Electronic Supplementary Information (ESI)

Enzyme-catalysed reaction for long-term fluorescent observation of single DNA molecules

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Experimental details

T4 DNA (Nippon Gene, Tokyo, Japan) was used for DNA samples. To observe DNA molecules under a fluorescence microscope, YOYO-1 (Invitrogen, San Diego, CA) and YOPRO-1 (Invitrogen) were used for fluorescence dyes with a ratio of basepairs/dye = 5. DNA samples were diluted to 5 ng/mL using a concentrated buffer (1×TE: 10 mM Tris-HCl, 1 mM EDTA, pH=8.0, Nippone Gene). Finally dithothreitol (DTT; 10 mM; Sigma-Aldrich Co., St. Louis, MO) was added to the DNA sample buffer as an anti-photobleaching agent.

Eclipse TE300 (Nikon, Tokyo, Japan) equipped with 488 nm laser (FLS-448-20; Sigma Koki Co., Ltd, Tokyo, Japan) was used for the single DNA molecule observations. To avoid any undesirable photocleavage of DNA molecules, the output power of the laser was set to 6.1 W/m^2. DNA molecule images were captured with an EB-CCD (C7190-43, Hamamatsu Photonics K.K., Hamamatsu, Japan) through 100×/1.40 NA objective lens (Nikon) and recorded in a DV-CAM (DSR-11, Sony, Tokyo, Japan). Those recorded images were analysed with Adobe Premire (Adobe Systems Inc., California, USA) and image processing software (Cosmos32; Library, Tokyo, Japan).

Coated cover glasses on which DNA molecules were stretched were first prepared as follows. Cover glasses (60×25 mm; Matsunami Glass Ind., Ltd., Osaka, Japan) were treated in piranha solution (H_2SO_4:H_2O_2 = 4:1), then two cover glasses were stuck together with poly-L-lysine solution (Sigma-Aldrich) and dried. After drying, the cover glasses were peeled apart, leading to coated cover glasses. DNA molecules were stretched on a coated cover glass using molecular combing. A 5 µL DNA sample was dropped onto a coated cover glass which was then protected with an uncoated cover glass (18×18 mm; Matsunami Glass Ind., Ltd.). To avoid drying out of sample solution, nail polish was applied around the edges of the protecting cover glass to seal the sample in.

The glucose and catalase (GODase) enzyme reaction was done as follows: glucose (10 mM; Wako Pure Chemical Industries, Ltd., Osaka, Japan), glucose oxidase (500 nM; Sigma-Aldrich), and catalase (64 nM; Sigma-Aldrich) were dissolved in 1×TE buffer; YOYO-1 dyed T4 DNA (5 ng/mL) was added into the mixture; the first enzyme reaction was carried out at 35 ºC for 5 min; and the second enzyme reaction was carried out at 25 ºC for 5 min.

The protocatechuate-3,4-dioxygenase (PADase) enzyme reaction was done as follows: protocatechuic acid (2.5 mM; Wako Pure Chemical Industries, Ltd.) and protocatechate-3,4-dioxigenase (300 nM; Sigma-Aldrich) were dissolved in 1×TE buffer; YOYO-1 dyed T4 DNA (5 ng/mL) was added into the mixture; and the enzyme reaction was carried out at 37 ºC for 5 min.

Movie

Movie of the photobleaching process of a YOYO-1 stained DNA molecule with no treatment, DTT, PADase, and GODase