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ORIGINAL ARTICLE

A novel amylomaltase from *Corynebacterium glutamicum* and analysis of the large-ring cyclodextrin products

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Abstract Amylomaltase catalyzes the formation of large-ring cyclodextrins (LR-CDs) from starch. This study aims to construct the recombinant amylomaltase from *Corynebacterium glutamicum* and to characterize the purified enzyme with the emphasis on the profile of LR-CDs production. A novel amylomaltase from *Corynebacterium glutamicum* ATCC 13032 was cloned and expressed in *Escherichia coli* BL21 (DE3) using the expression vector pET-19b. The open reading frame of amylomaltase gene of 2,121 bp (encoding the polypeptide of 706 amino acid residues) was obtained with the N-terminal His-tag fragment of 69 bp attached before the start codon of the amylomaltase gene. The deduced amino acid sequence showed a low sequence identity (20–25%) to those thermostable amylomaltases from *Thermus* sp. The maximum enzyme activity was obtained when the recombinant cells were cultured at 37 °C for 2 h after induction with 0.4 mM isopropyl thio-*β*-D-galactoside (IPTG). The enzyme was 11-fold purified with a yield of 30% by a HiTrap affinity column. The purified amylomaltase showed a single band of 84 kDa on a 7.5% SDS-PAGE. When the enzyme acted on pea starch, it catalyzed an intramolecular transglucosylation (cyclization) reaction that produced LR-CDs or cycloamyloses (CA). The product profile was dependent on the incubation time and the enzyme concentration. Shorter incubation time gave larger LR-CDs as principal products. At 4 h incubation, the product was composed of a mixture of LR-CDs in the range of CD19–CD50, with CD27–28 as products with highest amount. It is noted that CD19 was the smallest product in all conditions tested. The enzyme also catalyzes intermolecular transglucosylation on various malto-oligosaccharides, with maltose as the smallest substrate.

Keywords Large-ring cyclodextrin · Amylomaltase · Transglucosylation · Cyclization · *Corynebacterium*

Abbreviation

LR-CD	Large-ring cyclodextrins
CA	Cycloamylose
CD	Cyclodextrin
4αGTase	4-α-Glucanotransferase
IPTG	Isopropylthio-*β*-D-galactoside
SDS-PAGE	Sodium dodecylsulfate-polyacrylamide gel electrophoresis
TLC	Thin-layer chromatography
HPAEC	High performance anion exchange chromatography

Introduction

Amylomaltase (EC 2.4.1.25) is an intracellular enzyme of the 4-α-glucanotransferase (4αGTase) family. This enzyme catalyzes the transfer of α-1,4-D-glucan units via an intermolecular and intramolecular transglucosylation reaction.

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The intermolecular glucan transfer reaction (disproportionation) catalyzes glucosyl transfer from a donor molecule to the non-reducing end of an acceptor, which may be a short-chain oligosaccharide or another glucan chain, and this results in longer linear products. In addition, the cyclic glucan products, cycloamylose or large-ring cyclodextrins, with a degree of polymerization of 16 upwards is obtained from an intramolecular glucan transfer reaction within a single linear glucan molecule, the so-called cyclization reaction [1]. LR-CDs are highly soluble in water, assumed to form a single helical V-amylose conformation and a toroidal shape, with an anhydrophilic channel-like cavity [2]. They can form inclusion complexes with inorganic [3] and organic molecules [1] and have several potential applications in pharmaceuticals, food science and biotechnology [4, 5]. LR-CDs have been suggested for application in the paper industry as an improved paper coating material [6]. For pharmaceutical application, LR-CDs could play important roles in stabilization and solubilization of large and insoluble or unstable drug molecules [5]. Recently, application of LR-CDs in biotechnology as an artificial chaperone for protein refolding is recognized, they are added as an ingredient in a commercial protein refolding kit [7].

In microorganisms, amylomaltase was first found in *Escherichia coli* as a maltose-inducible enzyme that is essential for the metabolism of maltose [8]. Amylomaltases have been later reported in *Clostridium butyricum* NCIMB 7423 [9], hyperthermophilic archaeon *Thermococcus litoralis* [10], *Thermus aquaticus* ATCC 33923 [11], *Aquifex aeolicus* [12] and *Thermus brockianus* [13]. In some organisms, such as *Corynebacterium glutamicum* [14, 15], although the putative gene has been found there are no supporting studies on the enzyme activity and characteristics. Interestingly, we found that the reported nucleotide sequence of amylomaltase from *C. glutamicum* has a low sequence similarity to those from *T. aquaticus* [11] and *A. aeolicus* [12], in which the enzymatic production of cycloamyloses from starch have already been reported. This raises the issue of whether the distant amylomaltase homologue from *C. glutamicum* would display similar or novel activities. Thus, the aim of the present study was to produce the recombinant amylomaltase from *C. glutamicum* and to characterize the purified enzyme with the emphasis on the profile of LR-CDs produced.

Materials and methods

Bacterial strains, plasmids, and chemicals

C. glutamicum ATCC 13032 was obtained from Thailand Institute of Scientific and Technological Research (TISTR). *E. coli* BL21 (DE3) and pET-19b expression vector were from Novagen (Germany). Restriction enzymes, DNA ligase and polymerase were the products of New England Biolabs Inc. (USA). HisTrap FF^{TM} affinity column was from GE Healthcare (UK). Pea starch was kindly provided by Emsland-Stärke GmbH (Emlichheim, Germany) while soluble starch (potato) was a product of Scharlau (Spain). All other chemicals used were of analytical grade.

Culturing of *C. glutamicum*

C. glutamicum ATCC 13032 was cultivated in the Nutrient Broth medium containing 0.3% (w/v) beef extract and 0.5% (w/v) peptone. The culture was grown at 37 °C for 14–18 h. Cells were then harvested by centrifugation at 5,000×*g* for 15 min.

Cloning and expression of amylomaltase gene

Genomic DNA was extracted from *C. glutamicum* cells using a standard method [16]. Amylomaltase gene of *C. glutamicum* (*CGAM*) was PCR amplified using the designed forward and reverse primers containing *Nde*I and *Xho*I sites, respectively. (f-CGAM : GGG AAT TCC ATA TGA CTG CTC GCA GAT TTT TGA ATG and r-CGAM : CCG CTC GAG CTA ATC TCG CTT GCT TGC CTT TGC C). These primers were designed from the known sequence deposited in Genbank (accession number NP_601 497). PCR conditions were : an initial denaturation at 95 °C for 2 min; followed by 35 cycles of amplification, each at 94 °C for 1 min, 58 °C for 30 s, 72 °C for 3 min; and a final extension step at 72 °C for 5 min. Amplified PCR products were analyzed by electrophoresis on a 0.7% agarose gel in 1× TAE buffer (40 mM Tris-HCl, 40 mM acetate and 1 mM sodium EDTA, pH 8.0) with a constant potential of 100 V, followed by ethidium bromide staining. A 1 kb standard DNA ladder (New England Biolabs Inc, USA) was coresolved for estimation of the product size. Then the amplified DNA fragment was digested with *Nde*I and *Xho*I and inserted into the pET-19b vector preceding His-taq sequence, creating pET-CGAM. Transformation into *E.coli* BL 21 (DE3) strain was then performed. Transformants with pET-CGAM were selected on LB plates containing 100 μg/ml ampicillin, and randomly selected positive clones were checked by sequencing.

For the expression of amylomaltase, recombinant *E.coli* cells were grown in LB medium containing 100 μg/ml ampicillin at 37 °C for 14–18 h. Amylomaltase expression was induced by adding 0.4 mM IPTG when the A_{600} of the culture reached 0.4. After 2 h of IPTG induction, cells were harvested and sonication was performed using a Bandelin Sonoplus (Bandelin, Germany). Bacterial debris was

removed by centrifugation at 12,000×*g*, 4 °C for 30 min. The supernatant which contains the crude amylomaltase was collected.

Purification of amylomaltase

Crude amylomaltase (containing His-tag fragment) was loaded onto a 1 ml × 2 Hitrap affinity (HisTrap FF^{TM}) column (GE Healthcare, UK), then washed with 20 mM phosphate buffer containing 0.5 M NaCl and 20 mM imidazole, pH 7.4. The purified enzyme was eluted by the same buffer but supplemented with 500 mM imidazole.

Assay of amylomaltase

Amylomaltase activity was measured by the iodine method, modified slightly from Park et al. [17]. One ml of reaction mixtures containing 250 µl of 0.2% (w/v) soluble starch (potato), 50 µl of 1% (w/v) maltose, 0–100 µl of enzyme, and 0.6 ml of 50 mM Tris-HCl pH 7.4 was incubated at 30 °C for 10 min and the reaction was stopped by boiling for 10 min. Then 100 µl aliquots were withdrawn and mixed with 1 ml iodine solution (0.02% I_2 in 0.2% KI, (w/v)) and the absorbance at 600 nm was measured. One unit is defined as the amount of enzyme that produces a one percent reduction in the intensity of the color (absorbance) of the starch-iodine complex per min under the described conditions.

Electrophoresis and protein determination

Sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE), with a 5% stacking and a 7.5% separating gels was carried out on a Bio-Rad Mini-Protein III gel apparatus (Bio-Rad Laboratories, Hercules, MA, USA) using the Laemmli buffer system (0.25 M Tris, 1.92 M Glycine, 1% (w/v) SDS, pH 8.3) [18]. The molecular weight markers used were phosphorylase b (97.4 kDa), bovine serum albumin (66.3 kDa), ovalbumin (45.0 kDa), lactate dehydrogenase (36.5 kDa), carbonic anhydrase (31.0 kDa), trypsin inhibitor (21.5 kDa) and lysozyme (14.4 kDa) (GE Healthcare, UK). The protein concentrations were measured by the Coomassie blue G method [19] using bovine serum albumin as the standard.

Disproportionation activity on linear malto-oligosaccharides

Purified recombinant amylomaltase (2.5 U/ml) was incubated with 0.2% (w/v) of linear malto-oligosaccharide substrates (G1, glucose to G7, maltoheptaose) in 50 mM phosphate buffer pH 6.0 at 30 °C for 2 h. The reaction mixture was boiled for 10 min to stop the reaction, then analyzed by Silica gel 60 thin-layer chromatography (TLC). The mobile phase was a solvent mixture of n-propanol:ethyl acetate:water (7:1:2, by volume). Detection was by spraying with sulphuric acid:ethanol (1:9, by volume) followed by heating at 110 °C for 15 min.

Cyclization activity on starch

Purified recombinant amylomaltase (0.15 or 0.5 U/ml) was incubated with 0.2% (w/v) pea starch in 50 mM phosphate buffer pH 6.0 at 30 °C for 30 min to 24 h. The reaction mixture was boiled for 10 min, then treated with glucoamylase (1 U/ml, from *Rhizopus* sp., (Sorachim, Switzerland)), centrifuged, and analyzed by high performance anion exchange chromatography with pulsed amperometric detector (HPAEC-PAD, Dionex-300, USA). A CarboPac PA-100 column (250 × 4 mm, Dionex) was used. Elution was by a linear gradient of sodium nitrate (0–2 min, increasing from 4 to 8%; 2–10 min, increasing from 8 to 18%; 10–20 min, increasing from 18 to 28%; 20–40 min, increasing from 28 to 35%; 40–55 min, increasing from 35 to 45%; 55–60 min, increasing from 45 to 63%) in 150 mM NaOH with a flow rate of 1 ml/min. The size of LR-CD products were compared with standard LR-CDs which are size approved by MALDI-TOF.

Results and discussions

Cloning and expression of amylomaltase

The restriction patterns of the undigested pET-CGAM from recombinant clones and the *Nde*I-*Xho*I digested vector revealed that the gene fragment inserted into the pET-19b expression plasmid had a size of around 2.2 kb. This corresponds to the 2,121 bp size of amylomaltase (4αGTase) reported for *C. glutamicum* ATCC 13032 [14, 15] plus the His-tag fragment from pET-19b. Sequencing of the inserted fragment revealed a nucleotide sequence of 2,190 bp comprised of the 69 bp His-tag fragment before the start codon of the cloned *CGAM* gene (2,121 bp). This ORF sequence of 2,121 bp matched with the sequence of the *C. glutamicum* 4αGTase deposited at Genbank (accession number NP_601497) [14, 15].

From the ORF of *CGAM,* the amino acid sequence of 706 residues was deduced. Based on the deduced amino acid sequence, *CGAM* exhibited low level of sequence identity with previously reported amylomaltases, especially the well-characterized enzymes from *Thermus* and *Aquifex* (23.9% identity with *T. aquaticus* [11], 24.1% identity with *T. thermophilus* [11] and 21.4% identity with *A. aeolicus* [12] (Fig. 1). The recombinant cells were able to express a maximum level of activity of CGAM when the cells were

Fig. 1 Alignment of amino acid sequences for amylomaltases. *TA Thermus aquaticus* (EED09753), *TT Thermus thermophilus* (YP_144527), *AA Aquifex aeolicus* (AAC06897.1) and *Corynebacterium glutamicum* (NP_601497). Catalytic residues are shown in the *boxes*. Amino acid conservation across the aligned sequences is shown as (*asterisk*) identical, (*colon*) conserved substitutions and (*dot*) semi-conserved substitutions

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TA  -----------------------------------MELPRAFGLLLHPTSLPG------- 18
TT  -----------------------------------MELPRAFGLLLHPTSLPG------- 18
AA  --------------------------------------MRLAGILLHVTSLPS------- 15
CG  MTARRFLNELADLYGVATSYTDYKGAHIEVSDDTLVKILRALGVNLDTSNLPNDDAIQRQ 60
                                           *  *: *. :.**.

TA  -----------PYGVGVLGLEARDFLRFLKGAGGR------------------------- 42
TT  -----------PYGVGVLGQEARDFLRFLKEAGGR------------------------- 42
AA  -----------PYGIGDLGKEAYRFLDFLKECGFS------------------------- 39
CG  IALFHDREFTRPLPPSVVAVEGDELVFPVHVHDGSPADVHIELEDGTQRDVSQVENWTAP 120
               *   . :. *.  ::  ::  .

TA  ---------------------FWQVLPLG-----------------PTG--YGDSP---- 58
TT  ---------------------YWQVLPLG-----------------PTG--YGDSP---- 58
AA  ---------------------LWQVLPLN-----------------PTSLEAGNSP---- 57
CG  REIDGIRWGEASFKIPGDLPLGWHKLHLKSNERSAECGLIITPARLSTADKYLDSPRSGV 180
                          *: * *                  .*.    :**

TA  ----YQALSAFAG---------------------------------------NPYLIDLR 75
TT  ----YQSFSAFAG---------------------------------------NPYLIDLR 75
AA  ----YSSNSLFAG---------------------------------------NYVLIDPE 74
CG  MAQIYSVRSTLSWGMGDFNDLGNLASVVAQDGADFLLINPMHAAEPLPPTEDSPYLPTTR 240
        *.  * ::                                        .  *   .

TA  PLAEKGYLVLKDPG-FPQGRVDY-----GWLYAWKWPALKAAYQGFLERAPRKEREDFLA 129
TT  PLAERGYVRLEDPG-FPQGRVDY-----GLLYAWKWPALKEAFRGFKEKASPEEREAFAA 129
AA  ELLEEDLIKERDLKRFPLGEALY-----EVVYEYKKELLEKAFKNFRR------FELLED 123
CG  RFINPIYIRVEDIPEFNQLEIDLRDDIAEMAAEFRERNLTSDIIERNDVYAAKLQVLRAI 300
     : :   :  .*   *   .             ::   *

TA  FREKEASWLKDYALFMALKAQHGGLPWNRWPLPLRRREEKALKEAEAALAEEVAFHAWTQ 189
TT  FREREAWWLEDYALFMALKGAHGGLPWNRWPLPLRKREEKALREAKSALAEEVAFHAFTQ 189
AA  FLKEHSYWLRDYALYMAIKEEEG-KEWYEWDEELKRREKEALKRVLNKLKGRFYFHVFVQ 182
CG  FEMPRSSEREANFVSFVQREGQGLIDFATWCADRETAQSESVHGTEPDRDELTMFYMWLQ 360
    *   .:   .   : :. :  .*   :  *    .  :.:::: .         *: : *

TA  WLFFEAWKALKEEAEALGIQIIGDMPIFVAEDSAEVWAHPEWFHLDEEGRPTVVAGVPPD 249
TT  WLFFRQWGALKAEAEALGIRIIGDMPIFVAEDSAEVWAHPEWFHLDEEGRPTVVAGVPPD 249
AA  FVFFKQWEKLRRYARERGISIVGDLPMYPSYSSADVWTNPELFKLDGDLKPLFVAGVPPD 242
CG  WLCDEQLAAAQKRAVDAGMSIG----IMADLAVGVHPGGADAQNLSHVLAPDASVGAPPD 416
    ::  .     :  *   *: *     :      .     .:  :*.    *   .*.***

TA  YFSETGQRWGNPLYRWDVLEREGFSFWIARLAKALELFHLVRVDHFRGFEAYWEIPASCP 309
TT  YFSETGQRWGNPLYRWDVLEREGFSFWIRRLEKALELFHLVRIDHFRGFEAYWEIPASCP 309
AA  FFSKTGQLWGNPVYNWEEHEKEGFRWWIRRVHHNLKLFDFLRLDHFRGFEAYWEVPYGEE 302
CG  GYNQQGQDWSQPPWHPVRLAEEGYIPWRNLLRTVLRHSGGIRVDHVLGLFRLFVMPR-MQ 475
     :.: ** *.:* :.     .**:  *   :   *.    :*:**. *:   : :*

TA  TAVEGRWVKAPGEKLF--DRIQEVFGQVPILAEDLGVITPEVEALRDRYGLPGMKVLQFA 367
TT  TAVEGRWVKAPGEKLF--QKIQEVFGEVPVLAEDLGVITPEVEALRDRFGLPGMKVLQFA 367
AA  TAVNGRWVKAPGKTLF--KKLLSYFPKNPFIAEDLGFITDEVRYLRETFKIPGSRVIEFA 360
CG  SPATGTYIRFDHNALVGILALEAELAGAVVIGEDLGTFEPWVQDALAQRGIMGTSILWFE 535
    :.. * :::   : *.    :   :    .:.**** :   *.       : *  :: *

TA  FDDGMENPFLPHNYPAHGRVVVYTGTHDNDTTLGWYRTASPHERAFLERYLADWGISFRQ 427
TT  FDDGMENPFLPHNYPAHGRVVVYTGTHDNDTTLGWYRTATPHEKAFMARYLADWGITFRE 427
AA  FYD-KESEHLPHNVEENN--VYYTSTHDLPPIRGWFENLGEESRKRLFEYLG----REIK 413
CG  HSPSQPGPRRQEEYRPLA--LTTVTTHDLPPTAGYLEGEHIALRERLGVLNTDPAAELAE 593
    .     .    .:       :  . ***  .  *: .      :  :            :

TA  EEEVPWALMGLCMKS-------------------------------AARLAIYPVQDVLA 456
TT  EEEVPWALMHLGMKS-------------------------------VARLAVYPVQDVLA 456
AA  EEKVNEELIRLVLIS-------------------------------RAKFAIIQMQDLLN 442
CG  DLQWQAEILDVAASANALPAREYVGLERDQRGELAELLEGLHTFVAKTPSALTCVCLVDM 653
    : :    :: :   :                                :  *:  :  :

TA  LGSEARMNYPGRP---SGNWAWRLLPG------QLTQEHAARLLAMAEATGRA 500
TT  LGSEARMNYPGRP---SGNWAWRLLPG------ELSPEHGARLRAMAEATERL 500
AA  LGNEARMNYPGRP---FGNWRWRIKEDY-----TQKKEFIKKLLGIYGREV-- 485
CG  VGEKRAQNQPGTTRDMYPNWCIPLCDSEGNSVLIESLRENELYHRVAKASKRD 706
    :*.:   * ** .     **   :  .        . .       :
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cultured at 37 °C for 2 h after induction with 0.4 mM IPTG and yielded a specific activity of 2.2 U/mg protein for the crude enzyme preparation.

Purification of recombinant amylomaltase

The recombinant amylomaltase was efficiently purified by a HisTrap FF affinity column. The crude enzyme was purified 11-fold to a specific activity of 24 U/mg protein with a 30% yield (Table 1). SDS–PAGE analysis (Fig. 2) showed that the recombinant enzyme was highly expressed in *E. coli* and was efficiently purified to only a single protein band of 84 kDa after affinity column compared with the at least 15 bands present in the crude extract fraction. The size of this enzyme is significantly larger than the 4αGTases of *T. aquaticus* YT-1 (58 kDa) [10], and *T. brockianus* (57 kDa) [12]. Our CGAM worked best at 30 °C while the *Thermus* enzymes showed optimum temperature around 70 °C [11].

Disproportionation activity

From TLC analysis (Fig. 3), the recombinant amylomaltase was able to catalyze transglucosylation reaction from the malto-oligosaccharides G2 to G7 but that glucose (G1) could not act as a substrate. Various malto-oligosaccharides of different lengths were produced from each reaction and when relatively long malto-oligosaccharides (G5, G6 and G7) were used as a substrate, high molecular mass oligosaccharides were observed. The result suggests that the recombinant amylomaltase can transfer glucose units from one 1,4-α-glucan to another and that the enzyme requires at least one maltose unit for the disproportionation reaction. This property is the same as that reported for the enzymes from other bacteria e.g. *T. litoralis* [10], *T. aquaticus* (although at a lower rate) [11], *A. aeolicus* [12] and *T. brockianus* [13]. On the contrary, the characterized plant D-enzymes from potato tuber [20] and pea chloroplast [21] showed different enzymatic activities on malto-oligosaccharides in which the maltotriose (G3) was the smallest substrate for disproportionation reaction.

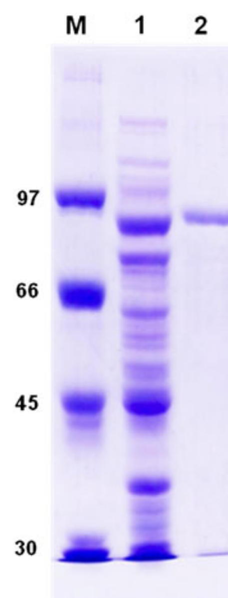


Fig. 2 SDS-PAGE analysis of the recombinant amylomaltase from *C. glutamicum*. *Lane M* molecular weight marker, *lane 1* crude extract (15 μg), *lane 2* purified enzyme after HiTrap FF column chromatography (3 μg)

Cyclization activity and the synthesis of large-ring cyclodextrins

The recombinant amylomaltase not only catalyzed intermolecular transglucosylation, but also catalyzed intramolecular transglucosylation. The ability of the recombinant amylomaltase to produce LR-CDs was examined by using pea starch as a substrate. To analyze cycloamyloses, the reaction mixture was treated with glucoamylase since cycloamylose is resistant to this enzyme. When cyclization products of the recombinant amylomaltase were analyzed by HPAEC, a unique LR-CDs profile was obtained. The cyclization products obtained were dependent on the incubation time and the amount of enzyme used. At 0.15 U/ml enzyme, the main products obtained changed from larger LR-CDs (principally CD31) at shorter incubation time (30 min) to smaller sized ones (principally CD27-28 and CD25) at 4 and 24 h, respectively (Figs. 4, 5a). The amount of products formed was higher at longer incubation time and when using a higher concentration of the enzyme (0.5 U/ml). However, the product pattern of the two enzyme concentrations was not much different. They were consisted of mixtures of LR-CDs that ranged from about CD19 to CD50, with CD27–28 as the products with highest

Table 1 Purification of the recombinant amylomaltase from *C. glutamicum*

Purification step	Total protein (mg)	Total activity[a] (U)	Specific activity (U/mg)	Purification fold	Yield (%)
Crude extract	740	1633	2.21	1	100
HisTrap FF	20.7	493	23.8	10.8	30.2

[a] Assayed by starch-iodine method

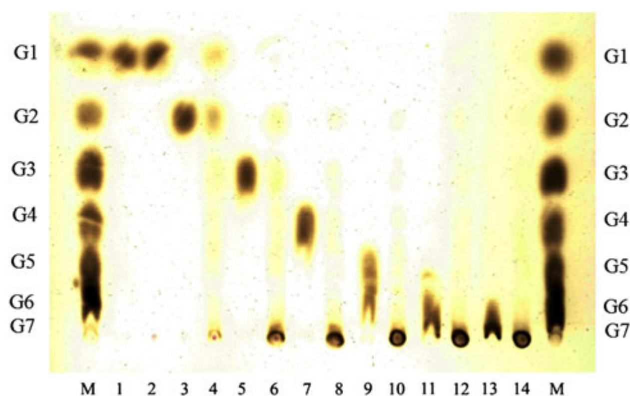
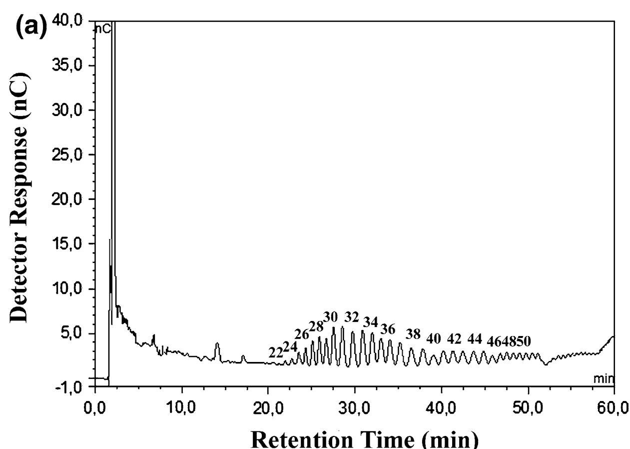


Fig. 3 TLC chromatogram of the reaction products of the recombinant amylomaltase incubated with linear malto-oligosaccharides. The enzyme solution (2.5 U/ml) was incubated with 0.2% (w/v) of each substrate at 30 °C for 2 h, and then 10 µl was loaded onto the TLC plate. *Lane M* G1–G7 standard; *lanes 1&2* G1 without/with enzyme, *lanes 3&4* G2 without/with enzyme, *lanes 5&6* G3 without/with enzyme, *lanes 7&8* G4 without/with enzyme, *lanes 9&10* G5 without/with enzyme, *lanes 11&12* G6 without/with enzyme; *lanes 13&14* G7 without/with enzyme



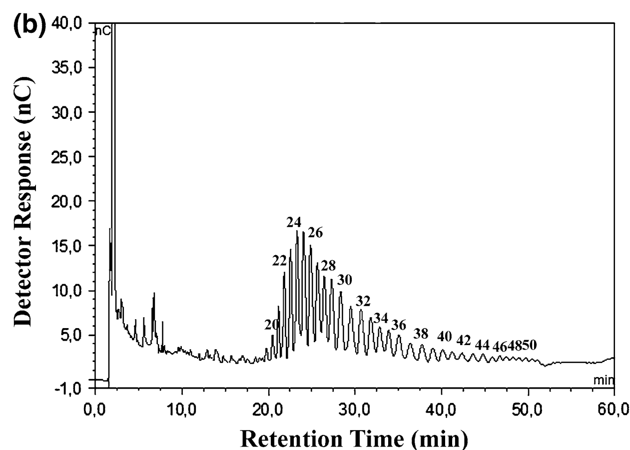
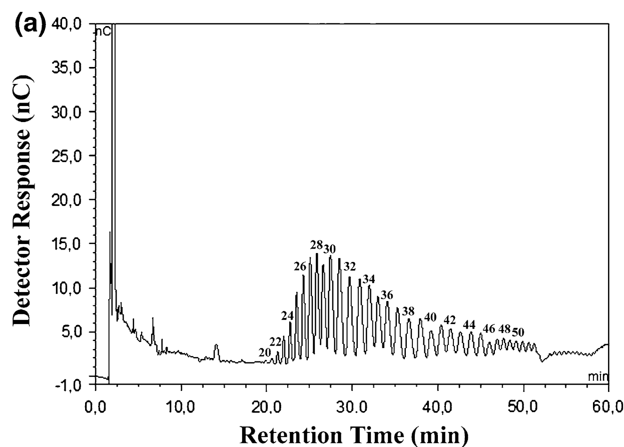


Fig. 4 HPAEC analysis of the cycloamylose (CA) synthesized by the recombinant amylomaltase at different incubation times. Pea starch at 0.2% (w/v) was incubated with 0.15 U/ml enzyme at 30 °C for **a** 30 min and **b** 24 h. Peak numbers indicate the degree of polymerization of the identified LR-CDs



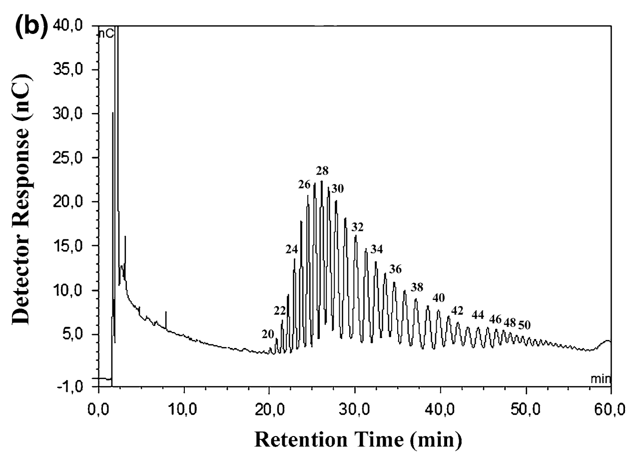


Fig. 5 HPAEC analysis of CA synthesized by the recombinant amylomaltase at different amount of enzyme. Pea starch at 0.2% (w/v) was incubated with **a** 0.15 U/ml and **b** 0.5 U/ml enzyme at 30 °C for 4 h. Peak numbers indicate the degree of polymerization of the identified LR-CDs

amount obtained at 4 h incubation time (Fig. 5). It was found that CD19 was detected as smallest LR-CD at all conditions tested. When compared with other amylomaltases, our cloned CGAM is different from the well characterized *T.aquaticus* ATCC 33923 amylomaltase and the potato D-enzyme in the smallest LR-CD products obtained. CGAM gave CD19 while CD22 [11] and CD17 [22] were reported as smallest products of *T.aquaticus* and potato enzymes, respectively. CD19 as smallest LR-CD product had been reported to be formed by the chimeric enzyme TAαGT-DE containing amylomaltase from *T.aquaticus* YT-1 and the starch binding domains (domain D and E) of *Bacillus stearothermophilus* ET1 CGTase [17]. The LR-CDs produced from 2% (w/v) amylose substrate after a 12 h incubation with the chimeric enzyme were in the range of CD19–CD35, with CD25 as the main product. From these differences, our cloned CGAM thus showed a unique property in the synthesis of LR-CDs.

Conclusions

A novel amylomaltase from *C.glutamicum* ATCC 13032 was cloned and expressed in *E.coli.* The recombinant enzyme was purified to homogeneity by a one step affinity column chromatography. This enzyme was unique in its LR-CD production profile. CD19 was the smallest LR-CD product. A series of LR-CDs from CD19 to CD50 with CD27–28 as the products with highest amount was obtained from pea starch at 4 h incubation with 0.5 U/ml enzyme. This enzyme might be of advantage for LR-CDs production at mild temperature.

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