SUPPLEMENTAL DATA

A novel α-glucosidase from the acidophilic archaeon, *Ferroplasma acidiphilum* Y with strong transglycosylation activity and an unusual catalytic nucleophile

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Supplemental Materials and Methods

Materials and strains of microorganisms

Potato starch, amylose, amylopectin, pullulan, glucose, maltose, maltotriose, iso-maltose, iso-maltotriose, trehalose, maltooligosaccharides (from G4 to G8), *p*-nitrophenyl alpha/beta-D-glucopyranoside (pNPG), *p*-chloromercuriphenylsulphonic acid (PCMPS), N-ethylmaleimide, iodoacetate, *p*-chloromercuribenzoate (CMB), tosylphenylalanylchloromethane (TPCK), diethyl pyrocarbonate (DEPC), 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide (EDC) and tetranitromethane (TNM) were from Sigma Chemical Co (St. Louis, MO, USA). Kejibiose and nigeriose were from TCI (Tokyo, Japan). Sucrose and soluble starch (Paselli SA2) were provided by Merck and Avebe (Foxhol, The Netherlands), respectively. 5-fluoro-α-D-glucopyranosyl fluoride (5FαGlcF) was synthesized as described McCarter *et al*. [1]. Molecular mass markers for SDS-PAGE were provided by Novagen (Madison, Wisconsin, USA). DNA restriction and modification enzymes were from New England Biolabs (Beverly, Massachusetts, USA). DNase I grade II and pepsin (porcine mucosa), were obtained from Boehringer Mannheim (Mannheim, Germany). Chromatographic media and LMW calibration kit for native electrophoresis were from Amersham Pharmacia Biotech (Little Chalfont, United Kingdom).

Cloning, expression of *αgluFa* from *F. acidiphilium* Y^T and purification of the recombinant protein

Cloning and detection of *F. acidiphilum* glucosidases was performed after establishing genome library of the *F. acidiphilum* Y in bacteriophage lambda, using ZAP Express Kit (Stratagene, La Jolla, California, USA), and phage infection of *E. coli* XL1-Blue MRF'. α*gluFa*-harbouring clone was detected among glucosidase-positive clones as follows. After infection of *E. coli* XL1-Blue MRF' and consequent incubation, the plates (22.5 x 22.5 cm) containing 2% (w/v) sucrose and 10 μM $FeCl_2$ in the soft and bottom NZY agar, with about 10,000 phage clones per plate, were incubated overnight and then overlaid with 50 ml of iodine solution (Sigma). Positive clones exhibiting a violet halo were picked and the separate positive clones were isolated after consequent phage particles dilution, *E. coli* infection and halo detection. From one of the selected phage colonies, the pBKGluFa phagemid has been derived using helper phage excision procedure (Stratagene) and transferred to *E. coli* XLOLR

cells. The complete nucleotide sequence of the DNA fragment, coding for α*gluFa* reported in this paper, was submitted to EMBL/GenBank/DDBJ Databases under the accession number AJ 717661.

For the expression of α*gluFa*, the *E. coli* cells were grown at 37°C in LB medium containing 50 μg of kanamycin/ml and 10 μM $FeCl_2$. When the OD_{600} reached 1.0, isopropyl-β-D-galactopyranoside (IPTG) was added to a final concentration of 1 mM to induce expression. Cells were harvested 3 h after induction, and resuspended in buffer A [10 mM sodium citrate buffer, pH 3.0] containing one protease inhibitor cocktail tablet (Roche) and 10 μg/ml DNase I grade II (Roche), incubated on ice for 30-45 min, and then sonicated for 4 min total time. The soluble fraction was separated from insoluble debris by centrifugation (10,000 x g, 30 min, 4°C), dialyzed overnight against buffer A, concentrated by ultrafiltration on a Centricon YM-10 membrane (Amicon, Millipore) to a total volume of 1000 μl, and purified as follows. Sample was applied to a HiPrep 16/10 SP XL (Amersham Pharmacia Biotech) column and washed with buffer A. αGluFa was eluted with a linear gradient of NaCl (total volume, 200 ml; 0 to 0.2 M). Active fractions were pooled and dialyzed against 50 mM sodium citrate, pH 3.0, 1 M $(NH_4)_2SO_4$). 1000 μl of Centricon YM-10-concentrated and filtered sample (0.22 μm) containing αGluFa were loaded on a Resource 15PHE hydrophobic chromatography column (PE 4.6/100), previously equilibrated with the same buffer. The column was washed with the equilibrium buffer and the enzyme was eluted with a linear gradient of $(NH_4)_2SO_4$ (total volume of 25 ml, 1.0 to 0 M). The eluted enzyme was dialyzed against buffer A overnight, and concentrated by ultrafiltration on a Centricon YM-10 (Amicon, Millipore), to a total volume of 1000 μl. Sample was further purified on a Superose 12 HR 10/30 gel filtration column pre-equilibrated with 10 mM sodium citrate (pH 3.0), 150 mM NaCl. Separation was performed at 4°C at a flow rate of 0.5 ml/min. The purified recombinant α-glucosidase was dialyzed *vs.* buffer A overnight and stored at –20°C, at a concentration of 10 μM, until use.

Expression level of αGluFa

For quantification of protein expression, cell free extracts, prepared as described above, were examined using SDS-PAGE with 12-15% (v/v) acrylamide. Proteins in the gel were stained by Coomassie brilliant blue R-250 (BIOMOL GmbH Hamburg, Germany), and the gel region corresponding to the αGluFa size was examined for rough estimation of the expressed protein quantity in the total protein fraction. A Molecular Dynamics densitometer was used to scan the Coomassie-stained gel, and ImageQuant software was used to quantify the intensity of the bands by volume integration.

Hydrolytic assay

The hydrolytic activity towards sucrose, starch, amylose, amylopectin, pullulan and dextrin was

determined by measuring the release of reducing sugars from 1% (w/v) substrate solutions using the dinitrosalicylic acid (DNS) method [2]. The reaction mixture (50 µl) was composed by 1% (w/w) substrate in the corresponding buffer and the enzyme (0.25 µg). A calibration curve was obtained with a 2 g/l glucose solution. The microplate was incubated at 200 rpm for 30 min in an orbital shaker (Stuart Scientific). Then, 50 µl of 10 g/l DNS were added to each well, heated at 85ºC for 30 min and cooled to room temperature. Finally each well was diluted with 150 µl water and absorbance at 540 nm measured. Hydrolytic activity using kejibiose, nigeriose, iso-maltose, iso-maltotriose, trehalose and maltooligosaccharides from G4 to G7 was studied by HPLC assay (see below). The reaction mixture (1 ml) contained the substrate (1% w/v), and 5 µg αGluFa. Reaction was followed for 30 min, and then stopped by heating 15 min at 80ºC, before HPLC analysis. Sample blanks were used to correct for spontaneous release of reducing sugar. In all cases, one enzyme unit was defined as that liberating 1 µmol of glucose (or equivalent reducing groups) per minute. Activity towards *p*-nitrophenyl alpha/beta-D-glucopyranoside (pNPG) was measured spectrophotometrically in a UV/visible spectrophotometer by following the increase in A_{346} of a reaction mixture (3 mL) containing 15 µg of enzyme and 2 mM of pNPG as the substrate. A molar extinction coefficient of 4800 $mol^{-1} \cdot dm^3 \cdot cm^{-1}$ was used. One unit of activity was defined as the amount of enzyme hydrolyzing 1 µmol of *p*-nitrophenol per min under these conditions.

Standard hydrolytic assay and determination of kinetic parameters

Unlike otherwise indicated, the standard assay used in the present study was performed at 50°C in 100 mM sodium citrate buffer (pH 3.0) and 1% (w/v) sucrose, as substrate (see conditions above). Kinetic parameters (k_{cat} and K_m) were determined at 50ºC in 100 mM sodium citrate buffer (pH 3.0). Substrate concentration was varied in the range 0.1 – 20.0 mM and the activity measured as described above. Kinetic parameters were calculated fitting the initial rate values to the Hanes-Woolf transformation of the Michaelis-Menten equation.

Transglycosylation assay and HPLC conditions

The transglycosylation assay, using 5 µg purified αGluFa/ml, was carried out at 50ºC in 0.2 M sodium citrate buffer (pH 3.0) supplemented with 600 g/l maltose. The reaction was allowed to proceed during 180 min. Aliquots were taken at intervals, the enzyme was inactivated by heating 15 min at 80ºC, and then the sample diluted 1:5 (v/v) with water, centrifuged and filtered using Ultrafree-MC filter (0.45 µm) devices (Millipore). Analyses were carried out by HPLC using a 4.6 x 250 mm Lichrospher-NH_2 column (Merck, Darmstadt, Germany). Acetonitrile:H_2O 75:25 (v/v) was used as mobile phase at 0.7 ml/min. Detection was performed using a refraction index detector (Varian). The column was kept constant at 25°C. Integration was carried out using the Millennium software.

pH and thermal activity and stability

The optimal pH for enzyme activity was measured at 50ºC incubating the enzyme substrate mixture (using sucrose as substrate) at pH ranging from 0.8 to 10. The buffers (100 mM) used were: citrate (pH 1.0-4.5), acetate (pH 4.5-5.5), MES (pH 5.5-7.0) and HEPES (pH 7.0). The enzyme was allowed to react for 30 min after which, the enzymatic activity was measured using the DNS method [25]. The optimal temperature for activity was determined by incubating the enzyme-substrate mixture (in 100 mM sodium citrate buffer, pH 3.0) at temperatures ranging from 15 to 80°C, after equilibration of solution for 2 min. The pH and thermal stabilities were estimated by preincubating the enzyme, at pH and temperatures in the range 1.5-6.5 and 40-80°C, respectively. Aliquots (100 µl) were taken at intervals and the remaining hydrolytic activity was measured using the standard α-glucosidase assay, after adding the substrate (sucrose).

Effect of various chemicals on αGluFa activity

The influence of cations on enzyme activity was analyzed by adding the chloride salts to the standard α-glucosidase substrate mixture to final concentrations ranging from 0 to 125 mM. Activity measurements were made immediately and after 30 min of incubation. Residual activity was expressed as percent of the control value obtained without addition of cation. All values were determined in triplicate and were corrected considering the spontaneous hydrolysis of the substrate.

For inactivation kinetics, the enzyme (final concentration, 0.1 mg/ml) was preincubated with a range of concentration of the inhibitors (0-10.0 mM) at 50ºC in 100 mM sodium citrate buffer, pH 3.0, and 50 µl aliquots of the sample were withdrawn at time intervals, stopped by chilling on ice, and analyzed using the standard α-glucosidase assay. Residual activity was expressed as percent of the control value obtained without addition of chemical. Values for the inactivation rate contants (k_i) and the dissociation constants for the inactivators (K_i) were determined by fitting to Equation 1.

$$K_{obs} = k_i[I] / (K_i + [I]) \qquad \text{(Eq. 1)}$$

where K_{obs} is the measured rate constant, and I is the concentration of inhibitor.

Modification of αGluFa with PCMPS, DFP, TNM and EDC

α-Glucosidase was subjected to modification of specific amino acids by using standard protocols [3-5]. Briefly, purified recombinant αGluFa (100 µg) was treated with PCMPS in 10 mM sodium citrate buffer, pH 3.0, for 120 min at 25ºC, or with DFP for 30 min at 37ºC. Following treatment, the reaction mixture was dialyzed against 10 mM sodium citrate buffer, pH 3.0, containing 300 mM NaCl, 10% glycerol and 0.1% taurodeoxycholate, and stored at –20ºC. Alternatively, purified αGluFa (100 µg) was incubated at 25ºC in 1 ml 10 mM sodium citrate buffer, pH 3.0, with an ethanolic TNM solution (final concentration range: 0-20 mM) (total ethanol added: 10 µl). The reaction was allowed to

proceed for 18ºC. Modified proteins were separated from that unmodified by high-performance liquid chromatography (HPLC) using a Bio-Sil SEC 400 column (Bio-Rad) preequilibrated with 10 mM sodium citrate buffer, pH 3.0. Separation was performed at 25ºC at a flow rate of 0.4 ml/min. Fractions containing bound fractions were pooled and stored for further investigation. αGluFa was subjected to chemical modification of carboxylic groups as follows. Purified αGluFa (2.0 mg) was incubated in 5 mL of 10 mM MES buffer (pH 4.0) containing 10 mM KCl at 25ºC with solid amino nucleophile (taurine: 198 mM final concentration) and 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide (EDC) (19.8 mM final concentration), as described by Alcalde *et al*. [5]. Reaction was maintained at pH 4.0 with 0.2 N NaOH using a pH-stat (Radiometer) for 3 hours, after which the sample was treated with 0.5 M hydroxylamine (pH 7.0) for 5 h at 25ºC. Then, sample was dialyzed against 10 mM MES buffer (pH 4.0) at 4ºC to remove salt and excess of reagents. Activity measurements were made immediately and after 30 min of incubation. In all cases, residual activity after modification was measured immediately, in three independent assays, and expressed as percent of the control value obtained without addition of inhibitor.

Construction of αGluFa protein variants containing Glu, Asp and His mutations

Point mutations were introduced into the phagemid pBKGluFa using the QuickChange SDM procedure (Stratagene) with the oligonucleotide pairs synthesized at Sigma-Genosys Ltd. (Pampisford, Cambs, UK) and success of mutagenesis was confirmed by DNA sequencing using the sequencing primers CH4: 5'-AAC TCA TTA TAT ATA TTG AAT C-3' and CH1158: 5'-ATT AGT GTT CCA TGA CGG TAA A-3'. Eight Glu residues at positions 54, 258, 311, 426, 450, 452, 516 and 531 were replaced by Gln residues (αGluFa E/Q). Seventeen Asp residues at positions 40, 125, 172, 179, 185, 202, 250, 276, 334, 346, 391, 397, 428, 476, 502, 504 and 510 were also replaced by Gln (αGluFa D/Q). Finally the His residues at positions 9, 41, 47, 243, 275, 325, 377 and 390 were replaced by Ala (αGluFa H/A). Oligonucleotides used for mutagenesis are listed in Table S1. The resulting mutated plasmids were transformed into *E. coli* DH5α electrocompetent cells (Invitrogen), which were plated on LB agar supplemented with 50 μg/ml kanamycin. Mutations were confirmed by DNA sequencing using the sequencing primers CH4: 5'-AAC TCA TTA TAT ATA TTG AAT C-3' and CH1158: 5'-ATT AGT GTT CCA TGA CGG TAA A-3'. Mutant proteins were purified using the same protocol as for the wild type protein.

Detection of the catalytic nucleophile

To identify the catalytic nucleophile of *F. acidiphilum* α-glucosidase, the enzyme was labelled with 5FαGlcF, and then subjected to proteolysis and electrospray ionization MS (ESI-MS) to identify labelled peptides. Briefly, a stock solution of the enzyme (50 μl, 10 mg/ml) was incubated with

5FαGlcF (50 µl, 10 mM) at 37ºC for 5 min. The sample was diluted with 50 mM phosphate buffer (pH 2.0, 200 µl) and incubated with pepsin (15 µl, 1 mg/ml) for 15 min at room temperature. The sample was then frozen quickly and analyzed immediately upon thawing. A control sample in the absence of 5FαGlcF was prepared according to the same procedure. Mass spectra were recorded on a VG AutoSpec spectrometer equipped with and ESI ion source. ESI-MS in the negative mode was performed in a QTOF-MS. Neon served as the collision gas for high energy collision-induced dissociation (CID). Peptides were separated by reversed-phase HPLC using a C_{18} column (Análisis Vínicos, Spain; 4.6mm×150mm) and a refraction index detector (Spectra-Physics), and eluted with a gradient of 0–60% eluting solvent (0.045% trifluoroacetic acid/80% acetonitrile in water) over 60 min at a flow rate of 100 µl/min. The temperature of the column was kept constant at 25ºC. Total ion chromatograms of the labelled and unlabelled enzyme digests were compared to find the fraction containing the labelled peptide fragments, which was collected and analyzed by MS/MS fragmentation analysis.

Circular dichroism and inductively coupled plasma-mass spectroscopy

Circular dicroism (CD) spectra of αGluFa at a concentration of 10 mg/ml at pH values ranging from 0.8 to 7.0, were measured with a Jasco J-720 spectropolarimeter equipped with a constant-temperature cell holder (40ºC), and 0.1-cm cell. Spectra were measured in the following buffers (100 mM): citrate (pH 1.0-4.5), acetate (pH 4.5-5.5), MES (pH 5.5-7.0), and HEPES (pH 7.0). When necessary, the enzyme solution was incubated with 1 mM EDTA before absorption spectra were recorded. The metal ion content of αGluFa variants was determined using a Perkin-Elmer Life Sciences ICP-MS (model PE ELAN 6100 DRC). The metal content was determined by dilution of 50 µg of enzyme with 5 ml of 0.5% (v/v) HNO_3 to digest the protein and release the metal ions and this solution was used without any further manipulation.

Assays and other methods

The protein concentration was determined by the Bradford dye-binding method with a Bio-Rad Protein Assay Kit with bovine serum albumin as standard [6]. SDS-PAGE and native electrophoresis were performed according to Laemmli [7].

Supplemental References

1. Mc Carter, J. D., and Withers, S. G. (1996) 5-Fluoro Glycosides: A New Class of Mechanism-Based Inhibitors of Both α-and β-Glucosidases. J. Am. Chem. Soc. **118,** 241-242
2. Summer, J. B., and Howell, S. F. (1935) A method for determination of invertase activity. J. Biol. Chem. **108,** 51-54

3. Villette, J. R., Helbecque, N., Aibani, J. R., Sicard, P. J., and Bouquelet, S. J.-L. (1993) Cyclomaltodextrin glucanotransferase from *Bacillus circulans* E 192: nitration with tetranitromethane. Biotechnol. Appl. Biochem. **17,** 205-216
4. Tenno, M., Toba, S., Kézdy, F. J., Elhammet, A. P., and Kurosaka, A. (2002) Identification of two cysteine residues involved in the binding of UDP-GalNAc to UDP-GalNAc:polypeptide N-acetylgalactosaminyltransferase 1 (GalNAc-T1). Eur. J. Biochem. **269,** 4308-4316
5. Alcalde, M., Plou, F. J., Pérez-Boada, M., García-Arellano, H., Valdés, I., Méndez, E., and Ballesteros, A. (2003*)* Chemical modification of carboxylic residues in a cyclodextrin glucanotransferase and its implication in the hydrolysis/transglycosylation ratio of the α-amylase family. J. Mol. Catal. B. **26,** 57-67
6. Bradford, M.M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72,** 248-254
7. Laemmli, U. K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature **227,** 680-685

Supplemental Tables

Supplemental Table S1. Oligonucleotides used for site-directed mutagenesis[a]

Mutation	Forward and reverse oligonucleotides
Glu54Gln	5'-AACGTGAGCCAGGTGCCGAAA-3' and 5'-TTTCGGCACCTGGCTCACGTT-3'
Glu258Gln	5'-AACATGACCCAGCGTATT-3' and 5'-AATACGCTGGGTCATGTT-3'
Glu311Gln	5'-TATAAATATCAGGTGCCGGTG-3' and 5'-CACCGGCACCTGATATTTATA-3'
Glu426Gln	5'-AACGGCATTCAGACCGATAGC-3' and 5'-GCTATCGGTCTGAATGCCGTT-3'
Glu450Gln	5'-AGCCTGCAGTTTGAAAAC-3' and 5'-GTTTTCAAACTGCAGGCT-3'
Glu452Gln	5'-AGCCTGCAGTTTCAGAACTTT-3' and 5'-AAAGTTCTGAAACTGCAGGCT-3'
Glu516Gln	5'-ATGACCGTGCAGGTGTAT-3' and 5'-ATACACCTGCACGGTCAT-3'
Asp40 Gln	5'-AACCCGACCCAGCATCTG-3' and 5'-CAGATGCTGGGTCGGGTT-3'
Asp125 Gln	5'-ATTAACCTGCAGAGCAAC-3' and 5'-GTTGCTCTGCAGGTTAAT-3'
Asp172 Gln	5'-CTGAACACCCAGAACATT-3' and 5'-AATGTTCTGGGTGTTCAG-3'
Asp179 Gln	5'-ACCGGCACCTTTCAGAAAAACATT-3' and 5'-AATGTTTTTCTGAAAGGTGCCGGT-3'
Asp185 Gln	5'-ATTACCATTCAGCTGGGCAAA-3' and 5'-TTTGCCCAGCTGAATGGTAAT-3'
Asp202 Gln	5'-AAACTGCAGGGCGCGGTGTATAGCTAT-3' and 5'-ATAGCTATACACCGCGCCCTGCAGTTT-3'
Asp250 Gln	5'-AACAACCAGACCGCGCGTTTT-3' and 5'-AAAACGCGCGGTCTGGTTGTT-3'
Asp250 Gln	5'-AACAACCAGACCGCGCGTTTT-3' and 5'-AAAACGCGCGGTCTGGTTGTT-3'
Asp276 Gln	5'-CATCAGAACAGCAGCGTGGCGAACAGC-3' and 5'-GCTGTTCGCCACGCTGCTGTTCTGATG-3'
Asp334 Gln	5'-AACTTTGGCGTGCAGGTGACC-3' and 5'-GGTCACCTGCACGCCAAAGTT-3'
Asp346 Gln	5'-AAAACCATTCAGCCGTATCTG-3' and 5'-CAGATACGGCTGAATGGTTTT-3'
Asp391 Gln	5'-TTTCATCAGGGCAAAATT-3' and 5'-AATTTTGCCCTGATGAAA-3'
Asp397 Gln	5'-AAAATTGTGTATCAGAACACCATT-3' and 5'-AATGGTGTTCTGATACACAATTTT-3'
Asp428 Gln	5'-ATTGAAACCCAGAGCACCAAC-3' and 5'-GTTGGTGCTCTGGGTTTCAAT-3'
Asp476 Gln	5'-AGCTATCCGCTGCAGAGCGGCCTG-3' and 5'-CAGGCCGCTCTGCAGCGGATAGCT-3'
Asp502 Gln and Asp504 Gln	5'-AAACTGGTGTATCAGAACCAGAGCAAA-3' and 5'-TTTGCTCTGGTTCTGATACACCAGTTT-3'
Asp510 Gln	5'-AGCACCCAGGGCCGTATGACC-3' and 5'-GGTCATACGGCCCTGGGTGCT-3'
His9Ala	5'-AACAGCCTGTATATTCTGAACGCGAAAGTGATT-3' and 5'-AATCACTTTCGCGTTCAGAATATACAGGCTGTT-3'
His41Ala	5'-TATAACCCGACCGATGCGCTGATT-3' and 5'-AATCAGCGCATCGGTCGGGTTATA-3'
His47Ala	5'-AAAGCGCCGATTAGCAACGT-3' and 5'-ACGTTGCTAATCGGCGCTTT-3'
His243Ala	5'-AACAGCAGCGTGATTGCGTATAAC-3' and 5'-GTTATACGCAATCACGCTGCTGTT-3'
His275Ala	5'-AGCGCGGTGCCGCTGGCGGAT-3' and 5'-ATCCGCCAGCGGCACCGCGCT-3'
His325Ala	5'-TATAGCAACGCGGCGTATGTGAGCAAA-3' and 5'-TTTGCTCACATACGCCGCGTTGCTATA-3'
His377Ala	5'-AACGGCATTGCGATGGCGACC-3' and 5'-GGTCGCCATCGCAATGCCGTT-3'
His390Ala	5'-CTGGTGTTTGCGGATGGCAAAATT-3' and 5'-AATTTTGCCATCCGCAAACACCAG-3'
Thr212Gln	5'-GCTATAACTGGGTGCAGAACGGCA-3' and 5'-TGCCGTTCTGCACCCAGTTATAGC-3'
Asn213Gln	5'-GACCCAGGGCAGCGGCatgGCGA-3' and 5'-TCGCcatGCCGCTGCCCTGGGTC-3'

[a]Primers were designed using software Primer ExpressTM using αgluFa coding sequence.

Supplemental Table S2. Transglycosylation of maltose by αGluFa[a]

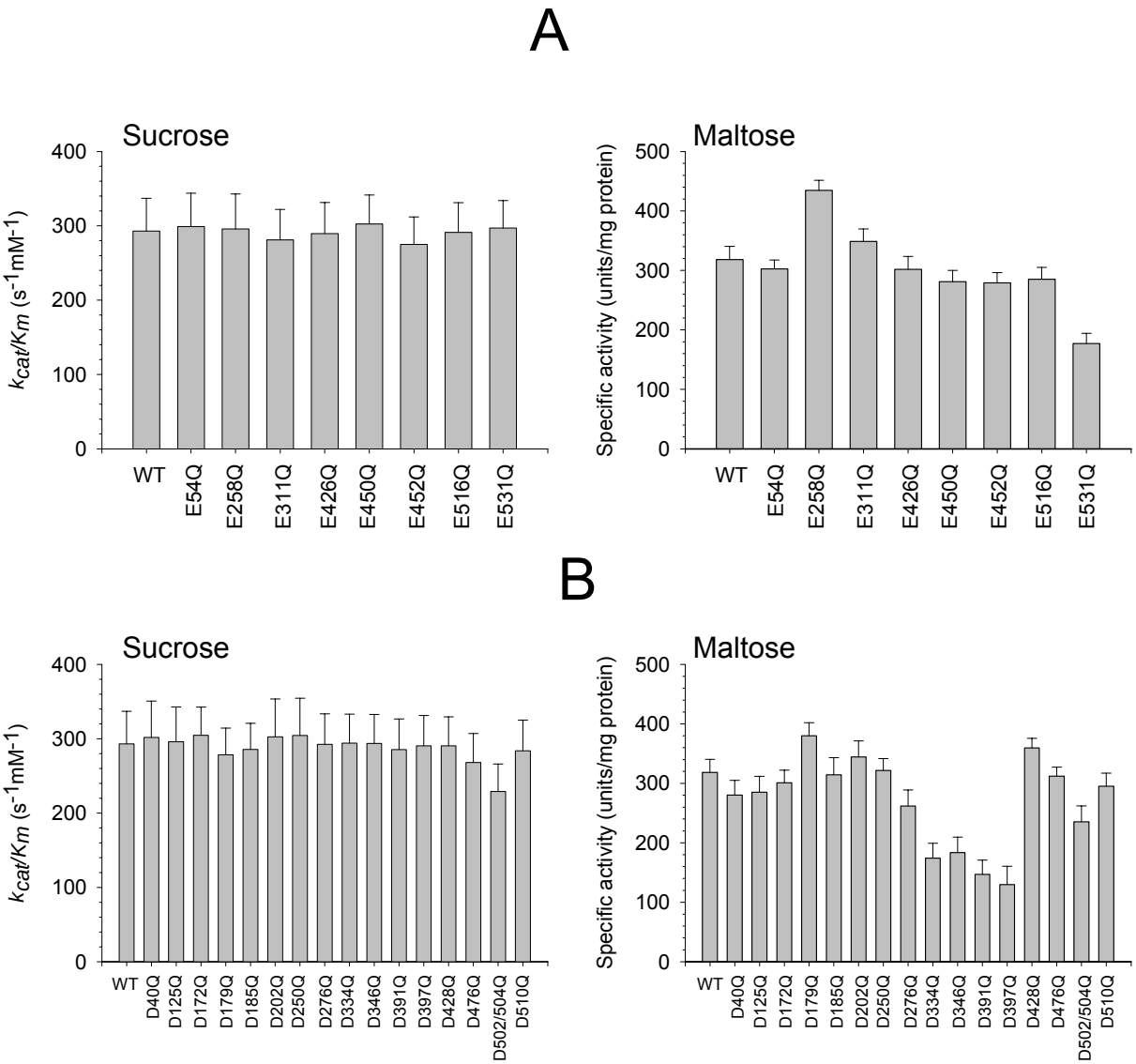
Reaction time (min)	Glucose (g/l)	Maltose (g/l)	Maltotriose (g/l)	maltose transglycosylated (%)
0	0	600	0	-----
5	59	456	88	70.3
10	76	402	122	72.7
20	112	302	186	74.5
30	153	199	248	73.3
60	165	159	276	74.7
180	185	111	304	74.0

[a]Reaction conditions: 600 g maltose/l in 0.2 M sodium citrate buffer (pH 3.0), 5 μg purified αGluFa/ml, 50ºC.

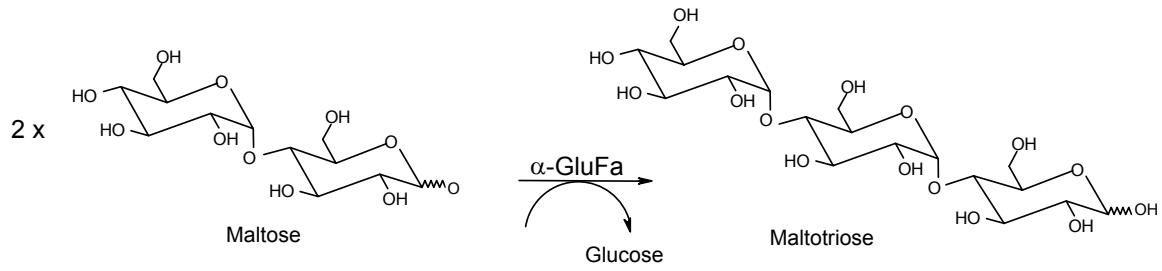
Supplemental Figures

Supplemental Figure S1. Catalytic specificity (k_{cat}/K_m) of wild type, E/Q and D/Q mutant α-glucosidases from *F. acidophilum* for hydrolysis of sucrose (A and B, left site) and apparent specific activities on maltose (A and B, right site)

Activity was measured at 50ºC in 100 mM sodium citrate buffer, pH 3.0, using standard the assays.



Supplemental Figure S2. Scheme of the transglycosylation process catalyzed by αGluFa, using maltose as substrate.



Supplemental Figure S3. Circular dichroism of αGluFa variants.

The secondary structure CD was measured at wavelength between 200 and 250 nm. The CD spectra were measured for recombinant wild type and Thr212Gln, His390Ala and Thr212Gln/His390Ala variants.