanatory drawings of individual events corresponding to respective reaction groups.
More importantly, scatter plots of $t_s$ in Figure 3C indicated a direct correlation. The time required for the first trapping determines the time for the second trapping. It probably depends on an initial binding position in the encounter complex. This behavior is suggestive of a weakly populated intermediate state in which the two molecules are held together by fluctuating short-range interactions as they search for mutually favorable orientations. Scatter plots of $t_s$ in Figure 3D shows stochastically-variable characteristics of the interaction. An intramolecular transfer model in the previously published papers [1, 2] supports the insights obtained from the analysis.

CONCLUSION

The single-molecule DNA tracking device described here enabled detailed observation of the binding-induced sequence-searching in restriction enzyme digestion. It revealed transient structures and stepwise processes that would have hidden in both bulk and single-molecule experiments using existing instrumentation. The device successfully captured the actual moment of single DNA digestion in real time by the introduction of dual molecular tagging. Moreover, the capability of the molecular tagging provides the ability to study weakly bound complexes and fragments which usually dissociate quickly at single-molecule concentrations.

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