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Supporting information for article:

Crystallization and crystallographic analysis of branching enzymes from *Cyanothece* sp. ATCC 51142

Mari Hayashi, Ryuichiro Suzuki, Christophe Colleoni, Steven G. Ball, Naoko Fujita and Eiji Suzuki

Table S1 Substrate preference of bacterial (GH13_9) and plant (GH13_8) BEs.

	Enzyme	**Family**	**Substrate preference**	**Reference**
Cyanothece sp. ATCC 51142	BE1, BE2, BE3	GH13_9	Amylose > Amylopectin	This study
Escherichia coli	EcBE	GH13_9	Amylose > Amylopectin	Guan *et al*., 1997
Deinococcus geothermalis	DgBE	GH13_9	Amylose > Amylopectin	Palomo *et al*., 2009
Deinococcus radiodurans	DrBE	GH13_9	Amylose < Amylopectin	Palomo *et al*., 2009
Oryza sativa L	OsBEI	GH13_8	Amylose > Amylopectin	Nakamura *et al*., 2010
	OsBEIIa, OsBEIIb	GH13_8	Amylose < Amylopectin	
Zea mays	ZmBEI	GH13_8	Amylose > Amylopectin	Guan *et al*., 1997
	ZmBEIIb	GH13_8	Amylose < Amylopectin	

Specific activity or kinetic parameter of recombinant BEs for the amylose and the amylopectin was compared. Substrate preference was indicated by inequality sign.

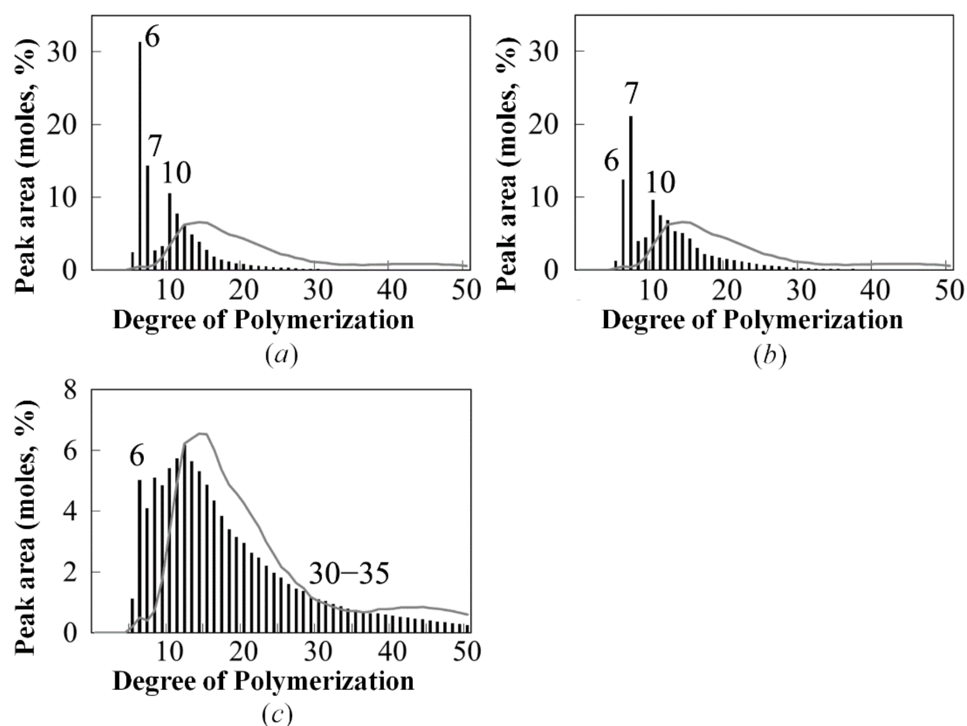

Figure S1 Chain-length distributions of debranched enzymatic reaction products of BE1 (*a*), BE2 (*b*) and BE3 (*c*) using an *ae*-amylopectin as a substrate. An *ae*-amylopectin is purified from the BEIIb-deficient rice endosperm (Nishi *et al*., 2001; Ustumi & Nakamura, 2006). The *ae*-amylopectin from rice has fewer branched chains per molecule as compared to the wild-type amylopectin due to defects of BEIIb (Nakamura *et al*., 2002; Nishi *et al*., 2001). In this respect, *ae*-amylopectin was used as a suitable substrate for the BE reaction. The reaction mixtures (100 µl) containing 100 m*M* HEPES-NaOH buffer (pH 7.0), 64 µg of *ae*-amylopectin, and enzyme (BE1, 1.04 µg; BE2, 0.74 µg; or BE3, 5.00 µg) were incubated at 303 K for 3 h. After debranching treatment and fluorescent labeling, the linear glucan chains were resolved by capillary electrophoresis using P/ACE MDQ Carbohydrate System (Beckman-Coulter) according to the previously described protocol (O'Shea *et al*., 1998). Grey lines in each panel indicate chain-length distributions of *ae*-amylopectin. Black bars in each panel indicate chain-length distributions after reaction with *ae*-amylopectin. The x axis and the y axis represent DP of each chain and molar percentage of each chain respectively. Representative data are shown from three independent experiments. Standard deviations for each molar percentage between the experiments were 0.7% or smaller.

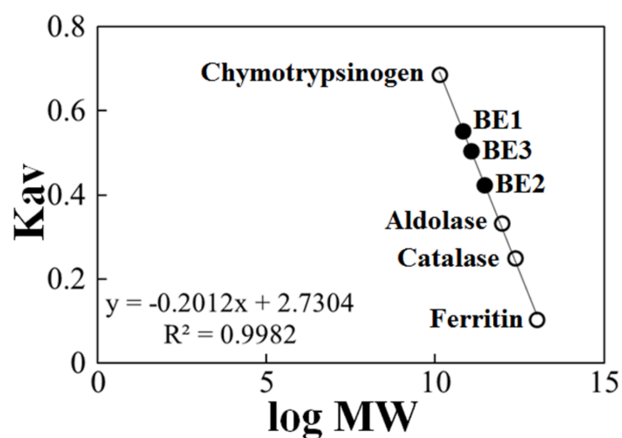

Figure S2 Molecular mass estimation of BE1, BE2 and BE3 by gel-filtration chromatography using Hiload 16/60 Superdex 200 pg column (GE Healthcare) and Gel Filtration Calibration Kits (LMW and HMW; GE Healthcare). Blue dextran 2000 (2000 kDa) was used to measure a void volume. The calibration curve was constructed by plotting Kav values (y axis) versus log molecular masses (x axis) of protein standards (open circles), Ferritin (440 kDa), Catalase (232 kDa), Aldolase (158 kDa) and Chymotrypsinogen (25 kDa) according to the manufacturers' instructions (GE Healthcare). Molecular masses of BE1, BE2 and BE3 (closed circles) were calculated from a linear regression equation.