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Supporting information for article:

**X-ray crystallographic structural studies of α -amylase I from
*Eisenia fetida***

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Tamada**

Ef-AmyI	QYFGSYYCQPGRDVIVHLFEWKWTDIERECQ-WLADHNYCGVQVSPNEHRIIVTDPPYPWWQRYQPVSYTMNSRSGSETQ	96
Ef-AmyII	QYFGSYYCLPGRDVIVHLFEWKWTDIERECQ-WLADHNYCGVQVSPNEHRIIVTDPSYPWWQRYQPVSYKMNSRSGTEAQ	96
Pp-Amy	QY--APQTQSGRTSIVHLFEWRWVDIALECEERYLGPKGGFGVQVSPNENIVVTNPSRPWWERYQPVSYKICTRSGNE	93
Hp-Amy	QY--SPNTQQGRTSIVHLFEWRWVDIALECEERYLAPKGGFGVQVSPNENVAIYNPFRPWWERYQPVSYKICTRSGNE	93
Tm-Amy	---KDNFAFASGRNSIVHLFEWKWNDIADECEERFLQPQGGFGVQVSPNENYLVVA--DGRPWWERYQPVSYIINTRSGDESA	76
Ef-AmyI	FRNMVTTCCNNLGVYIYVDVINVHMTGGGSSTGSD---GNSFDGDSLOYGVPVYGPNDFFHSDDDCSTSDGIEHDYNNPTEV	173
Ef-AmyII	FRNMVSTCCNNLGVYIYVDVINVHMTGGGSSTGSD---GSFFADALEFPQVYGPNDFFHTDDCPTSDQIEHDYNNPTEV	173
Pp-Amy	FRDMVTRCANNVGVRIYVDVINHMCAGSAAAGTGTTCGSGYCNPGRNRFPAVPSAWDFNI--GKCKTASGDIENYNDPYQV	172
Hp-Amy	FRNMVTRCANNVGVRIYVDVINHMCAGNAVAGTSTTCGSGYFNPSRDFPAVPSAWDFNI--GKCKTASGDIENYNDPYQV	172
Tm-Amy	FTDMVTRCNDAGVRIYVDVINHMTGMNGVG---TSGSSADHDGMNYPVAVPYGSGDFHSP-----CEVNNYQDADNV	145
Ef-AmyI	RNCRLSGLRDLDDGSANYVRDVEAEFFANRLIGWVAGFRLLDAKHMWPGDLEAILEGRNLNQLNTQW-FPAGR AIIYQEVVID	252
Ef-AmyII	RNCRLVGLRDLDDGSANYVRDMEAEFFANRLIGWVAGFRLLDAKHMWPGDLEAILEGRNLNQLNTQW-FPAGS AYVFCQEVVID	252
Pp-Amy	RDCQLVGLLDDLALEKDYVRSMIADYLNKLIIGVAGFRLLDAKHMWPGDIKAVLDKLNLSNTNW-FPAGS PFIFQEVVID	251
Hp-Amy	RDCRLTGLLDDLALEKDYVRSKIAEYMNHLIIGVAGFRLLDAKHMWPGDIKAVLDKLNLSNTNW-FPAGS PFIIYQEVVID	251
Tm-Amy	RNCELVGLRDLNQGSDYVRGVLDIDYMNHMIIGLVAGFRVDAKHMSPGDLSEVIFSGLNKLNLTQYGFADGA PFIIYQEVVID	225
Ef-AmyI	LGGEAVKATEYTYLGRVTEFKHGQLGNVVRKKNQORLANLVNFGEAGWQLSFDALVFIDNHDNQRGHGAGGFGTILTF	332
Ef-AmyII	MGGEPIATASEYTYLGRVTEFKHGQLANVIRKKNQORLAYLVNFGEAGWQLNFDALVFIDNHDNQR T---GHGTILTF	328
Pp-Amy	LGGEAVQSSEYFCNGRVTEFKYCALGTVVRKWSCEKMSYLNKNGEGWGFMPDRALVFDNHDNQRCHCACGA-SILTF	330
Hp-Amy	LGGEPIKSSDYFCNGRVTEFKYCALGTVVRKWSCEKMSYLNKNGEGWGFVMPDRALVFDNHDNQRGHGAGGA-SILTF	330
Tm-Amy	LGGEAVSKNEYTGFGCVEFQFGVSLGNAPQ---GGNQLKLANWGPWGLLELDALVVFVNDNHDNQR TGG-----SQILTY	299
Ef-AmyI	FEARMYKIATFEELAWDYGHVRLMSSSYNWRNIVNGVDVTDNDWVGPP T-NGGGDT-----DVECFNGEWICEHRWRREYNM	407
Ef-AmyII	FEARMYKIATFEELAWDYGHVRLMSSSYWRNIVVDGVDINDWVGPP T-NGGGDT-----DVECFNGEWICEHRWRRIENM	403
Pp-Amy	WDARLYKVAVGFMLAHPYGFTRVMSSYRWARNFVNGQDVNDWVGPPN-NGGVIKVITINADTTCGMDWVCEHRWRRIENM	409
Hp-Amy	WDARLYKMAVGFMLAHPYGFTRVMSSYRWRFQFNGQDVNDWVGPPN-NGGVIKVITINADTTCGMDWVCEHRWRRIENM	409
Tm-Amy	KNPKPVKMAIFMLAHPYGTTRVMSSFDFTDND-----QGGPQDSSGNLISFGINDNTCSNGYVCEHRWRQVYGM	370
Ef-AmyI	VRFHNVVIGNP SNWWDNCFQAIAFGRGRGFIFINNEDEFAITQTLQTLPLPGGEYCDVISCDNNRPPCG SGGACRAVI	487
Ef-AmyII	VRFHNVVIGNP ANWWDNCFHAIAFGRGRGFIFINNEDEFAITQSLQTLPLPGGEYCDVISCDNNRPPCG SGGACRAVI	483
Pp-Amy	VWFRNVVDGQP ANWWDNCSNQVAFGRGRGFIFVFNDDWQLSSTLQTLPLPGTYCDVISGDKVGNST-----IIVY	483
Hp-Amy	VIFRNVVDGQP TNWWDNCSNQVAFGRGRGFIFVFNDDWSFSLTQTLPLPGTYCDVISGDKINGNCT-----IIVY	483
Tm-Amy	VGFRNAVEGTQ ENWWDNDNQIAFSRGGQGFVAFVNGG-DLNLQNLNTGLPAGTYCDVISGELSGGSC T-----KIVT	443
Ef-AmyI	VNGDGAATFDVFN-GENPNI AIIHV --- 510	
Ef-AmyII	VNGDGAATFNVPN-GEDPNI AIIHV --- 506	
Pp-Amy	VSDDGAQFSIS SAEDPNI AIIH ESKL 511	
Hp-Amy	VSDDGAHFSIS SAEDPNI AIIH ESKL 511	
Tm-Amy	VGDNGADISLG AEDDGI LAIH NAKL 471	

Figure S1 Sequence alignment of α -amylases. The multiple sequence alignment of Ef-Amy I, II, Pp-Amy, Hp-Amy and Tm-Amy was performed using the Clustal Omega server (Sievers *et al.*, 2011), but was slightly modified based on the 3D structures. The completely, highly and weakly conserved residues are shown in red, dark pink and light pink characters. The catalytic nucleophile (Asp) and acid/base catalyst (Glu) are indicated by a green box. The highly conserved Asp residue involved in the substrate binding at the -1 subsite is indicated by a light blue box. The Gly-rich loop is indicated by a black box. The deleted and inserted loops in Ef-Amy I are indicated by red and blue boxes.

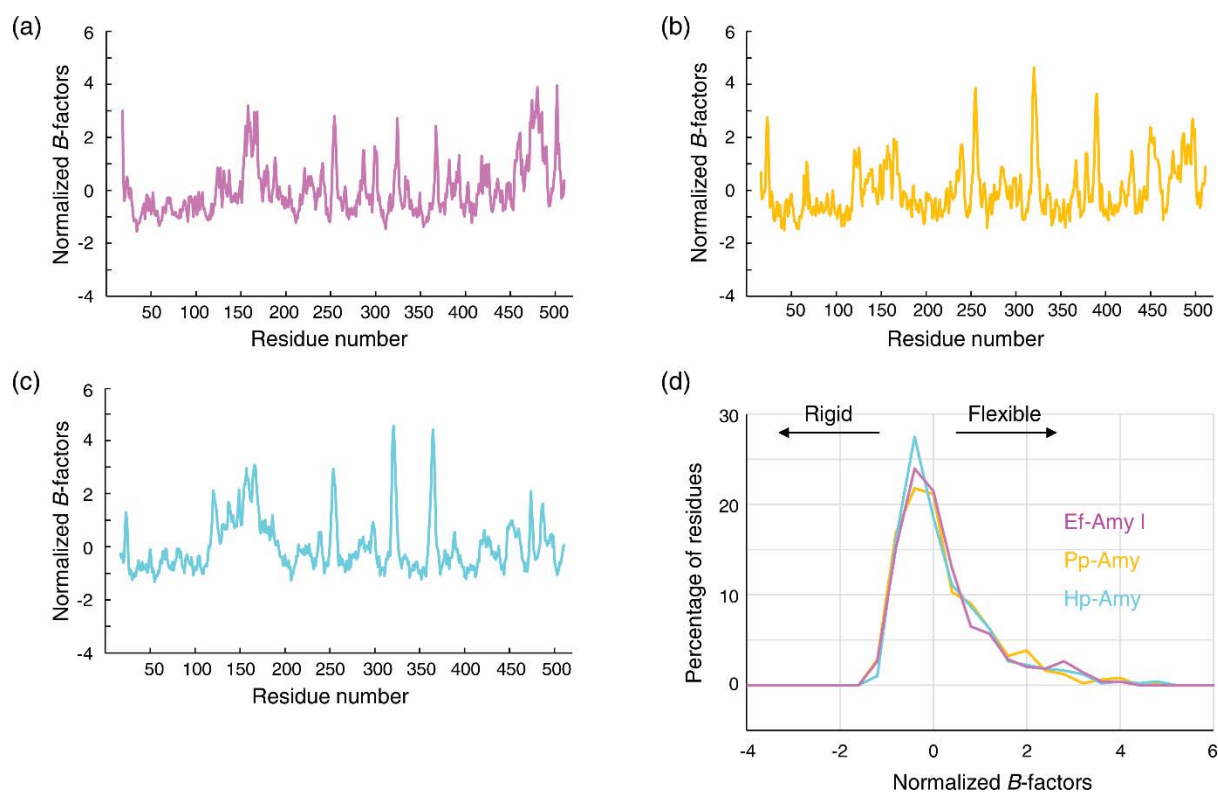


Figure S2 Normalized B -factors. Normalized B -factors were calculated using the equation $B_{\text{norm}} = (B_{\text{coord}} - B_{\text{ave}}) / B_{\text{sig}}$, where B_{norm} is normalized B -factor, B_{coord} is B -factor of C_{α} atoms in the coordinate files, B_{ave} and B_{sig} are average and standard deviation of B_{coord} . (a) The relative B -factors against residues in Ef-Amy I. (b) The relative B -factors against residues in Pp-Amy. (c) The relative B -factors against residues in Hp-Amy. (d) Percentage of residues with B -factors in a bin size of 0.4 for Ef-Amy I (wild type, pink), Pp-Amy (1VAH, orange) and Hp-Amy (1HNY, light blue).

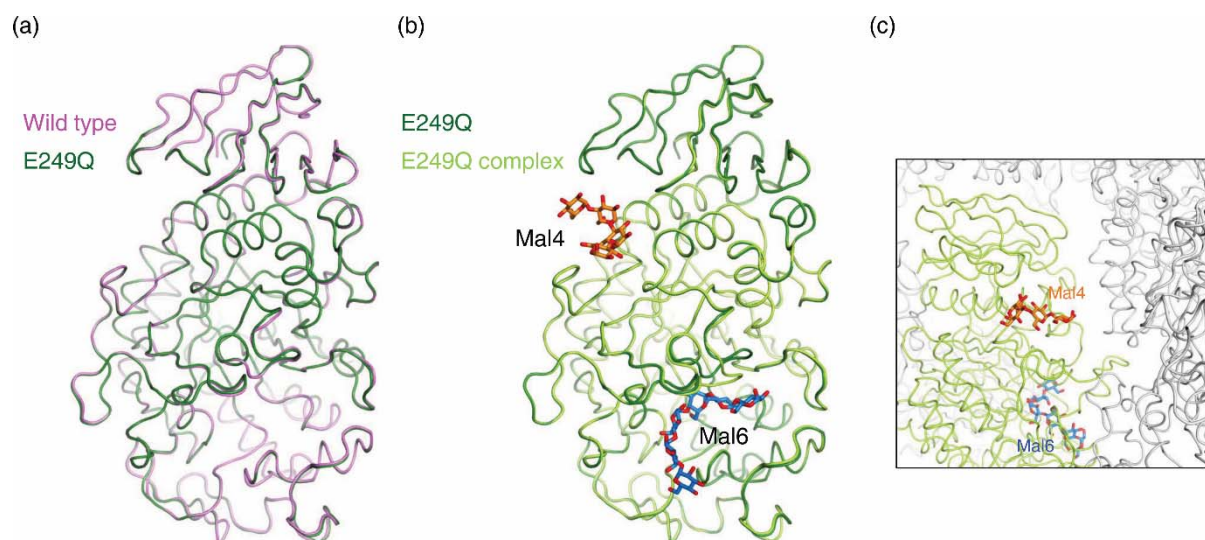


Figure S3 Superposition of the Ef-Amy I structure. (a) Superposition between the wild type (pink) and substrate-free E249Q (dark green) structures. (b) Superposition between substrate-free (dark green) and substrate complex (green) structures of the E249Q mutant. The Mal6 and Mal4 molecules in the substrate complex structure are shown as stick models. (c) Symmetry-related molecules in the crystal of Ef-Amy I: The protein in the asymmetric unit is shown as green ribbon and Mal4 and Mal6 are shown as stick models. The symmetry related proteins within the distance of 20 Å from the molecule in the asymmetric unit are shown as grey ribbons.