14f Real-Time Visualization of One-Dimensional Diffusion of a Fluorescently Labeled Protein along Single DNA Molecules Aligned on a Surface

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Sliding of TRITC-stained EcoRI proteins along YOYO-stained lambda DNA molecules is directly visualized by epi-fluorescence microscopy. While in earlier work by Kabata et al.[1], this sliding motion was observed on dielectrophoretically aligned DNA molecules stretched between two electrodes, we observed similar sliding on aligned DNA molecules bound to substrates by "dynamic molecular combing" through contact-line motion. The dynamic molecular combing uses a receding meniscus to stretch out the DNA on a hydrophobic surface such as polystyrene- or PMMA- (polymethylmethacrlate) treated cover glass. We then exposed these DNA molecules to flow through a custom-built flow cell that convects proteins in a direction orthogonal to the DNA alignment direction, permitting observation of the sliding motion as a diffusive movement along the DNA contour orthogonal to the flow direction. Type II restriction endonuclease EcoRI is shown to be catalytically active on YOYO- stained DNA molecules which are anchored onto a substrate, suggesting that neither surface binding nor staining makes the DNA inaccessible for interaction with proteins. EcoRI molecules are fluorescently labeled with TRITC (tetramethyl rhodamine isothiocyanate) or fluorescent nano particles for visualization. We estimate the one-dimensional diffusion coefficient (D_1) by plotting the MSD (mean-square displacement,) versus the time interval over which the displacement took place and taking the slope of the linear regime. The MSD is calculated by tracking positions of the enzyme after the enzyme made contact with the DNA. The estimated one-dimensional diffusion coefficient (D_1) of fluorescently labeled EcoRI is estimated to be 1.5×10^{-8} cm^2/s for TRITC-labeled EcoRI, and 8.0×10^{-10} cm^2/s for EcoRI bioconjugated to nano particles in 50% (v/v) glycerol solution, respectively similar to the values we measure for three-dimensional diffusion in free solution. Diffusion measurements, dynamic light scattering, and AFM (atomic force microscopy) observation reveal that the optically visible proteins are aggregates of size ranging up to 140 nm in diameter.

[1]Kabata H.; Okada W.; Washizu M. Jpn. J. Appl. Phys. 2000, 39, 7164-7171.