Single Molecule Study on the Direct Transfer of E. coli Single-Stranded Binding protein between Single-Stranded DNA Molecules

TECKLA AKINYI, Department of Physics, Xavier University, Cincinnati, OH, I-REN LEE, TAEKJIP HA, Department of Physics, University of Illinois at Urbana-Champaign — Single molecule fluorescence resonance energy transfer (smFRET) techniques allow a direct study of the mechanism of the spontaneous transfer of Escherichia coli Single-Strand Binding (SSB) protein from single-stranded DNA to a competitor single-stand (ss)DNA. This investigation attempts to understand the kinetics of dissociation and ultimately figure out how long can SSB remain bound to ssDNA in midst of competitor free ssDNA. Application of single molecule techniques as described by Taekjip Ha, (Ha. Methods 25, 78–86 (2001)) allow the quantification of the rapid dissociation of SSB from ssDNA as a function of ssDNA length and concentration. We also examined, whether the dissociation occurs with the SSB sub-units released simultaneously or consecutively with the possibility of an intermediate state. The variation of dissociation time with DNA length and concentration of the competitive ssDNA demonstrate direct proportionality implying SSB is transferred between ssDNA molecules with a ratio of 1:1, with an abrupt transition from a high FRET state to a low FRET state indicating instantaneous dissociation limited by our time resolution.

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