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Physiochemical properties and kinetics of glucoamylase produced from deoxy-D-glucose resistant mutant of *Aspergillus niger* for soluble starch hydrolysis

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Abstract

Glucoamylases (GAs) from a wild and a deoxy-D-glucose-resistant mutant of a locally isolated *Aspergillus niger* were purified to apparent homogeneity. The subunit molecular mass estimated by SDS–PAGE was 93 kDa for both strains, while the molecular masses determined by MALDI-TOF for wild and mutant GAs were 72.876 and 72.063 kDa, respectively. The monomeric nature of the enzymes was confirmed through activity staining. Significant improvement was observed in the kinetic properties of the mutant GA relative to the wild type enzyme. Kinetic constants of starch hydrolysis for *A. niger* parent and mutant GAs calculated on the basis of molecular masses determined through MALDI-TOF were as follows: $k_{cat} = 343$ and 727 s^{-1} , $K_m = 0.25$ and 0.16 mg mL^{-1} , k_{cat}/K_m (specificity constant) = 1374 and $4510 \text{ mg mL}^{-1} \text{ s}^{-1}$, respectively. Thermodynamic parameters for soluble starch hydrolysis also suggested that mutant GA was more efficient compared to the parent enzyme.

Keywords

Glucoamylase; *Aspergillus niger*; Activity staining; Starch hydrolysis; Purification

1. Introduction

Glucoamylases (GAs) are exo-acting enzymes that catalyse the hydrolysis of α -1,4 and α -1,6 glucosidic linkages from the non-reducing ends of starch and related oligo-, and polysaccharides into short chain saccharides (Dubey, Suresh, Kavitha, Karanth, & Kumar, 2000). Mould GAs are of great commercial importance. They are used in the production of glucose from starch and dextrans, the making of wines, beers and other alcoholic beverages, and biofuel production (Boel et al., 1984). Consequently, they are extensively used in textile, food, paper and pharmaceutical industries (Marlida, Hassan, Radu, & Baker, 2000).

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GA is a multidomain glycoprotein containing about 640 amino acids. The N-terminal part of the enzyme is the larger catalytic domain (CD) and comprises amino acids 1-470. The C-terminal part, comprising amino acids 509-640 is the starch-binding domain (SBD) which is linked to the CD by a linker region which comprises amino acids 471-508. The SBD has a cleft in which the substrate is strongly attached. The catalytic domain catalyses the hydrolysis of α -1,4- and α -1,6-glycosidic linkages of the substrate, producing free glucose residues. The SBD plays no role in the catalytic activity of the enzyme. Like SBD, the linker region also has no role in the activity of the enzyme but gives structural integrity to the enzyme (Chou, Pai, Liu, Hsiung, & Chang, 2006).

A problem with traditionally used enzymes is that, at high concentrations, some of the glucose produced condenses to form various by-products such as isomaltose, disaccharides and trisaccharides (Reilly & Ames, 1999). These are undesirable because they are not as sweet as glucose and cannot easily be further processed to fructose (Nigam & Singh, 1995). Moreover, the process has a high energy cost in lowering the temperature of the starch slurry from the liquefaction stage to that used for saccharification with thermally unstable GAs (Sun et al., 2010). Interest in thermostable enzymes that can cope with a robust industrial environment has increased tremendously recently, and resistance to thermal inactivation has become a desirable property in many industrial applications (Zheng et al., 2010). Thus, an increased thermal stability of GAs would allow a higher process temperature, which could well minimise the production of by-products, lower the production time and reduce the risk of contamination (Thorsen, Johnsen, Josefsen, & Jensen, 2006). Hence, the ultimate objective of this work is to produce GAs having both higher activity and thermostability.

It is through recombinant DNA technology that we can manufacture enzymes that are better adapted to the processing conditions being applied in modern food production. However, there is still a place for the older methods, through which we can prepare such enzymes: intensive screening of microbes from samples taken from diverse environments, metal modification and chemical modification. By the application of such methods either singly or in combination it should be possible to change properties like temperature, activity, stability, substrate inhibition or activation mechanisms, substrate specificity or other kinetic characteristics of the enzymes (Adrio & Demain, 2006). Changing the pH optimum significantly, although highly desirable, has so far proved to be an elusive goal (Fersht, 1985).

Nowadays, the mutation of fungal spores is an interesting research topic. Variants of *Aspergillus* spp. and *Penicillium* spp. can be obtained after UV irradiation. Mutagenesis or combinatorial biosynthesis offers an easy approach to generate new enzymatic activities, resulting in modified products (Awad, Florence, Yannick, & Lebrihi, 2005).

The current work presents the purification and characterisation of GAs from *Aspergillus niger* and a deoxy-D-glucose (DG) resistant mutant, with respect to their ability to catalyse the conversion to glucose of a soluble starch. Thermodynamic parameters for soluble starch hydrolysis and irreversible thermostability of these enzymes are also compared. The study provides a basis by which to determine the suitability of the mutant GA for industrial application.

2. Materials and methods

2.1. Microbial strain development

A local strain of *A. niger* NIBGE-1 was obtained from Industrial Biotechnology Division, NIBGE, Faisalabad and a mutant (M-7) was selected after intensive screening of γ -ray-mutated cells of the fungus on deoxy-D-glucose containing growth media (Data not shown).

2.2. Production and isolation of GA

The parent and mutant *A. niger* were grown in submerged conditions; crude enzymes were extracted from growth media by filtration through Whatman No. 1 filter paper and centrifuged at 13,000 rpm (25,900g) for 15 min at 4 °C to remove suspended particles.

2.3. GA assay

The activity of GA was determined as described previously (Riaz, Perveen, Javed, Nadeem, & Rashid, 2007). Briefly, an appropriate aliquot of the enzyme was reacted with 1% (w/v) soluble starch solution in 50 mM Na-acetate buffer (pH 5.0) at 40 °C for 40 min unless otherwise stated. The reaction was quenched by placing the tubes in boiling water for 5 min, and then immediately cooled in ice. The released glucose was measured using a glucose measuring kit (Fluitest® GLU, Biocon, Bangalore, India). A unit of GA activity is defined as the amount of enzyme that releases 1 μmol of glucose min^{-1} from soluble starch at defined conditions of pH and temperature.

2.4. Protein assay

Total proteins were estimated by Bradford method (Bradford, 1976) using bovine serum albumin as a standard.

2.5. Purification of GAs

The harvested crude enzymes were subjected to a three-step purification procedure comprising ammonium sulphate precipitation, HiLoad anion exchange and hydrophobic interaction chromatographies by FPLC as described earlier (Riaz et al., 2007).

2.5.1. Ammonium sulphate precipitation—Solid ammonium sulphate was added bit by bit to the crude enzyme supernatant to 30% (parent) and 60% (mutant) saturation at 0 °C, and left overnight at 4 °C. The enzyme solutions were centrifuged at 25,900g for 20 min and the pellets were discarded. Further ammonium sulphate was added to the supernatants to achieve final concentrations of 80% and 90% saturation at 0 °C, respectively. The enzyme preparations were again kept overnight and centrifuged under the same conditions as above. This time the supernatants were discarded and pellets containing GA were dissolved in distilled water and dialysed over night against several changes of distilled water at 4 °C to remove salt.

2.5.2. HiLoad anion-exchange chromatography—The dialysed sample was loaded onto a HiLoad anion exchange 26/10 column (Q-Sepharose HP), using a 50-mL superloop at a flow rate of 2 mL min^{-1} . A linear gradient of NaCl (0–1 M) in 20 mM Tris-HCl, pH 7.5, was used as elution buffer. The fractions containing GA were pooled and dialysed against distilled water at 4 °C.

2.5.3. Hydrophobic interaction chromatography—Solid ammonium sulphate was added to the dialysed sample from the previous step to a final concentration of 2 M. The enzyme solution was filtered through a 0.45- μm Amicon filter and was loaded onto a hydrophobic interaction (Phenyl-Superose) FPLC 10/10 column at a flow rate of 0.5 mL min^{-1} . The elution was carried out with a linear gradient of ammonium sulphate (2–0 M) in 50 mM sodium phosphate buffer, pH 7. Active fractions were collected and dialysed against distilled water at 4 °C.

Note: Total enzyme units and total protein were estimated after each step of the purification procedure.

2.6. Molecular mass determination

Purity of the enzyme and its sub-unit molecular mass was determined by 10% SDS-PAGE as described (Laemmli, 1970). Protein markers from Fermentas having molecular masses in the range 10–200 kDa were run as standard. The gel containing enzyme and molecular markers was stained with Coomassie Brilliant Blue R-250 solution. For the true molecular mass, apparently pure GAs (0.5 μ L of 0.5 mg mL⁻¹) from parent and mutant *A. niger* were separately spotted onto a MALDI plate. The same quantity of sinapinic acid was mixed with the protein drops. The mixtures were allowed to dry for 2-3 min. The sample plate was loaded into the Voyager DE STR MALDI-TOF MS (Applied Biosystems, Foster City, CA). The molecular masses of the enzymes were determined using VOYAGER software (Franco et al., 2000).

2.7. Activity staining

The purified samples of GA were separated on a 10% native polyacrylamide gel. The gel was then immersed in 2% soluble starch solution prepared in 50 mM Na acetate buffer pH 5 and incubated at 50 °C for 2 h. After incubation the gel was washed with 50 mM Na-acetate buffer, pH 5, and immersed in iodine solution for 30 min at room temperature. White coloured activity bands of GAs appeared on the gel.

2.8. Optimum pH

The various biological buffers including glutamic acid/HCl (pH 2–2.9), Na acetate/acetic acid (pH 3.2–5.3), MES/KOH (pH 5.6–6.5), MOPS/KOH (pH 6.8–7.4), HEPES/KOH (pH 7.7–8.3), glycine/NaOH (pH 8.6–9.8) and CAPSO/NaOH (pH 10.1–11.0) were used to maintain the pH of the reaction mixtures. The change in the enzymatic activity of the enzymes while incubating in these reaction mixtures was indicative of their pH optima. The pKa of ionisable groups of essential active site residues involved in the catalysis were determined by plotting a graph of pH versus $V_{\max}$ values. Afterwards, 0 slope (on the top of bell shaped curve), +1 and –1 slope lines were drawn. The intersection points of +1 and –1 slope lines on the 0 slope line represented the pKa₁ and pKa₂, respectively (Dixon & Webb, 1979).

2.9. Optimum temperature, activation energy and temperature quotient (Q₁₀)

The optimum temperature of GA was determined by incubating an aliquot of the enzyme with 1% soluble starch at various temperatures ranging from 25–65 °C in 50 mM Na-acetate buffer for 40 min at pH 4.4. The activation energy (E_a) was calculated by using an Arrhenius plot (Siddiqui, Azhar, Rashid, & Rajoka, 1996). The effect of temperature on the rate of reaction was expressed in terms of temperature quotient (Q_{10}), which is the factor by which the rate increases due to a raise in the temperature by 10 °C. Q_{10} was calculated by using the equation given below as described (Dixon & Webb, 1979).

$$Q_{10} = \text{antilog}_e (E \times 10/RT^2)$$

$$E = E_a = \text{activation energy}$$

2.10. Kinetics of starch hydrolysis

The kinetic constants ($V_{\max}$, K_m , k_{cat} and k_{cat}/K_m) for soluble starch hydrolysis were determined by incubating a fixed amount of GA with varied concentrations of soluble starch as a substrate ranging from 0.005% to 0.075% (w/v) at 60 °C, pH 4.4, as described (Saleem et al., 2005).

2.11. Thermodynamics of starch hydrolysis

The thermodynamic parameters for substrate hydrolysis were calculated by rearranging the Eyring's absolute rate equation derived from the transition state theory (Eyring & Stearn, 1939):

$$k_{\text{cat}} = (k_b T / h) e^{(-\Delta H^* / RT)} \cdot e^{(\Delta S^* / R)} \quad 2$$

where k_b Boltzmann's constant (R/N) = $1.38 \times 10^{-23} \text{ J K}^{-1}$, T absolute temperature (K), h Plank's constant = $6.626 \times 10^{-34} \text{ J s}$, N Avogadro's number = $6.02 \times 10^{23} \text{ mol}^{-1}$, R gas constant = $8.314 \text{ J K}^{-1} \text{ mol}^{-1}$, ΔH^* enthalp of activation, ΔS^* entropy of activation.

$$\Delta H^* = E_a - RT \quad 3$$

$$\Delta G^* \text{ (Free energy of activation)} = -RT \ln (k_{\text{cat}} h / k_b \cdot T) \quad 4$$

$$\Delta S^* = (\Delta H^* - \Delta G^*) / T \quad 5$$

3. Results and discussion

3.1. Purification of GA

The crude GAs from *A. niger* parent and DG-resistant mutant having specific activities of 8.5 and 16.2 IU mg^{-1} , respectively were purified to apparent homogeneity in a three-step purification procedure. First the enzymes were subjected to ammonium sulphate precipitation followed by gel filtration FPLC on a HiLoad Q-Sepharose column. Finally, the parent and mutant enzymes were purified on a Phenyl-Superose column. Purifications of 25.4 and 30.6-fold were obtained with final yields of 20% and 31%, respectively (Table 1). The GAs were purified to apparent homogeneity and the purity was confirmed on 10% SDS-PAGE which displayed single bands. These results are in keeping with published results, in which the GA from *Rhizopus oryzae* mutant 4U2 was purified nearly 3-fold with a yield of 15%, using five step purification procedure (Suntornsuk & Hang, 1997), and a GA from *Arachnionotus citrinus* was purified 63-fold with a recovery of about 33%, using a four-step purification procedure (Niaz et al., 2004). The GA from culture filtrate of *Aspergillus niveus* was 2.53-fold purified with a recovery of 52% by two chromatographic steps using DEAE-Fractogel column and Concanavalin A-Sepharose affinity column (Silva et al., 2009).

3.2. Molecular mass

The subunit molecular mass for GAs from both of the strains determined by SDS-PAGE was found to be the same, i.e., 93 kDa (Fig. 1). The purity of the enzymes was confirmed by activity staining of the native gel and SDS-PAGE showing single bands (Fig. 1). SDS-PAGE is also indicative of the monomeric nature of the enzymes. The molecular masses of GAs have been evaluated by a number of workers from a variety of microbial sources. The molecular mass of GA from *A. niger* NRRL-3135 was estimated to be 90 kDa (Vandersall, Cameron, Nairn, Yelenosky, & Wodzinski, 1995). The native and sub-unit molecular masses were almost the same for GA produced from *A. citrinus* (Niaz et al., 2004). Similarly, the subunit and native molecular masses of GA from *Humicola* spp. were also the same (Riaz et al., 2007). Silva et al. (2009) had also reported about the monomeric nature of GA from *A. niveus* and its subunit and native molecular mass 77 and 76 kDa, respectively, which was determined by SDS-PAGE and gel filtration chromatography.

The molecular masses of parent and mutant GAs determined by MALDI-TOF were 72.876 and 72.062 kDa (Fig. 2), respectively. Variation in molecular mass (814 Da) suggested that the mutant enzyme was structurally different compared to the parent enzyme. The full-length G1 isoform of glucoamylase from *A. niger* has been found to comprise 640 amino acid residues and giving a molecular mass of 68.309 kDa on the basis of the sequence. This difference in molecular masses from that calculated by sequence analysis and actually determined by MALDI-TOF was most likely due to the extensive *O*-linked and *N*-linked glycosylation in the linker region of the enzymes (Voisin et al., 2005). The difference in molecular masses of parent and mutant calculated by sequence analysis suggested some 25 more glycosylated mannose units in the parent as compared to the mutant GA. As the sequence of the mutant is not yet known, it is not possible to say whether the glycosylation has been changed between native and mutant GA. Moreover, the differences in the molecular masses when analysed by SDS-PAGE and by MALDI-TOF are most probably due to the effect that the glycosylation has upon the apparent hydrodynamic radius of the enzymes (Stoffer et al., 1993). MALDI-TOF is a more accurate and sensitive method for molecular mass determination of biomolecules, so the kinetics of starch hydrolysis of GAs was calculated on the basis of molecular mass determined through this technique.

3.3. pH optimum

GAs from *A. niger* parent and mutant showed same pH optima and were highly stable in the pH range of 2.3–6.2 and 2.9–5.9, respectively. Similar types of results for optimal activity of GAs in the pH range 3.0–6.0 were reported by various workers from different microbial sources (Marlida et al., 2000; Michelin et al., 2010; Niaz et al., 2004). The GA of *A. niger* worked optimally at pH 5.0–5.5, while the enzyme remained stable for at least 2 h in the pH range of 4.0–9.5 (Silva et al., 2009). The pK_a refers to ionisation constant, which describes the dependence of an enzyme's activity or a chemical shift upon pH of a reaction. The pK_a values for the ionisable active site residues for ES^* -complex were determined after plotting the log maximum velocity versus pH (Fig. 3). Results revealed that pK_{a1} of proton-donating ionisable group were the same (3.4) while the pK_{a2} of proton-receiving group increased to 6.50 for the mutant from 6.2 for the parent. It is known that the GA active site contains three Glu and one Asp residues and it is tempting to conclude that our pK_a values are those of the acid, Glu 203, and the base, Glu 424 which have been assigned to those roles (Lee & Paetzal, 2011). No change in pK_{a1} revealed that γ -ray mutation of *A. niger* had no effect on the former Glu residue but the slight shift from 6.2 to 6.5 in pK_{a2} might be due to the altered environment around Glu 424. The shifts in pK_a from the expected 2.1 and 4.1 to 3.4 and 6.2 are expected from the constrained environment of the enzyme active site.

3.4. Temperature optimum, temperature quotient and activation energy

Thermophilicity is the capability of enzymes to work at elevated temperatures in the presence of substrates, while thermostability is the ability of an enzyme to resist thermal unfolding in the absence of substrate (Georis et al., 2000). The temperature dependence of the enzymes was determined between 25 and 70 °C. Like molecular mass and pH optima, the GAs from both fungal strains showed the same temperature optima and worked optimally at 60 °C. The temperature quotient for GAs from both of the strains was about same with a difference of 0.01. The activation energies, E_a , for the two enzymes were 44.27 kJ mol⁻¹ and 48.89 kJ mol⁻¹, determined from the Arrhenius plot (Fig. 4A). The plot demonstrated that the enzyme from both organisms had a single conformation up to the transition temperature (60 °C), whereafter it showed a decline. The higher energy required by the mutant enzyme to make the transition-state complex indicated that the ES^* -complex formation by parent enzyme was more efficient. The temperature optimum of the purified intracellular GA from *Lactobacillus amylovorus* ATCC 33621 was 45 °C (James, Borger, & Lee, 1997). Similarly, it was reported that *A. niger* NCIM-1248 worked optimally at 60 °C,

pH 4.4 (Selvakumar, Ashakumary, & Pandey, 1998). The temperature optimum of *A. niveus* GA was 65 °C and it retained 100% activity after 240 min at 60 °C (Silva et al., 2009).

3.5. Kinetics of substrate hydrolysis

The Michaelis-Menten constants were determined from Lineweaver-Burk plots (Fig. 4B). The $V_{\max}$ (U mg⁻¹ protein), k_{cat} (s⁻¹) and K_m (mg mL⁻¹) for the *A. niger* parent GA were 283, 343 and 0.25, while the values for mutant enzyme were 606, 727 and 0.16, respectively. The specificity constant (k_{cat}/K_m) determined for *A. niger* parent was 1374 whereas for its DG-resistant mutant it was 4510 mg mL⁻¹ s⁻¹. Thus, the kinetic properties of mutant were significantly improved, compared to those of the parent: the $V_{\max}$ and k_{cat} of the mutant GA were increased more than 2-fold. The decrease in the Michaelis constant and increase in specificity constant for the mutant GA confirmed the increase in efficiency of mutant GA towards soluble starch hydrolysis. K_m and $V_{\max}$ values of 0.6 mg mL⁻¹ and 8.33 U mg⁻¹ were reported for soluble starch hydrolysis from *Sclerotium rolfsii* (Kelkar & Desphande, 1993). Similarly, K_m values for soluble starch of 3.5 and 12.2 mg mL⁻¹ were reported for the GAs from *A. niger* NCIM-1248 and *R. oryzae* mutant 442, respectively (Selvakumar et al., 1998; Suntornsuk & Hang, 1997). Kinetic properties of the *A. niger* parent and mutant GA were also much better than the native and chemically modified GA from *Fusarium solani* (Bhatti et al., 2007). Silva et al. (2009) calculated Michaelis-Menten kinetic constants for soluble starch hydrolysis by GA of *A. niveus*, and its k_m and $V_{\max}$ values were 0.32 mg mL⁻¹ and 237 U mg⁻¹, respectively, while K_{cat} was equal to 14.2 s⁻¹. The kinetic properties of GAs from *A. niger* parent, as well as that of mutant, suggest that the enzyme may be utilised in the industry efficiently.

3.6. Thermodynamics of substrate hydrolysis

The enthalpy (ΔH^*) of parent GA for the formation of the transition state or activated complex between enzyme-substrate was lower than that of the mutant (Table 2). However, the lower Gibbs free energy (ΔG^*) of mutant GA (Table 2) suggested that the conversion of its transition complex into products was more spontaneous than that of the parent. It was found that the mutant enzyme required more E_a as compared to the parent for the formation of activated complex with the substrate, however once ES^* -complex existed, an equilibrium stage was developed as a result; product formation was more favourable, i.e. the ΔG^* was lower. The entropy of the mutant GA was higher than that of the parent, which might be explained as the transition complex having a less well ordered arrangement and hence, higher rate of product formation.

4. Conclusions

γ -Ray-mediated mutagenesis of *A. niger* did not result in major change in the physiochemical properties of the glucoamylase (GA). However, random mutagenesis made the DG-resistant mutant GA highly efficient in substrate hydrolysis, as compared to the parent. Moreover, the mutant enzyme was slightly more stable when exposed to temperatures greater than 60 °C. Kinetic and thermodynamic properties of GAs from parent and mutant suggested that they may be used commercially for the production of glucose in starch processing as well as in the food industry. Mutant GA however, seems to have more capability to withstand higher temperature with the efficient hydrolysis of starch. Our future plans are to investigate the type and extent of glycosylation in the *A. niger* parent and mutant glucoamylase and its role in the stability of the enzyme. Moreover, engineering of the mutant GA through the chemical modification of surface carboxyl groups and the effect of metals on the stability-function relationship of the GA will also be determined.

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Fig. 1. 10% Polyacrylamide gel electrophoresis (A) Native-PAGE for activity staining of glucoamylase from *Aspergillus niger* with Lane 1, parent and Lane 2, DG-resistant mutant. (B) SDS-PAGE for molecular mass determination of the glucoamylase from *Aspergillus niger* with Lane 3, parent; Lane 4, DG-resistant mutant and Lane 5, MW markers.

Fig. 3.

Dixon plot of glucoamylase from *Aspergillus niger* parent (open circles) and DG-resistant mutant (closed circles) at 40 °C for the determination of pK_a values of activesite residues that control $V_{\max}$. Slopes 0 (top of bell-shaped curve), +1 (left of bell-shaped curve) and -1 (right of bell-shaped curve) were drawn. The intersection points of +1 and -1 slope lines on the 0 slope line represented the pK_{a1} and pK_{a2} , respectively for mutant glucoamylase. The values for parent pK_{a1} and pK_{a2} may be compared with mutant glucoamylase. All data points were means of three replicates.

Fig. 4.

(A) Arrhenius plot for the determination of activation energy (E_a) of glucoamylases, when $E_a = -\text{slope} \times R$, where R (gas constant) = $8.314 \text{ J K}^{-1} \text{ mol}^{-1}$. (B) Lineweaver-Burk plot for the determination of kinetic constants of glucoamylases, where intercept on the y-axis corresponds to $1/V_{\max}$ and the intercept on the x-axis to $-1/K_m$. Open and closed circles correspond to *Aspergillus niger* parent and DG-resistant mutant, respectively. All data points were means of three replicates.

Table 1

Purification of glucoamylases from *Aspergillus niger* parent and its DG-resistant mutant.

Strain	Treatment	Activity (U)	Protein (mg)	Specific activity (U mg ⁻¹)	Purification fold	Yield %
Parent	Crude	23,135	2724	85	1.00	100
	(NH ₄) ₂ SO ₄ precipitation	16,230	670	24	2.8	70
	HiLoad column chromatography	7863	75	105	12.3	34
	Hydrophobic interaction column chromatography	4532	21	216	25.4	20
Mutant	Crude	22,976	1725	16.2	1.00	100
	(NH ₄) ₂ SO ₄ precipitation	18,632	372	50.1	3.1	81
	HiLoad column chromatography	9986	46	217.1	13.4	43
	Hydrophobic interaction column chromatography	7032	14	495.2	30.6	31

All quoted values were taken after dialysis against distilled water.

Table 2

Thermodynamics of soluble starch hydrolysis by glucoamylases from *Aspergillus niger* parent and its DG-resistant mutant at 60 °C, pH 4.4.

Properties	<i>A. niger</i> parent	<i>A. niger</i> mutant
ΔH^* (kJ mol ⁻¹)	41.50	46.12
ΔG^* (kJ mol ⁻¹)	65.69	63.62
ΔS^* (J mol ⁻¹ K ⁻¹)	-72.65	-52.53