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Single-Molecule Force Spectroscopy for Studying Kinetics of Enzymatic Dextran Elongations

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Supporting Information

ABSTRACT: Catalytic elongation of dextran by a single molecule of dextransucrase (DSase) was directly monitored by observing the movements of the positions of a rupture peak, which represented the adhesive force between an isomaltoheptaose (dextran 7-mer)-immobilized probe and a DSase-immobilized mica surface. This was initiated with the addition of sucrose monomers. From the histograms of the rupture peaks after elongation reactions on each individual enzyme and the continuous peak shift of certain single enzymes, the catalytic elongation rate constant (k_{cat}) was ascertained to be 1.2−2.7 s^{-1}.

Biorecognition between a ligand and a receptor is a significant event in various biological phenomena.[1] Direct measurement of the intermolecular forces between biomolecules using atomic force spectroscopies, optical tweezers, and magnetic beads has been explored and applied to the characterization of the dynamic response of an individual ligand and receptor complex to external forces.[2] In the past decade, single-molecule force spectroscopies have been used to analyze the energy landscape of the interaction between ligands and receptors.[3] From the attractive and repulsive forces of atomic force spectroscopy (AFM), adhesive strength and bond distances can be directly observed at a single-molecule level by immobilizing a receptor on a substrate and a ligand on a probe.[4] Single rupture forces were measured as 150 pN for the strong interaction between streptavidin and biotin[4] and 50 pN for the weak interaction between concanavalin A and mannose ligand.[5] However, there have been very few reports on the observation of dynamic catalytic reactions of enzymes by force-curve measurements. There have been only a few studies in which the static interaction between enzymes and substrates was observed by surface force measurements.[7−9]

In this communication, we report the observation and dynamic kinetic analysis of dextran elongation catalyzed by dextransucrase (DSase) using single-molecule force spectroscopy. DSase (sucrose:1,6-α-D-glucan-6-α-D-glucosyltransferase [EC 2.4.1.5]) is a glucosyltransferase that catalyzes the transfer of a D-glucose unit in sucrose to a D-glucose at the nonreducing end of a dextran acceptor (Figure 1A).[10] We directly observed the elongation process from the shifts of rupture peaks between the isomaltoheptaose (dextran 7-mer)-immobilized probe and the DSase-immobilized mica surface in the presence of sucrose as a monomer (Figure 1B).

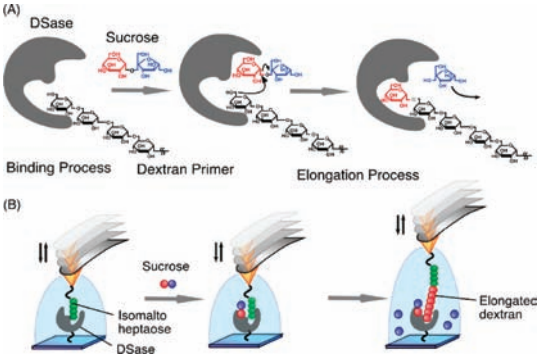

Figure 1. Illustrations of (A) dextransucrase (DSase)-catalyzed sugar elongation of dextran primer using sucrose as a monomer and (B) single-molecule force-curve measurements of dextran elongation between a DSase-immobilized mica surface and an isomaltoheptaose (dextran 7-mer)-immobilized probe using an eight-branched PEG_{40} linker (precise chemical structures are shown in Figure S1).

The DSase-immobilized mica surface and the isomaltoheptaose-immobilized Si_3N_4 probe with an eight-branched PEG_{40} linker were prepared as follows. The precise chemical structures and the procedure are shown in Figure S1 in the Supporting Information. A freshly cleaved mica surface and a Si_3N_4 probe were first treated with aminopropyltriethoxysilane in the vapor form. The amino-functionalized mica surface was reacted with the eight-branched PEG_{40} linker [hexaglycerol octa(*N*-hydroxysuccinimidylglutaryl) poly(ethylene glycol), $n \approx 40$, M_w = 15 000, NOF EUROPE (BELGIUM) NV] bearing eight activated *N*-hydroxysuccinimidyl esters at the terminal carboxyl ends. DSase was immobilized through the amino groups on its surface by the activated esters of the eight-branched PEG_{40} linker. The surface coverage was confirmed to be ∼10% by AFM images (see Figure S2). Isomaltoheptaose was immobilized through Schiff base formation between the reducing end of the glucose unit and the amino group of *O*-[2-(aminoethoxy)ethyl]hydroxylamine. The other amino group was reacted with the activated succinimidyl ester of the eight-branched PEG_{40} linker.

Figure 2A shows typical force−distance curves for both the approach and release processes involving the DSase-immobilized mica surface and the dextran primer-immobilized probe. During the release process, the rupture was observed at a distance of 50 nm and a force of 80 pN because of the interaction between

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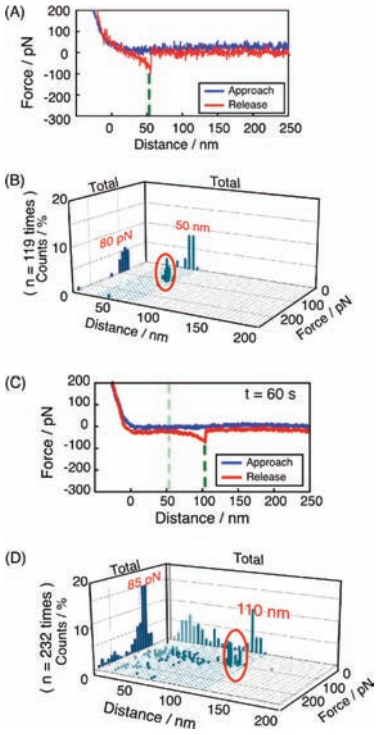

Figure 2. (A) Typical force−distance curves and (B) histograms of the peaks of the rupture forces between the isomaltoheptaose-immobilized probe and the DSase-immobilized mica surface (24 hits were observed in 119 trials, and most of the peaks were acquired within a distance of 50 ± 5 nm and at a force of 80 ± 5 pN). (C) Typical force−distance curves and (D) histograms of the peaks after 60 s upon addition to the system of 10 mM sucrose as a monomer (78 hits were observed in 232 trials, and most of the peaks were acquired within a distance of 110 ± 10 nm and at a force of 85 ± 10 pN). Buffer: 10 mM acetate, 150 mM NaCl. *Z*-scan rate: 1 Hz. Spring constant: 0.02 N/m.

the DSase-immobilized mica surface and the dextran primer-immobilized probe. Figure 2B shows the histograms of the various rupture peaks, in which 24 hits were observed in 119 trials; most of the peaks were acquired within a distance of 50 ± 5 nm and at a force of 80 ± 5 pN. The single rupture force (F_{rup}) and dissociation constants (K_d) have been reported for the interaction between streptavidin and biotin (F_{rup} = 150 pN and $K_d = 10^{-15}$ M)[5] and for the interaction between concanavalin A and mannose (F_{rup} = 50 pN and $K_d = 10^{-6}$ M) at a loading rate of ~10 nN/s.[6] We reported a K_d value of 10^{-8} M for DSase and oligodextran obtained using an isomaltoheptaose-immobilized quartz-crystal microbalance (QCM) in an aqueous solution of DSase.[11] On the basis of the dissociation constant $K_d = 10^{-8}$ M for DSase and isomaltoheptaose determined by QCM, the obtained F_{rup} values of 80 ± 5 pN in this force spectroscopy are reasonable for a single-molecule interaction.

We used single-molecule force spectroscopy to examine the interaction between DSase and the dextran primer, allowing us to observe the enzymatic elongation of dextran catalyzed by DSase in the presence of sucrose monomers. Figure 2C,D shows that the rupture peak shifted from 50 ± 5 to 110 ± 10 nm after 60 s with the addition of 10 mM sucrose without any change in the force, which was 85 ± 10 pN. This indicates the enzymatic elongation of dextran. In Figure 2, the tip could contact somewhat different points. The histograms in Figure 2C reflect the rupture force based on interaction between the different complexes. The force curve did not shift in the control experiments, where lactose was not recognized by DSase because a substrate was added instead of sucrose (data not shown). When the dextran polymer (200-mer) was immobilized on the probe instead of the dextran 7-mer, the rupture force peak appeared at 150 nm (±30 nm) and 100 pN instead of at 50 nm and 80 pN (see Figure S3). Averages of the experimental extension length for dextran 200-mer were slightly smaller than the calculated one (180 nm) because the dextran polymer has a certain content of dispersibility in the chain length or the possibility that the dextran was stretched obliquely to the mica surface, as opposed to perpendicularly in the case of the polymer. These control experiments strongly suggest that the shift of the rupture force peak was because of the enzymatic elongation.

It should be noted that DSase-catalyzed elongation (β-glycosylation of the nonreducing end of the dextran oligomer) can occur only when the enzyme binds to the dextran primer and sucrose monomer during the contact time of the probe. We selected a scan rate of 1 Hz for the set point of 250 nm and a delay time of 1 s in the force measurements. The contact time between the enzyme and the substrate was calculated to be 1.1 s in one cycle (see the Supporting Information). In Figure 2C,D, the calculated actual reaction period during 78 hits in 232 trials is shown to be 1.1 s × 78 hits = 86 s. Thus, dextran was elongated by 60 ± 10 nm during 86 s [(60 ± 10)/86 = 0.7 ± 0.1 nm s^{-1}]. If the concentration of the sucrose monomer (10 mM) was sufficient for its reaction with dextran, k_{cat} can be estimated as 1.2 ± 0.1 s^{-1}, considering that one glucose unit is 0.6 nm in length. We reported that the same enzymatic elongation of dextran can be monitored on the isomaltoheptaose-immobilized QCM in the presence of DSase and sucrose in an aqueous solution.[11−13] By variation of the concentrations of DSase (0.1−50 nM) and sucrose (1−10 mM), the Michaelis constant for sucrose (K_m) and the catalytic elongation rate constant (k_{cat}) were obtained as 3.4 mM and 3.5 s^{-1}, respectively.[11] These results indicate that 10 mM sucrose was sufficient for DSase (K_m = 3.4 mM) and that the k_{cat} value of 1.2 ± 0.1 s^{-1} obtained from the AFM force measurement for a single-molecule reaction is consistent with the value k_{cat} = 3.5 s^{-1} obtained as the average from the many enzyme reactions that occur on the QCM plate.

In order to monitor real-time single-molecule enzyme reactions, the force−distance curves were continuously measured each second on a particular DSase molecule at the same point. Figure 3A shows the typical continuous force−distance curves of the isomaltoheptaose-immobilized probe on the DSase-immobilized mica surface. It was clearly observed that the rupture force peak shifted to the nanometer order for every few seconds of contact time. A linear slope of 1.6 nm s^{-1} = 2.7 mer s^{-1} (k_{cat} = 2.7 s^{-1}) was obtained when the elongation distance was plotted against contact time, as shown in Figure 3B. The k_{cat} value of 2.7 s^{-1} obtained for a particular single enzyme was also consistent with the value k_{cat} = 1.2 ± 0.1 s^{-1} obtained by averaging the values observed for several enzymes for a single-molecule reaction. These values were also in good agreement with the value k_{cat} = 3.5 s^{-1} obtained as the average from the many enzyme reactions on the QCM plate.

In conclusion, we were able to achieve kinetically a single-molecule enzyme reaction as a dextran elongation using the distance shift of the rupture peak for the dextran oligomer-immobilized probe and the DSase-immobilized mica surface.

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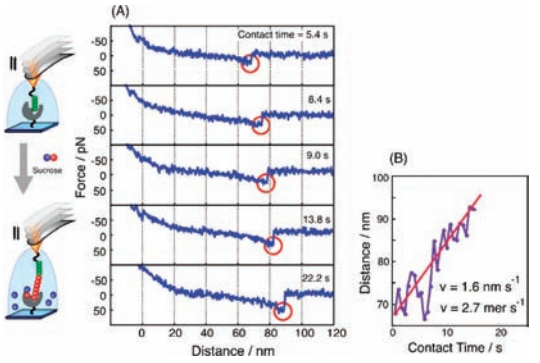

Figure 3. (A) Typical continuous force−distance curves of the elongation of dextran on a single molecule of DSase at each contact time in the presence of 10 mM sucrose as a monomer. (B) Relationship between the contact time and the shift in the distance of the adhesive peak. Buffer: 10 mM acetate, 150 mM NaCl. *Z*-scan rate: 1 Hz. Spring constant: 0.02 N/m.

This technique can also be applied to enzyme reactions on cell surfaces.

■ ASSOCIATED CONTENT

Supporting Information. Experimental procedure for surface modification, calculation of contact time, AFM image of DSase-immobilized mica surface, and the force−distance curve for the adhesion between the dextran polymer(200-mer)-immobilized probe and the DSase-immobilized mica surface. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ REFERENCES

(1) (a) Benoit, M. D.; Gabriel, D.; Gerisch, G.; Gaub, H. E. *Nat. Cell Biol.* **2000**, *2*, 313–317.(b) Lauffenburger, D. A.; Linderman, J. J. *Receptors: Models for Binding, Trafficking, and Signaling*; Oxford University Press: New York, 1996.

(2) (a) Neuman, K. C.; Nagy, A. *Nat. Methods* **2008**, *5*, 491–505. (b) Zlatanova, J.; Lindsay, S. M.; Leuba, S. H. *Prog. Biophys. Mol. Biol.* **2000**, *74*, 37–61.

(3) (a) Evans, E.; Ritchie, K. *Biophys. J.* **1997**, *72*, 1541–1555. (b) Bell, G. I. *Science* **1978**, *200*, 618–627.

(4) (a) Bizzarri, A. R.; Cannistraro, S. *Chem. Soc. Rev.* **2010**, *39*, 734–749. (b) Lee, C.-K.; Wang, Y.-M.; Huang, L.-S.; Lin, S. *Micron* **2007**, *38*, 446–461.

(5) (a) Yuan, C.; Chen, A.; Kolb, P.; Moy, V. T. *Biochemistry* **2000**, *39*, 10219–10223. (b) Merkel, R.; Nassoy, P.; Leung, A.; Ritchie, K.; Evans, E. *Nature* **1999**, *397*, 50–53.

(6) Ratto, T. V.; Langry, K. C.; Rudd, R. E.; Balhorn, R. L.; Allen, M. J. *Biophys. J.* **2004**, *86*, 2430–2437.

(7) Radmacher, M.; Fritz, M.; Hansma, H. G.; Hansma, P. K. *Science* **1994**, *264*, 1577–1579.

(8) Suzuki, T.; Zhang, Y.-W.; Koyama, T.; Sasaki, D. Y.; Kurihara, K. *J. Am. Chem. Soc.* **2006**, *128*, 15209–15214.

(9) Alegre-Cebollada, J.; Perez-Jimenez, R.; Kosuri, P.; Fernandez, J. M. *J. Biol. Chem.* **2010**, *285*, 18961–18966.

(10) Kitaoka, M.; Robyt, J. F. *Carbohydr. Res.* **1999**, *320*, 183–191.

(11) Nihira, T.; Mori, T.; Asakura, M.; Okahata, Y. *Langmuir* **2011**, *27*, 2107–2111.

(12) (a) Mori, T.; Sekine, Y.; Yamamoto, K.; Okahata, Y. *Chem. Commun.* **2004**, 2692–2693. (b) Mori, T.; Toyoda, M.; Ohtsuka, T.; Okahata, Y. *Anal. Biochem.* **2009**, *395*, 211–216. (c) Mori, T.; Ohtsuka, T.; Okahata, Y. *Langmuir* **2010**, *26*, 14118–14125.

(13) (a) Okahata, Y.; Mori, T.; Furusawa, H.; Nihira, T. *Springer Ser. Chem. Sens. Biosens.* **2007**, *5*, 341–369. (b) Nishino, H.; Nihira, T.; Mori, T.; Okahata, Y. *J. Am. Chem. Soc.* **2004**, *126*, 2264–2265. (c) Nishino, H.; Murakawa, A.; Mori, T.; Okahata, Y. *J. Am. Chem. Soc.* **2004**, *126*, 14752–14757.