Supporting Online Material for

Traffic Jams Reduce Hydrolytic Efficiency of Cellulase on Cellulose Surface

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Supporting Online Materials

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Materials and Methods

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Adsorption of *Tr*Cel7A on crystalline celluloses

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Materials and Methods

Cellulose and enzyme preparations- Cellulose from green alga *Cladophora* sp., having more than 95% crystallinity, was used in this study (*14, 26*). Cel7A from *T. reesei* was purified from a commercial cellulase mixture, Celluclast® 1.5L (Novozyme, available from Sigma-Aldrich) as described previously (*26*), and hydrolysis of highly crystalline celluloses from *Cladophora* was performed as described previously (*26*).

T. reesei Cel6A wild-type was purified from a *T. reesei* strain lacking the endogenous genes for the major endoglucanases Cel5A and Cel7B. The presence of contaminating cellulolytic activities was ruled out by measuring the activities towards $MeUmb(Glc)_1$, $MeUmb(Glc)_2$, and hydroxyethyl cellulose (HEC), as described earlier (*27*). Concentrations of purified wild-type protein were determined from UV absorbance at 280 nm using the molar extinction coefficient $\varepsilon = 104{,}000\ M^{-1}\ cm^{-1}$, based on the result of total amino acid analysis (*28*).

High-speed AFM observations- High-speed atomic force microscopic observations were carried out using a laboratory-built HS-AFM apparatus and a BL-AC10EGS-A2 small cantilever (Olympus Corporation), based on previous reports (*12, 29-31*). Two microliters of crystalline cellulose suspension in water (0.2 %) was dropped on a graphite sample disc, which was rinsed 3 times with 18 µl of 20 mM sodium acetate buffer, pH 5.0, after incubation for 5 minutes at 25˚C. Crystalline cellulose on the sample disc was initially observed without enzyme in 70 µl of the same buffer, followed by the addition of 20 µM enzyme solution with final concentration of 2.0 µM. The AFM images were taken at 1-4 frames sec^{-1} at 25˚C. Digital movies were constructed using Adobe ImageReady CS2 (Version 9.0, Adobe Systems Inc.). The linear movement of individual molecule was tracked and analyzed by the use of a newly developed routine in Igor Pro (Version 6.4, WaveMetrics, Inc.), which was basically designed to follow the center of the moving particle. AFM data analysis software Gwyddion

(Version 2.2, http://gwyddion.net/) was used for making the 3-dimensional movies in Fig. 4 and movies S8 and S9 as described previously (*32*).

Adsorption of Tr*Cel7A on crystalline celluloses- Cladophora* cellulose (either cellulose I_α or cellulose III_I, 0.1% w/v) was incubated with various concentrations of enzymes (final concentration, Abs_{280}=0.04-0.8) in 1 ml of 50 mM sodium acetate buffer, pH 5.0, at 30 ˚C with inversion for 120 min. The free protein concentration [*F*] (μM) at equilibrium was measured after centrifugation (15,000g x 30 sec) by measuring the absorbance at 280 nm of the supernatant (900 μl). An absorption coefficient at 280 nm of 88,250 $M^{-1}cm^{-1}$ was used for *Tr*Cel7A as described previously (*26*). The amount of adsorbed enzyme (*A*, nmol/mg-cellulose) was calculated by subtraction of the amount of free enzyme from the amount of added enzyme. The amount of adsorbed enzyme was plotted versus free enzyme concentration, based on a two-binding-site model for *Tr*Cel7A analysis, using the following equation:

$$A=A_1 \bullet [F]/(1/K_{ad1}+[F])+A_2 \bullet [F]/(1/K_{ad2}+[F])$$

where A_1 and A_2 are the adsorption maxima of high- and low-affinity binding (nmol/mg-cellulose); K_{ad1} and K_{ad2} are the adsorption constants of the high- and low-affinity binding sites (μM^{-1}).

*Synergistic hydrolysis of crystalline cellulose-Tr*Cel7A and *Tr*Cel6A (the *Tr*Cel6A/*Tr*Cel7A mixing ratio was 0/2.0, 0.5/1.5, 1.0/1.0, 1.5/0.5, or 2.0/0 μM) were incubated in 50 mM sodium acetate buffer, pH 5.0, with 0.1 % cellulose III_I (*14*) at 30˚C for 30 min, then centrifuged (x 15,000 *g*). The soluble products thus obtained were analyzed by HPLC (LC-2000 series; Jasco, Tokyo, Japan), using a Corona™ Charged Aerosol Detector™ (ESA Biosciences, Chelmsford, MA) as described previously (*33*). The supernatant was separated

on Shodex® Asahipak NH_2P-50 (Showa Denko K.K., Kanagawa, Japan) with a linear gradient of acetonitrile/H_2O (60/40 to 50/50, v/v). The amount of products was quantified using cellooligosaccharides with degree of polymerization (DP) = 2-7 (Seikagaku Corporation, Tokyo, Japan) as standards.

Captions for Supporting Online Movies

Movie S1. HS-AFM images of *Tr*Cel7A (final conc. 2.0 μM) sliding on crystalline cellulose I_α. x/y=150/90 nm, 10x playback, total time 153 s. Scale bar and height lookup table (LUT) of the images are shown in Fig. 1A.

Movie S2. HS-AFM images of *Tr*Cel7A (final conc. 2.0 μM) on cellulose III_I. x/y=180/100 nm, 10x playback, total time 466 s. Scale bar and height LUT of the images are shown in Fig. 2C.

Movie S3. HS-AFM images of *Tr*Cel7A (final conc. 2.0 μM) on cellulose III_I. x/y=72/40 nm, 5x playback, total time 154 s. Scale bar and height LUT of the images are included in the images.

Movie S4. HS-AFM images of *Tr*Cel7A (final conc. 2.0 μM) on cellulose III_I. x/y=150/75 nm, 10x playback, total time 90 seconds. Scale bar and height LUT of the images are shown in Fig. 3B.

Movie S5. HS-AFM images of *Tr*Cel7A (final conc. 2.0 μM) on cellulose III_I peeling the surface of the same cellulose fiber of movie S4. x/y=150/75 nm, 10x playback, total time 372 seconds. Scale bar and height LUT of the images are shown in Fig. 3B.

Movie S6. HS-AFM images of cellulose III_I before the enzyme addition. x/y=250/125 nm, 10x playback, total time 18 seconds

Movie S7. HS-AFM images of crystalline cellulose III_I with the addition of *Tr*Cel6A (10 s)

and *Tr*Cel7A (505 s) with final concentrations of 2.0 μM each. x/y=250/125 nm, 10x playback, total time 832 seconds.

Movie S8. Three-dimensional HS-AFM images of cellulose III_I incubated with *Tr*Cel6A. This movie was created from the first 480 s of movie S7. *Tr*Cel6A was added (10 s) at a final concentration of 2.0 μM. Height LUT of these images is shown in Fig. 4.

Movie S9. Three-dimensional HS-AFM images of cellulose III_I incubated with *Tr*Cel6A+*Tr*Cel7A. This movie was created from the latter part of movie S7. After 495 s from the addition of *Tr*Cel6A (5 s from the initial frame in this movie), *Tr*Cel7A was added at a final concentration of 2.0 μM.

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