Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information for:

Preparation of fibrous cellulose by enzymatic polymerization using cross-linked mutant endoglucanase II

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General procedure. All reagents were purchased from commercial sources and used as received. The NMR measurement was performed by a Bruker DPX-400 NMR spectrometer (400 MHz).

Synthesis of bisNTA.

BisOSu. To the DMF (0.8 mL) solution of dis-dPEG$^{TM}_5$ acid (30 mg, 0.089 mmol), *N*,*N*-dicyclohexyl carbodiimide (DCCI, 38 mg, 0.18 mmol) was added. After 15 min stirring of the reaction mixture at room temperature under Ar atmosphere, *N*-hydroxy succinimide (41 mg, 0.36 mmol) dissolved in DMF (0.2 mL) was dropwised, and stirred more 36 h, the reaction mixture was concentrated. The crude product was dissolved into ethyl acetate, and dicyclohexylurea was removed by filtration. Crude bisOSu was obtained after evaporation of the solvent.

BisNTA. To the solution of crude bisOSu in dimethyl sulfoxide (DMSO, 5 mL), AB-NTA free acid (47 mg, 0.18 mmol) and triethylamine (74 mL, 0.53 mmol) mixed DMSO solution (2 mL) was added, and the mixture was stirred at room temperature under Ar atmosphere for 6 h. The reaction mixture was purified by Sephadex LH-20 column chromatography using DMF as an eluent to afford pure bisNTA as colorlesssyrup (60 mg, 2 steps: 82%). ^{1}H NMR (400 MHz, DMF-d7) δ 7.95 (2H, s, N*H*CO), 3.90–3.70 (28H, m, -C*H*$_2$COOH, -O-C*H*$_2$-), 3.65 (2H, dd, C*H* (C-1) of 5-amino-1-carboxypentyl), 3.33 (4H, m, C*H*$_2$ (C-5) of 5-amino-1-carboxypentyl), 2.59 (4H, t, -C*H*$_2$CONH), 1.96 (2H, m, C*H* (C-2a) of 5-amino-1-carboxypentyl), 1.83 (2H, m, C*H* (C-2a) of 5-amino-1-carboxypentyl), 1.77–1.56 (8H, m, C*H*$_2$ (C-3, 4) of 5-amino-1-carboxypentyl).

Purification of $EGII_{core2H}$. Transformed yeast cell possessing plasmid coding $EGII_{core2H}$ was cultivated and secretory enzyme was collected by centrifugation. The collected crude $EGII_{core2H}$ was concentrated by stirred ultrafiltration cell (membrane; NMWL 30,000, Millipore). The supernatant was purified by a metal immobilized affinity chromatography (Ni-NTA agarose beads, QIAGEN) eluted with 20 mM phosphate buffer (500 mM NaCl, pH 7.4) containing 100 mM imidazole. The fractions, which showed the cellulase activity, were collected and concentrated by ultrafiltration. Then, the product was purified by a gel-permeation chromatography (Sephacryl S-200) using 20 mM phosphate buffer (200 mM NaCl, pH 7.4). The fractions, which showed the cellulase activity, were collected and concentrated by ultrafiltration. Both molecular weight and purity of the purified $EGII_{core2H}$ were confirmed by SDS-PAGE on precast 10% polyacrylamide gel (PAGEL NPU-10L, ATTO Corp.). The gel was stained by silver staining. The result of SDS-PAGE was shown in Fig. S1.

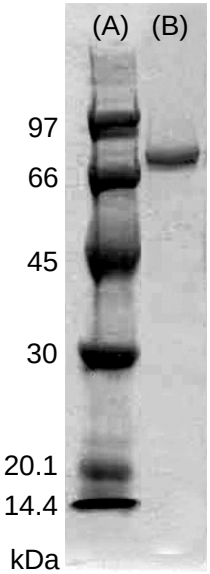

Fig. S1 SDS-PAGE of (A) protein molecular weight marker and (B) purified $EGII_{core2H}$.

Cross-linking of $EGII_{core2H}$ in the solution. $EGII_{core2H}$ was cross-linked by using Ni-chelated bisNTA (bisNTA-Ni). First, bisNTA was chelated by Ni ion as follows. To a $NiSO_4$ aqueous solution, a bisNTA aqueous solution was added dropwise and the mixture solution was incubated at room temperature. The mole ratio is bisNTA : Ni ion = 1 : 25. The final concentration of bisNTA was 5 mM. After 24 h, unreacted Ni ion was removed by Sephadex G-10 column chromatography with water as eluent (bisNTA-Ni). Construction of bisNTA-Ni was confirmed by UV-vis spectroscopic analysis at 280 and 395 nm (specific absorption of bisNTA and Ni ion, respectively). Then, $EGII_{core2H}$ solution (20 mM phosphate buffer, 500 mM NaCl, pH 7.4) was added dropwise to the obtained bisNTA-Ni solution (excess amount against $EGII_{core2H}$). The mixture solution was incubated at 4 ^{o}C to introduce bisNTA-Ni into His-tag of $EGII_{core2H}$. After 24 h, excessive amount of bisNTA-Ni was removed by ultrafiltration (NMWL; 30,000) and bisNTA-Ni bound $EGII_{core2H}$ was collected. Finally, to $EGII_{core2H}$ solution, prepared bisNTA-Ni bound $EGII_{core2H}$ solution was added dropwise and incubated at 4 ^{o}C (cross-linked $EGII_{core2H}$). After 24 h, obtained solution was analyzed by Sephacryl S-200 column chromatography. The fractions which showed absorption at both 280 and 395 nm were collected and concentrated by ultrafiltration (NMWL; 30,000).

The two other obtained elution patterns by gel-permeation chromatography at 280 nm of cross-linked $EGII_{core2H}$ are shown in Fig. S2. In both patterns, main absorption peaks are observed at about 38 mL of elution volume which coincide with Fig. 2a. Moreover, the peak heights of these main absorption peaks were nearly the same (about 4×10^{-3}). Therefore, the cross-linked $EGII_{core2H}$ was reproducibly formed.

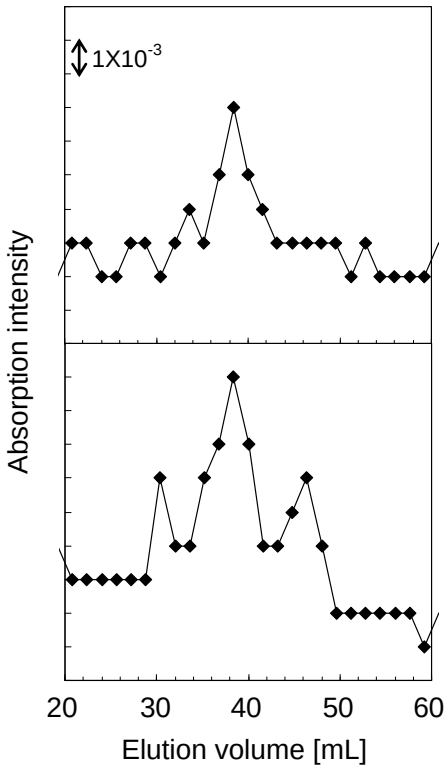

Fig. S2 Elution patterns of gel-permeation chromatography of cross-linked $EGII_{core2H}$.

Enzymatic polymerization using cross-linked $EGII_{core2H}$. Enzymatic polymerization of β-cellobiosyl fluoride (25 mM) was carried out in the acetonitrile/acetate buffer (50 mM, pH 5.0) = 3/1 v/v mixture solution with obtained cross-linked $EGII_{core2H}$ as catalyst at 30 ^{o}C. After 48 h, the solution was centrifuged to separate water-soluble part and water-insoluble part. The obtained precipitation was washed by acetonitirile/acetate buffer = 3/1 v/v solution for two times, and dispersed in acetonitrile.

TEM observation and MALDI-TOF MS measurement. TEM image was taken using JEOL JEM-2000EX microscope at an accelerating voltage of 100 kV.

Samples were dispersed into acetonitrile and putted on the carbon-coated grid without any staining.

MALDI-TOF MS spectra was taken using Bruker ultraflex III mass spectrometer. Samples dissolved in acetonitrile were mixed with the 10 mg/mL dihydroxybenzyoic acid (DHB) acetonitrile solution with 0.1 wt% trifluoroacetic acid (TFA), and air-dried on the sample plate.