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Purification and characterization of extracellular alpha-amylase from *Aspergillus fumigatus*

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Abstract

Extracellular alpha-amylase [(1 → 4)-α-D-glucan glucanohydrolase, EC 3.2.1.1] from *Aspergillus fumigatus* (*Aspergillus sp.* K-27) was purified to homogeneity by anion-exchange (DEAE-cellulose) and affinity (α-cyclodextrin-Sepharose) chromatography. The purified enzyme, a glycoprotein with 15% carbohydrate content, showed an isoelectric point of 3.7, a molecular weight of 65,000 (as estimated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis), and an amino acid composition with a high number of neutral hydrophobic residues. Alpha-amylase activity on α-D-glucans in solution was optimal at pH 5.5, and the enzyme was stable at 40°C. It hydrolyzed amylose and amylopectin, with respective K_m of 0.42 and 7.7 mg mL^{-1} and k_{cat}/K_m of 3.4 and 2.5 mL mg^{-1} min^{-1}. The major end-products of maltohexaose, degradation were glucose and maltose. Maltotriose, maltotetraose, and maltopentaose were formed as intermediate products with an α-anomeric configuration. Despite its ability to slowly degrade some α-(1 → 6) linkages, this purified enzyme should be classified as an alpha-amylase.

Keywords: Alpha-amylase; Enzyme purification; *Aspergillus fumigatus*; Characterization

1. Introduction

The main biological events in which starch degradation occurs, such as digestion and germination, are due to the action of alpha-amylases [(1 → 4)-α-D-glucan glucanohydrolase; EC 3.2.1.1)]. The susceptibility of starch granules to degradation by alpha-amylase depends on their botanical origins and sources of alpha-amylase [1,2]. Some native starches from potato or high-amylose cereals are known to be highly resistant to hydrolysis by common alpha-amylases from plants and animals. For these starches, the

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final figures after extensive hydrolysis are in the range 5–15% [1,2]. Considerable interest has been devoted to microbial amylases able to degrade these native starches completely. The industrial development of fermentation technologies for energy production has led researchers to look for glucose-producing amylases allowing direct production of ethanol.

Hizukuri and co-workers [3,4] obtained a raw-starch-digesting amylase from a strain of *Aspergillus fumigatus* deposited as *Aspergillus sp.* K-27. Two enzymes, a glucoamylase and an alpha-amylase, were purified and roughly characterized. Considerable attention was given to the synergistic action of alpha-amylase on the digestion of starch by glucoamylase. Further studies by Abe et al. [5] have been concerned with the efficiency of glucoamylase as affected by modification of its molecular structure. However this alpha-amylase would present a higher efficiency on native starches than common alpha-amylases. Its use could thus facilitate the study of long-term degradation within more reasonable time periods. The need for such enzymes in the biodegradation process is obvious, since microbial development is the bottleneck for long-term studies. The purpose of the present study was to propose a new purification procedure and to provide a more complete characterization of the amylolysis of soluble α-glucans and dextrins.

2. Experimental

Linear oligosaccharides (G_n, $n = 1–7$), bovine serum albumin (BSA), and alpha-amylase from *Bacillus subtilis* (Type II-A) were supplied by Sigma (St Louis, MO). Alpha-amylase from porcine pancreas was supplied by Merck (Darmstadt, Germany). Amylose and amylopectin from smooth pea and waxy corn starches, respectively, were extracted and purified in the laboratory according to Banks and Greenwood [6]. Cyclomalto-hexaose (cG_6), -heptaose (cG_7), and -octaose (cG_8) were obtained from Hayashibara (Japan). The *Aspergillus sp.* K-27 crude enzyme preparation was kindly supplied by Professor S. Hizukuri (Kagoshima University, Japan).

Enzyme assay.—Alpha-amylase activity. Alpha-amylase activity was measured in 50 mM NaOAc buffer, pH 5.5, at 35°C on a blocked *p*-nitrophenyl maltoheptaoside substrate. The procedure, as described by McCleary and Sheehan [7], was slightly modified and miniaturized (final total volume, 150 μL), using a microsample plate reader.

Glucoamylase activity. An aliquot (50 mL) of enzyme solution was added to a 2% (w/v) maltose solution (200 μL) in 50 mM NaOAc buffer, pH 5.5, for 15 min at 45°C. The reaction was then stopped by boiling for 10 min, and the resulting D-glucose was measured by the miniaturized D-glucose oxidase–peroxidase method [8] (final total volume, 150 μL).

Activity on maltose. An aliquot (10 μL for the maltose test, 500 μL for the isomaltose test) of an enzyme solution (1 mg mL^{-1}) was added to a 0.5% (w/v) maltose solution (1 mL) or a 1% (w/v) isomaltose solution (500 μL) in 50 mM NaOAc buffer, pH 5.5, at 35°C. Aliquots (200 μL) were taken at regular time intervals (0, 5, 10, 15, and 20 min), and the reaction was stopped by boiling for 10 min. The D-glucose released was measured by the D-glucose oxidase–peroxidase method [8].

Protein concentration was determined by Bradford's procedure using BSA as standard [9].

Enzyme purification.—All procedures were carried out at +4°C. First, the crude enzyme preparation was dialyzed against 50 mM NaOAc (pH 5.5) buffer, 5 mM $CaCl_2$, and then applied to a DEAE-Sephacel column (200 × 18 mm) (Pharmacia, Uppsala, Sweden) previously equilibrated with the same buffer. Elution was done at a flow rate of 0.5 mL min^{-1}. After recovering the baseline, adsorbed proteins were eluted with buffer containing 1 M NaCl. Fractions (3 mL) were collected, and their alpha-amylase and glucoamylase activities determined.

Fractions containing alpha-amylase activity were pooled and applied to a cG_6-Sepharose 6B column (100 × 50 cm) [10] previously equilibrated with 50 mM NaOAc buffer, pH 5.5. Adsorbed proteins were eluted by the same elution buffer containing 0.5% (w/v) cG_6. Elution was done at a flow rate of 0.5 mL min^{-1}, and fractions (2.5 mL) were collected and tested for amylase activities.

Fractions containing alpha-amylase were pooled, dialyzed against water, then lyophilized, and stored at +4°C.

Electrophoresis and molecular mass determination.—Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) was performed as described by Laemmli [11] in 10–20% acrylamide gels. Calibration proteins with a molecular weight ranging from 14,400 to 94,000 (low-molecular-weight calibration kit, Pharmacia) were used to determine the molecular weight of the purified enzymes.

Isoelectric point determination.—Isoelectric focusing-PAGE was done as described by Bertheau et al. [12]. The isoelectric point of purified alpha-amylase was determined with broad-range standard protein markers (Pharmacia) ranging from pI 3.0 to 10.0.

Hydrodynamic radius.—This was determined by dynamic light-scattering as described by Rani et al. [13].

Amino acid analysis.—The amino acid composition of the purified enzyme was determined after acid hydrolysis at 110°C for 24 h, as described by Bidlingmeyer et al. [14]. Separation and identification of PTC-derived amino acids was carried out using a PICO TAG C_{18} column (15 × 0.39 cm) at 38°C, with a curvy gradient (Waters, Milford MA). Buffer A was constituted by 0.14 M NaOAc buffer, pH 6.4, containing triethylamine (0.1%); and buffer B by a 40:60 mixture of buffer A and MeCN. The flow rate was 1 mL min^{-1}.

Carbohydrate analysis.—Enzyme (1–2 mg) was hydrolyzed with 4 M trifluoroacetic acid (0.5 mL) at 100°C for 4 h. After evaporation under an air stream (40°C), released monosaccharides were reduced in 2 M NH_3 2% $NaBH_4$ solution (0.5 mL). The reaction was stopped by AcOH (3 × 0.05 mL). After evaporation, the reaction product was solubilized by the addition of three successive portions (0.5 mL) of AcOH in MeOH (10%, v/v), followed by the addition of three successive portions (0.5 mL) of MeOH. Alditols were acetylated by a mixture of pyridine (0.2 mL) and Ac_2O (0.2 mL) for 20 min at 120°C. Alditol acetates were analyzed by GC at 190°C on a DB-1 capillary column (30 × 0.32 mm i.d.; 0.25-μm film; J and W Scientific, Folsom, TX), with H_2 as carrier gas [15].

Physicochemical properties of alpha-amylase.—The effect of temperature on enzyme stability was determined after incubation of enzyme solutions at various temperatures for

20 min in 50 mM NaOAc buffer, pH 5.5, followed by measurement of enzyme activity at the same temperature.

Calcium influence was studied by incubating the enzyme, in 1 mM steps, at 35°C in 50 mM NaOAc buffer, pH 5.5, containing up to 15 mM Ca^{2+} ions. Residual activity was measured at 35°C.

pH Stability was determined by incubating the enzyme (10 μg mL^{-1}, final concentration) solutions in 50 mM NaOAc buffer at various pH values for 30 min at 35°C, followed by measurement of residual activity. In this case, alpha-amylase activity was measured at 35°C on maltoheptaose (Sigma, St Louis, MO) (2 mg mL^{-1}, final concentration). The reaction was stopped by adding 1 M KOH (30 μL) per mL of enzyme digestion solution. The resulting reducing sugars were determined colorimetrically by the method of Nelson [16].

Hydrolysis of linear oligosaccharides.—For malto-oligosaccharides from G_1 to G_7, each oligosaccharide (1.25 mg mL^{-1}) was dissolved in 50 mM NaOAc buffer, pH 5.5, containing 1 μg mL^{-1} of alpha-amylase at 35°C. Aliquots (1 mL) were taken at regular time intervals, the reaction being stopped by 1 M KOH (35 μL). The composition of the resulting oligosaccharides was determined by anion-exchange chromatography with pulsed amperometric detection (Dionex) on a Carbopac PA1 column eluted with 160 mM NaOH, with a 10 to 50% gradient of 600 mM NaOH/NaOAc, for 15 min at a flow rate of 1 mL min^{-1}. Calibration was carried out using a mixture of the different linear malto-oligosaccharides from glucose to maltoheptaose.

The same procedure was followed for pullulan, with an enzyme concentration of 10 μL min^{-1} and a substrate concentration of 1.5 mg min^{-1}, but the column was eluted with 100 mM NaOH containing 15% NaOAc. The calibration was carried out using D-glucose, different linear malto-oligosaccharides from G_2 to G_7, isomaltose, and panose.

Anomeric configuration of reaction products.—High-performance liquid chromatography (HPLC) on a C_{18} silica gel column (25 × 0.46 cm, Merck, Darmstadt, Germany) was carried out with water at a flow rate of 0.9 mL min^{-1}. The column was calibrated with a mixture of the different malto-oligosaccharides from glucose to maltoheptaose. Alpha-amylase (10 μg) was added at room temperature to 1 mL of maltoheptaose solution (1 mg mL^{-1}) in water. Digestion aliquots (30 μL) were injected after 1 min of reaction. Digestion of the remaining solution was stopped by boiling for 10 min. After standing overnight at +4°C, this solution was injected.

Determination of kinetic constants.—Kinetic constants K_m and V_{max} were determined for the two major carbohydrate components of starch: amylose and amylopectin. Substrate concentrations ranged from 0 to 10 mg mL^{-1}, and the enzyme was used at 25 mg mL^{-1}. After 15 min at 35°C, the resulting reducing sugars were determined colorimetrically by the method of Nelson [16]. The Michaelis constant (K_m) and the reaction rate at an infinite substrate concentration (V_{max}) were determined by the method of Lineweaver and Burk [17].

3. Results and discussion

Enzyme purification.—Starting from 1.57 g of protein, the crude enzyme preparation [4] obtained after concentration [18] and dialysis against acetate buffer contained 3.9

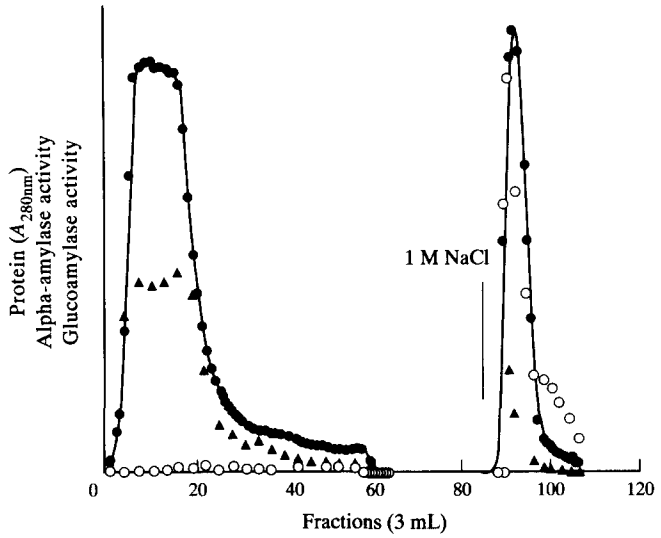

Fig. 1. Ion-exchange chromatography of the crude enzyme preparation of *Aspergillus fumigatus* (K-27) on a DEAE-cellulose column (200×18 mm); (●), protein ($A_{280\ nm}$); (○), alpha-amylase activity; (▲), glucoamylase activity.

nkat of alpha-amylase activity per mg of protein; the whole preparation represented 393 mg of protein. This protein extract was first purified by anion-exchange chromatography on a DEAE-Sephacel column. One large peak was obtained on elution with the buffer, and one on stepwise elution with the buffer containing 1 M NaCl (Fig. 1). This chromatography step isolated glucoamylase, which was recovered at homogeneity in fractions 0–60, in association with the first large protein peak.

Fractions 83–100, containing alpha-amylase activity, were combined and applied to a cG_6-Sepharose 6B column. One peak was obtained on elution with the buffer containing cG_6 (0.5% w/v) (Fig. 2). Fractions 1–36 were discarded, and fractions 37–47 obtained by affinity chromatography contained alpha-amylase at homogeneity (Fig. 3). These fractions were pooled, dialyzed against water, and then lyophilized.

The overall purification scheme is summarized in Table 1. This procedure gave pure amylase with a specific activity of 19.2 nkat per mg of protein, a purification factor of 21.3, and a yield of 1.5%.

Determination of the respective specific activities of both enzymes indicated that the crude enzyme preparation contained glucoamylase and alpha-amylase in a 70/30 ratio. Both enzymes were purified to homogeneity, as determined by SDS-PAGE analysis (Fig. 3). The SDS results in Fig. 3 would not detect the presence of 1% or less of other proteins.

This procedure was different from that described by Abe et al. [18] for the purification of glucoamylase and alpha-amylase from a crude enzyme preparation of *Aspergillus sp.* K-27. Considering that only glucoamylase could be adsorbed onto the matrix, they used cG_6-Sepharose 6B to isolate glucoamylase from alpha-amylase. In our

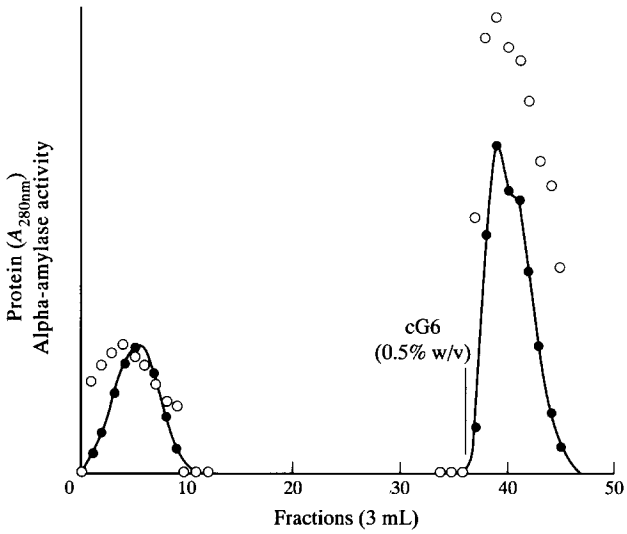

Fig. 2. Affinity chromatography of the alpha-amylase fraction on a cG_6-coupled Sepharose 6B column (100×50 cm): (●), protein ($A_{280\ nm}$); (○), alpha-amylase activity.

trials (data not shown), both glucoamylase and alpha-amylase were adsorbed onto the affinity matrix, regardless of whether cG_6, cG_7, or cG_8 was used. Our purification was improved by removal of glucoamylase before the affinity chromatography was performed. In these conditions, alpha-amylase was the only protein in the mixture and

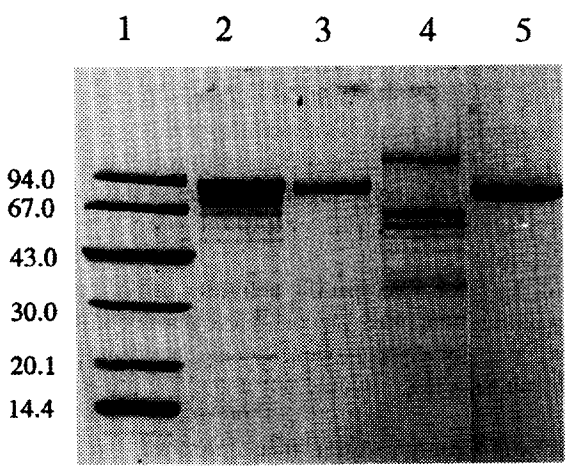

Fig. 3. SDS-PAGE (10–20%) of crude enzyme extract (2), purified glucoamylase (3), the fraction non-adsorbed by affinity chromatography (4), and purified alpha-amylase (5). Molecular weight markers (1) are indicated in thousands.

Table 1
Purification of the alpha-amylase of *Aspergillus sp.* K-27

	Protein (mg)	Recovery [a] (%)	Activity (10^3 nkat mL^{-1})	Specific activity (nkat mg^{-1})	Purification factor
Culture filtrate	1570	100	524	0.9	
Ammonium sulfate precipitation [b]	393	25	439	3.9	4.3
DEAE Sephacel column	84.5	5.4	205	14.2	15.7
cG_6-Sepharose column	23.5	1.5	120	19.2	21.3

[a] Recovery was assumed to be 100%.
[b] Results from Professor Hizukuri [18].

could be adsorbed on the cG_6 matrix. The overall procedure, involving three steps, is simpler than the previous one [18] with six steps.

Molecular properties.—Alpha-amylase, purified to homogeneity, showed a single band in SDS-PAGE. Its molecular weight was estimated to be 65,000 ($\pm$ 2,500) a value in agreement with the findings of Abe et al. [18]. Its isolectric point was found to be 3.7 by PAGE-isolectric focusing. These data are within the range of values reported for most alpha-amylases [19,20].

The enzyme was shown to be a glycoprotein. The carbohydrate moiety, representing 15% (w/w) of the protein, was composed of mannosyl (8.0%), glucosyl (4.0%), galactosyl (2.1%), and *N*-acetylglucosaminyl (0.9%) residues. This value is higher than those reported for other amylases, e.g., Taka-amylase from *Aspergillus oryzae*, which contains 0.65% of carbohydrates [21].

Analysis of amino acid composition showed no important differences between this enzyme and well-known alpha-amylases such as those from *Bacillus subtilis*, *Bacillus*

Table 2
Molecular composition of amino acids in the alpha-amylase from *Aspergillus fumigatus*

Amino acid residue	Relative %
Tryptophan	1.2
Phenylalanine	3.8
Proline	4.9
Alanine	10.4
Valine	7.4
Isoleucine	5.2
Leucine	7.7
Glycine	9.7
Threonine	11.4
Serine	12.5
Cysteine	0.8
Methionine	< 0.1
Aspartate	11.4
Glutamate	6.6
Histidine	1.5
Arginine	2.9

licheniformis, or pig pancreas (Table 2) [22,23]. Nevertheless, it is noteworthy that alpha-amylase from *Aspergillus fumigatus* contains a relatively large amount of neutral hydrophobic amino acids. As serine and threonine residues are known to be glycosylated [24], their presence may be related to the high carbohydrate content of the enzyme.

The hydrodynamic radius of this alpha-amylase, as determined in a solution by quasi-elastic light-scattering, was estimated to be 4 nm. Since alpha-amylases from *Bacillus subtilis* or porcine pancreas show similar values [25], this enzyme has no specific advantage in terms of diffusion.

Physicochemical properties.—Alpha-amylase from *Aspergillus fumigatus* displayed optimal activity at pH 5.5. Activity started at pH 3.5 and stopped at pH 9.0. After 20-min incubation at different temperatures, the enzyme showed optimal activity at 40°C followed by a sharp decrease leading to a loss of activity at 55°C. These findings are within the range of data reported for other alpha-amylases [18,19]. The Arrhenius law was respected between 20 and 35°C, and an activation energy of 38.9 kJ mol^{-1} was determined.

Compared to other alpha-amylases, such as that from barley malt [26], this enzyme displayed minor dependence on calcium ions at concentrations of 0–20 mM. Enzyme activity ranged from 4 to 5 10^{-6} kat mL^{-1}.

Catalytic properties.—Action of *Aspergillus fumigatus* alpha-amylase on linear malto-oligosaccharides (G_n, $n = 1$ to 7) was investigated. A very low enzyme concentration (1 μg mL^{-1}) was used to quantify and identify the products obtained during initial reaction stages. Figure 4 shows the relative percentage of oligosaccharides produced during the initial action of alpha-amylase on maltoheptaose (G_7). After a reaction time of 2 h, 71% of the substrate was hydrolyzed. The products were maltopentaose (G_5,

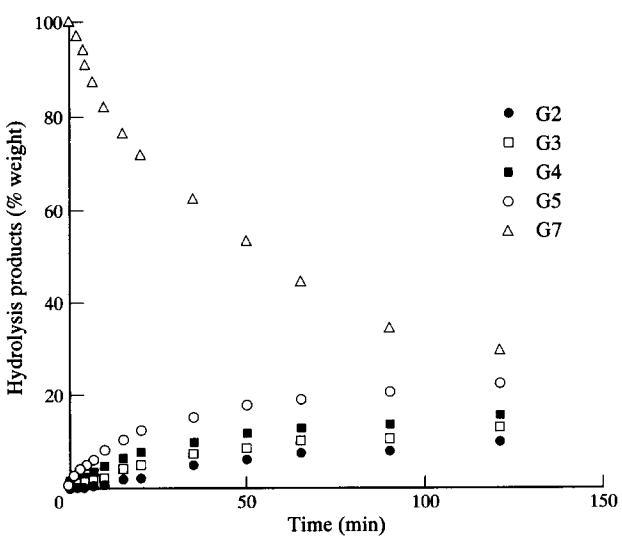

Fig. 4. Hydrolysis products of maltoheptaose with the action of the alpha-amylase (1 μg mL^{-1}) at 35°C in 50 mM NaOAc buffer, pH 5.5.

Table 3
Oligosaccharides produced after 24 h of hydrolysis of different linear maltooligosaccharides from G_1 to G_7 (1.25 mg mL^{-1}, 35°C ; enzyme concentration 1 $\mu g\ mL^{-1}$, 50 mM NaOAc buffer, pH 5.5)

Substrate	Oligosaccharide produced (% weight)			
	G_1	G_2	G_3	G_4
G_1	100			
G_2	8.7	91.3		
G_3	32.3	59.9	7.9	
G_4	10.2	79.6	10.2	
G_5	9.5	43.5	43.2	3.8
G_6	8.8	44.6	42.8	3.8
G_7	10.5	48.4	37.7	3.4

24%), maltotetraose (G_4, 18%), maltotriose (G_3, 14%), and maltose (G_2, 12%). No production of glucose (G_1) and maltohexaose (G_6) was detected during the initial action of this alpha-amylase on G_7. These results are consistent with the action of an endo-enzyme releasing oligosaccharides with different degrees of polymerization.

These observations confirm that the purified enzyme was an alpha-amylase, which is the only enzyme known to hydrolyze (1 → 4)-α-D-glucosidic linkages randomly. The absence of glucose during the first stages of hydrolysis demonstrates that, like other alpha-amylases, this enzyme hardly degraded terminal linkages either at the reducing or nonreducing ends of the polymer chain.

After 24 h of hydrolysis, G_4–G_7 oligosaccharides were completely hydrolyzed, whereas hydrolysis extents for G_2 and G_3 were 9 and 92%, respectively (Table 3). The hydrolysis rate of maltose was very low (17% in 72 h). When a higher enzyme concentration (5 $\mu g\ mL^{-1}$) was used for 72-h hydrolysis, the final end-products were G_1 and G_2, regardless of the starting oligosaccharide.

When this enzyme was incubated with G_1 in the same experimental conditions, no condensation reaction was detected, indicating that the above-mentioned variations in the qualitative composition of hydrolysis products could only be ascribed to successive hydrolysis reactions.

To control the absence of glucoamylase in the enzyme preparation, its activity on isomaltose was tested. The specific activity on this linkage was far lower (2.3 nkat mg^{-1} of protein) than on maltose (59.1 nkat mg^{-1} of protein). But all other alpha-amylases (pig pancreas, *Bacillus subtilis)* tested under the same conditions showed no activity towards isomaltose. Therefore alpha-amylase preparation could be contaminated by glucoamylase. Nevertheless, when pullulan was hydrolyzed, no glucose was detected but a large continuous distribution of oligomers was obtained (Fig. 5), indicating the absence of glucoamylase. Panose and isopanose were obtained, demonstrating an activity on α-(1 → 4) linkages. Furthermore maltotriose was also produced demonstrating a clear action on α-(1 → 6) linkages in pullulan.

Determination of kinetic constants.—This enzyme showed Michaelis-type kinetics when amylose and amylopectin, the main constitutive macromolecules of starch, were hydrolyzed. The apparent K_m and V_{max} are reported in Table 4, as calculated from

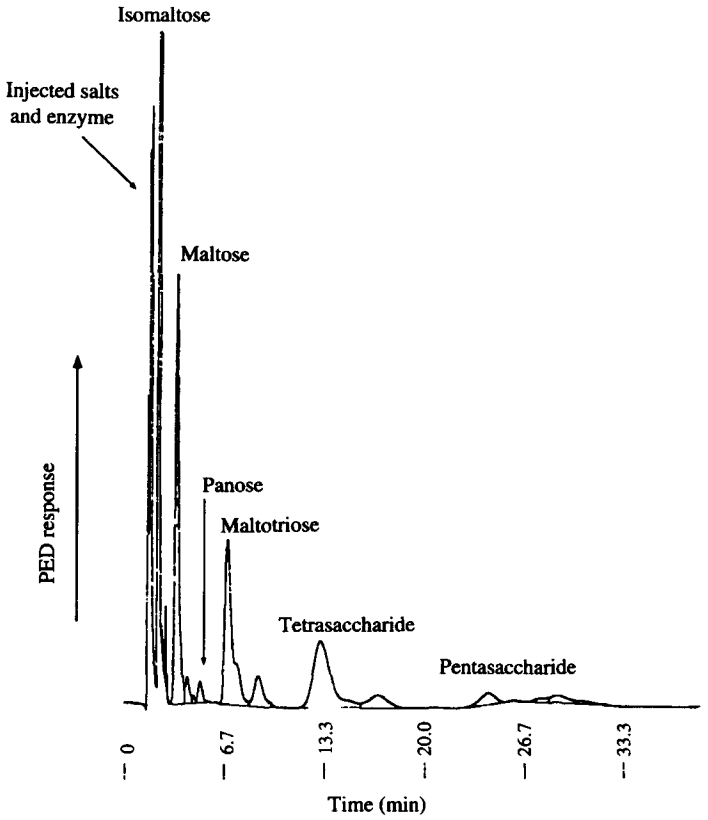

Fig. 5. Anion-exchange chromatography of pullulan hydrolysate (1.5 mg mL^{-1}) resulting from the action of alpha-amylase (10 μg mL^{-1}) in 50 mM NaOAc buffer, pH 5.5, for 6 h.

Lineweaver and Burk [17] plots. The k_{cat} and k_{cat}/K_m calculated with these values are also given.

The V_{max} values were significantly higher for amylopectin (0.38 split linkage min^{-1}) than for amylose (0.29 split linkage min^{-1}).

The K_m values were 4.2 and 7.7 mL mg^{-1} for amylose and amylopectin respectively. The lower K_m value for amylose reflects a higher number of hydrogen or

Table 4
Kinetic constants of *Aspergillus fumigatus* on amylose and amylopectin

Kinetic constants	Substrate	
	Amylose	Amylopectin
K_m (mg mL^{-1})	4.2	7.7
V_{max} (split linkages min^{-1})	0.29	0.38
k_{cat} (min^{-1})	14.5	19
k_{cat}/K_m (mL $mg^{-1}min^{-1}$)	3.4	2.5

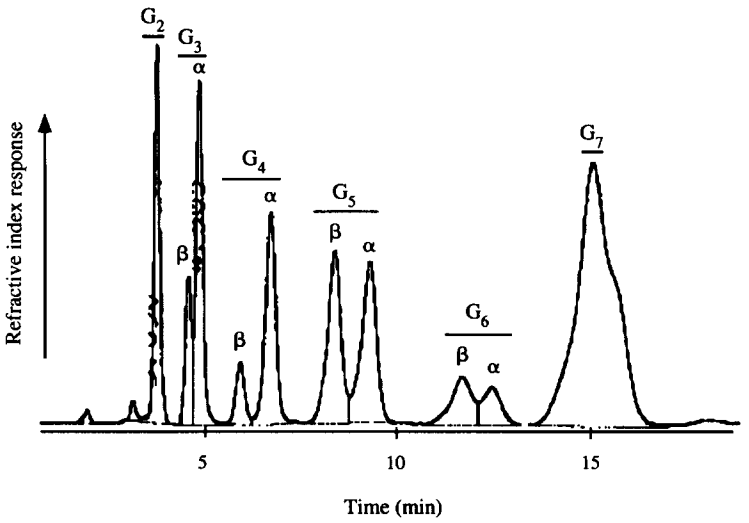

Fig. 6. C_{18} HPLC chromatography of maltoheptaose immediately after hydrolysis (1 min) by alpha-amylase in 50 mM NaOAc buffer, pH 5.5.

hydrophobic interactions between the substrate and active site residues. This enzyme has a better affinity for amylose than for amylopectin. Catalytic efficiency, estimated by k_{cat}/K_m, was lower for amylopectin than for amylose. This is in agreement with the behavior of alpha-amylase which preferentially hydrolyzes linear substrates composed of α-(1 → 4) linkages.

Anomeric configuration of hydrolysis products.—When chromatographed on a C_{18} column by HPLC, G_3, G_4, G_5, and G_6 all presented two peaks attributable to α- and β-anomeric forms at mutarotation equilibrium. Both α and β forms were observed in a ratio of 1:2, in agreement with known values for glucose at equilibrium. In contrast, G_1 and G_2 gave only one peak where α- and β-anomeric forms are mixed. A G_7 solution

Table 5
Anomeric configuration of hydrolysis products (G_3–G_6) from G_7 (1 mg mL^{-1}, enzyme concentration 10 μg mL^{-1}, 50 mM NaOAc buffer, pH 5.5) either immediately after hydrolysis or 16 h later for mutarotation equilibrium

Oligosaccharide	Time after hydrolysis (h)			
	0		16	
	Anomeric configuration (weight %)		Anomeric configuration (weight %)	
	β	α	β	α
G_3	27.9	72.1	58.8	41.2
G_4	20.1	79.9	60.6	39.4
G_5	48.9	51.1	61.5	38.5
G_6	59.4	40.6	68.1	31.9

(1 mg mL^{-1}) was hydrolyzed for one minute by this alpha-amylase and then immediately injected. The HPLC chromatogram of the reaction mixture demonstrated that mainly α-anomeric residues were released (Fig. 6) for G_3 and G_4, in contrast to minor β-anomeric residues. However the α- and β-anomeric residues for G_5 and G_6 were present in equal (1:1) proportion, probably because of associated rapid mutarotation during their longer HPLC reaction time (15 min instead of 5 min for G_3). In order to confirm this progressive mutarotation, a G_7 solution hydrolyzed for 1 min was boiled for 10 min, stored at +4°C overnight, and analyzed in the same conditions. The HPLC chromatogram revealed the predominance of the β form under conditions in which mutarotation was complete (Table 5). This retention of the α-anomeric configuration in hydrolysis products is in agreement with the endo-attack of amylose chains.

4. Conclusion

This purified enzyme showed similar molecular properties to the alpha-amylase described by Abe et al. [18]. Therefore, it cannot be assumed that there were variations in the composition of the crude enzyme extract. Our purification scheme is simpler than theirs and thus allows easier production. With respect to its behavior on linear α-(1 → 4)-linked oligosaccharides, this enzyme can be classified as an alpha-amylase. However this endo-enzyme was also shown to slowly degrade α-(1 → 6) linkages in isomaltose and pullulan. The ability to hydrolyze both α-(1 → 4) and α-(1 → 6) linkages has already been observed in other amylases [27]. However those enzymes were shown to degrade α-(1 → 6) bonds preferentially. In contrast to the enzyme purified from *Aspergillus fumigatus*, the hydrolysis pattern of these enzymes was closer to that of pullulanase, thus accounting for their designation as amylopullulanase [27].

Further studies are planned to investigate the degradation of solid-state substrates such as native starch granules.

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