Mechanical unfolding process of single collapsed DNA

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3価陽イオン（スペルミジン）により凝縮した1分子ＤＮＡの力学応答を測定するとともに，蛍光色素 (YOYO-1) でＤＮＡを可視化し伸張時の脱凝縮過程を観測した．凝縮状態ではＤＮＡ上に蛍光強度が高い部位が観測され，その強度は伸張にともない減少する．蛍光強度の減少は，伸張による凝縮部位の脱凝縮を示唆している．また，色素の結合によりＤＮＡの全長は増加するが，スペルミジン濃度の増加にともない再び減少する．これは，1分子ＤＮＡ上で色素－スペルミジンの対イオン交換が生じていることを示している．

DNA molecules in aqueous solution containing mono-valent cations repel each other, however, multivalent cations induce the DNA molecules to collapse or condense [1]. While an elastic response of a single DNA in an elongated coil state shows the entropic elasticity of an ideal worm-like chain (WLC), the response in a collapsed state is quite different from the WLC behavior [2]. At low concentration of trivalent cation, spermidine (SPD), force-extension (f-x) curves show force plateau in a wide range of the extension. With increasing the SPD concentration, a sawtooth pattern called as a stick-release pattern appears; the force first gradually increases with increasing extension, then abruptly decreased periodically during stretching. In the present study, we have visualized single DNA molecules using a fluorescent dye, YOYO-1, and measured elastic responses of single DNA molecules at various YOYO and SPD concentrations to elucidate the mechanism of collapsing and uncollapsing process at single molecule level.

Both ends of λ-Phage DNA of 48.5 kilobasepairs were attached to streptavidin-coated beads of 2.0 μm diameters, via biotinylated oligonucleotides that hybridized to the single-stranded ends of λDNA [3]. We used a dual-trap optical tweezers [2] to stretch individual DNA molecules tethered beads. First, the concentration of YOYO and SPD was varied by exchanging solutions, and f-x curves for an identical DNA was measured at each concentration. The contour length increases when the solution is exchanged to 0.1 μM YOYO because of the intercalation of YOYO [3]. However, the contour length decreases as the SPD concentration increases, though the same amount of YOYO is contained. The decrease of the contour length is caused by the detaching of YOYO from DNA, which indicates that the exchange of counterions on a single DNA molecule occurs. At 20 mM SPD and 0.1 μM YOYO, the stick-release response in stretching

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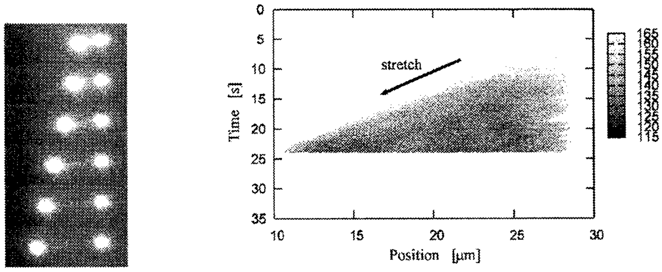

Figure 1: Fluorescence images of single DNA molecule in stretching (left) and time series of the fluorescence intensity profile (right) along the DNA molecule at no SPD and 0.1 μM YOYO. Gray scale (arbitrary unit) indicates the fluorescence intensity.

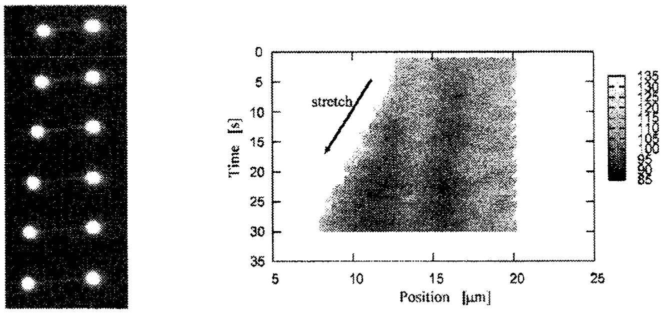

Figure 2: Fluorescence images (left) and time series of the fluorescence intensity profile (right) at 20 mM SPD and 0.1 μM YOYO.

and force plateau of about 1 pN in release can be observed repeatedly. It clearly indicates that the DNA molecule is in collapsed state at this condition. Next, fluorescence images of single collapsed DNA molecules in stretching were obtained to verify the mechanical unfolding processes. Figure 1 and 2 show the fluorescence images and time series of the fluorescence intensity profiles at 0 and 20 mM SPD, respectively. At no SPD, there are no bright spots at fixed positions in fluorescence intensity profile. At 20 mM SPD, however, we can see bright spots at around 14 and 18 μm in Fig. 2, which may correspond to the collapsing parts within single DNA molecule. The decrease of the fluorescence intensity of spots implies the mechanical unfolding of the collapsed parts, which are related to the stick-release response in f-x curves, though we need simultaneous measurements of fluorescence images and elastic responses to conclude.

We would like to thank Dr. Hirofumi WADA for helpful discussion.

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