Single-molecule Visualization of Binding Modes of Helicase to DNA on PEGylated Surfaces

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Binding modes of helicase to various DNA substrates were visualized by single-molecule fluorescence imaging on silicate coverslips coated with poly(ethylene glycol) (PEG) which minimizes the background noise from nonspecific protein adsorption. The results clearly show that the helicase has higher affinity for double-stranded DNA (dsDNA) with a single-stranded DNA (ssDNA) tail than that without one and that multiple helicases can bind to 4.7-kilonucleotide (knt) ssDNA. The study reported here will enhance the capabilities of single-molecule fluorescence studies on DNA–protein interactions.

Helicases are nucleic acid-dependent motor enzymes that couple nucleoside triphosphate binding and hydrolysis to translocation along single-stranded nucleic acids and unwinding of duplex DNA or RNA. As a consequence, they play major roles in almost every process in cells that involves nucleic acids, including DNA replication and repair.[1] *Escherichia coli* UvrD is a superfamily 1 DNA helicase which plays a crucial role in nucleotide excision repair and methyl-directed mismatch repair.[2] The enzyme unwinds a dsDNA from a 3′ end ssDNA tail, a gap, or a nick and translocates along ssDNA in a 3′–5′ direction. However, it has not been directly visualized how the enzyme interacts with DNA.

In this study, we used single-molecule fluorescence microscopy to understand the binding modes of UvrD to DNA in more detail. Single-molecule fluorescence imaging is a powerful method to elucidate fundamental biological processes without ensemble averaging.[3] Visualization of biological functions by single-molecule fluorescence techniques requires labeling of target biomolecules with a fluorophore, for example, chemical dyes such as cyanine dye (Cy3 or Cy5), green fluorescent protein (GFP), or quantum dot (Qdot). In particular, protein molecules, which play central roles in many biological functions, are the prime targets for fluorescence labeling in order to visualize their dynamics.

This observation requires suppression of the background noise arising from nonspecific adsorption of the fluorescently labeled molecules on the glass substrate, which is usually performed by a widely used PEG coating method, i.e., silanization followed by PEGylation.[4] We could further suppress the nonspecific adsorption by up to an order of magnitude on the silicate surface by using 50 mM MOPS (pH 7.5) for PEGylation instead of widely used 0.1 M sodium bicarbonate (pH 8.3) (see Table 1).

Table 1. Protein-nonadsorption capability on a silicate coverslip

	PEG (pH 7.5)	PEG (pH 8.3)	No treatment
Mean number of UvrD nonspecifically adsorbed[a] /nM /1000 μm^2	3.9 ±1.4 (30)[b]	39 ±4 (9)[b]	1.6×10^3 $\pm 0.5 \times 10^3$ (21)[b]
Ratio to the number for PEG	1	10	4.1×10^2

[a]The mean numbers of Cy5–UvrD nonspecifically adsorbed per 1000 μm^2 are normalized by the UvrD concentrations (nM) used for the experiments. [b]Values in parentheses are mean ± standard deviation (*n*).

This is crucial since silicate coverslips are extensively used for single-molecule fluorescence microscopy with epi-illumination,[5] highly inclined thin illumination[6] and total internal reflection fluorescence[7] microscopies (sometimes combined with other techniques such as optical tweezers[8]). The improvement in nonspecific adsorption using the new buffer condition for PEGylation was also observed on quartz, though to a lesser extent and with a Qdot-labeled protein (see Supplementary tables).[13] The key issue determining the nonadsorption properties of PEG-coated glass surfaces is their coverage by the polymer, which, we believe, is improved with the current method. PEG coating at pH 7.5 may be enhanced with respect to coating at higher pH owing to better stability of the NHS ester. Its lifetime is in the order of hours at physiological pH and decreases steeply at higher pH owing to increased hydrolysis.[9] The results indicate that our PEGylation method facilitates direct visualization of single-molecule interactions between fluorescently labeled protein molecules and DNA.

Indeed we could visualize how Cy5–UvrD interacts with a 18-bp dsDNA with or without an ssDNA tail immobilized on PEG-coated surfaces (for experimental details see Supporting Information).[13] Figure 1 shows a comparison of the number of Cy5–UvrD fluorescent spots observed. The comparison and fluorescence images clearly show that nonspecific adsorption of Cy5–UvrD on the surface was effectively suppressed enough to conclude that Cy5–UvrD has higher affinity for the dsDNA with an ssDNA tail than that without one, in agreement with a previous report.[10]

With the microscopy, we could compare the number of UvrD molecules bound to 18-nt ssDNA, 4.7-kbp dsDNA and 4.7-knt ssDNA immobilized on a PEG-coated silicate coverslip (for experimental details see Supporting Information[13]). Figure 2

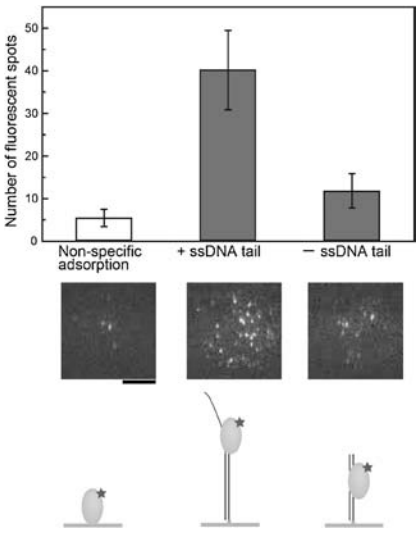

Figure 1. Comparison of the numbers of Cy5–UvrD interacting with 18-bp dsDNA with or without an ssDNA tail immobilized on a PEG-coated silicate coverslip. Below are the single-molecule fluorescence images. The Cy5–UvrD concentration used was 2 nM. Scale bar, 10 μm.

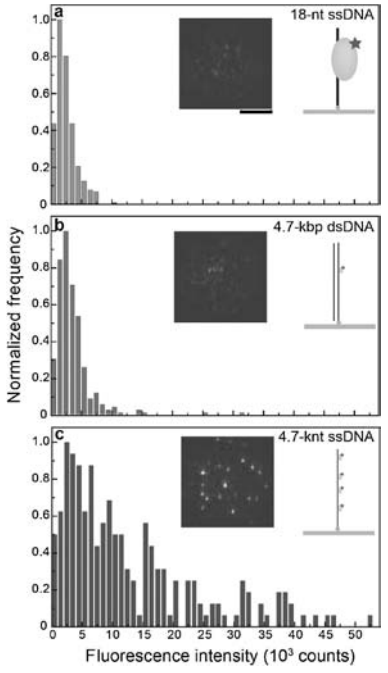

Figure 2. Comparison of fluorescence intensity distributions of Cy5–UvrD bound to (a) 18-nt ssDNA, (b) 4.7-kbp dsDNA, or (c) 4.7-knt ssDNA immobilized on a PEG-coated silicate coverslip. The insets are the single-molecule fluorescence images. The Cy5–UvrD concentration used was 2 nM. Scale bar, 10 μm.

shows a comparison of fluorescence intensity distributions of Cy5–UvrD bound to (a) 18-nt ssDNA, (b) 4.7-kbp dsDNA, or (c) 4.7-knt ssDNA. In agreement with the results in Figure 1, Cy5–UvrD had low affinity for dsDNA and thus the fluorescence intensity distribution for 4.7-kbp dsDNA peaked at a fluorescence intensity which corresponds to that of single Cy5–UvrD, which was validated by the observation that most of the fluorescent spots photobleached in a single step. This was also the case with the experiment using 18-nt ssDNA. For the experiment using 4.7-knt ssDNA, the fluorescence intensity distribution shifted to a larger value, indicating that multiple Cy5–UvrD attached to the ssDNA.

Direct visualization of single helicases interacting with various DNA substrates in this study unveiled the binding modes of the helicase to DNA. In particular, the interaction with ssDNA has not been well understood not only by biochemical studies but also other single-molecule techniques such as single-molecule fluorescence resonance energy transfer (FRET)[3,11] and single-molecule manipulation[12] because the interaction does not induce enough change of DNA physical properties to be detected by the techniques. Thus, the study reported here will promote our understanding of features of helicases that unwind dsDNA and translocate along ssDNA and also enhance the capabilities of single-molecule fluorescence studies on DNA–protein interactions.

We thank Pierre Nassoy (Institut Curie) for providing the basics of PEG coating, Yoshiyuki Arai (Osaka Univ.), Katsumi Uchida (Tokyo Univ. of Sci.), and Jun Nakanishi (NIMS) for technical advice. We also thank the lab members. This work was funded by JSPS (Postdoctoral Fellowships for Research Abroad and Fellowships for Young Scientists), the Mochida Memorial Foundation for Medical and Pharmaceutical Research, Nakayama Foundation for Human Science, Astellas Foundation for Research on Medical Resources, Kowa Life Science Foundation, The Uehara Memorial Foundation, Japan Science and Technology Agency to H.Y.

References and Notes

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1 M. R. Singleton, M. S. Dillingham, D. B. Wigley, *Annu. Rev. Biochem.* **2007**, *76*, 23.
2 a) C. J. Fischer, N. K. Maluf, T. M. Lohman, *J. Mol. Biol.* **2004**, *344*, 1287. b) E. J. Tomko, C. J. Fischer, A. Niedziela-Majka, T. M. Lohman, *Mol. Cell* **2007**, *26*, 335. c) T. M. Lohman, E. J. Tomko, C. G. Wu, *Nat. Rev. Mol. Cell Biol.* **2008**, *9*, 391.
3 a) S. Weiss, *Science* **1999**, *283*, 1676. b) T. Ha, *Methods* **2001**, *25*, 78. c) W. E. Moerner, *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 12596.
4 C. Joo, S. A. McKinney, M. Nakamura, I. Rasnik, S. Myong, T. Ha, *Cell* **2006**, *126*, 515.
5 a) T. Funatsu, Y. Harada, M. Tokunaga, K. Saito, T. Yanagida, *Nature* **1995**, *374*, 555. b) I. Sase, H. Miyata, J. E. Corrie, J. S. Craik, K. Kinosita, Jr., *Biophys. J.* **1995**, *69*, 323.
6 M. Tokunaga, N. Imamoto, K. Sakata-Sogawa, *Nat. Methods* **2008**, *5*, 159.
7 M. Tokunaga, K. Kitamura, K. Saito, A. H. Iwane, T. Yanagida, *Biochem. Biophys. Res. Commun.* **1997**, *235*, 47.
8 a) M. J. Lang, P. M. Fordyce, A. M. Engh, K. C. Neuman, S. M. Block, *Nat. Methods* **2004**, *1*, 133. b) S. Hohng, R. Zhou, M. K. Nahas, J. Yu, K. Schulten, D. M. Lilley, T. Ha, *Science* **2007**, *318*, 279.
9 G. T. Hermanson, *Bioconjugate Techniques*, Academic Press, **1996**, p. 140.
10 N. K. Maluf, C. J. Fischer, T. M. Lohman, *J. Mol. Biol.* **2003**, *325*, 913.
11 R. Roy, S. Hohng, T. Ha, *Nat. Methods* **2008**, *5*, 507.
12 C. Bustamante, Z. Bryant, S. B. Smith, *Nature* **2003**, *421*, 423.
13 Supporting Information is available electronically on the CSJ-Journal Web site, http://www.csj.jp/journals/chem-lett/index.html.

Published on the web (Advance View) February 28, 2009; doi:10.1246/cl.2009.308