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PURIFICATION, PROPERTIES, AND INDUSTRIAL SIGNIFICANCE OF TRANSGLUCOSIDASE FROM *Aspergillus niger*

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ABSTRACT

A transglucosidase, an α-D-glucosidase with a high transferase activity, has been purified to homogeneity from culture broths of *A. niger*. The enzyme, which gave a single protein band on SDS-gel electrophoresis (mol. wt. 116,000) and two protein bands on isoelectric focusing (pI values 5.1 and 5.0), is a glycoprotein, containing 27.6% of carbohydrate, most of which is mannose, has an optimal pH of 4.0–4.5, and is stable in the pH range 4.0–6.0 and at <50°. The transglucosidase acts on various substrates to give transfer products and glucose. The V_{max} and K_m values (mM) for the following substrates were obtained: maltose (73.5, 1.7), isomaltose (31.9, 7.7), nigerose (63.6, 20.0), kojibiose (22.1, 3.6), phenyl α-D-glucopyranoside (2.5, 2.4), *p*-nitrophenyl α-D-glucopyranoside (2.1, 0.7), and methyl α-D-glucopyranoside (1.0, 26.3). The effect of the enzyme on the conversion, by amyloglucosidase, of wort saccharides into glucose has been quantified. A procedure, based on the use of phenyl α-D-glucopyranoside as substrate, is recommended for the assay of this enzyme in crude culture filtrates.

INTRODUCTION

D-Glucosyltransferases (transglucosidases, EC 2.4.1.24) from fungal sources catalyse both hydrolytic and transfer reactions on incubation with α-D-gluco-oligosaccharides[1–4]. *Aspergillus niger* transglucosidase may transfer the non-reducing D-glucosyl residue of maltose to water (hydrolysis), to D-glucosyl residues released by hydrolysis, or to the non-reducing residue of maltose[5]. Transfer occurs most frequently to HO-6, producing isomaltose from D-glucose or panose (6-*O*-α-D-glucosylmaltose) from maltose. Less frequently, transfer occurs to the HO-2 or HO-3 of D-glucose, to form kojibiose or nigerose, or back to HO-4 to re-form maltose[6].

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Transglucosidase often contaminates preparations of fungal amyloglucosidase and since its action lowers the yield of glucose and produces oligosaccharides which interfere with the crystallisation of glucose and reduces fermentability, the activity is undesirable. Therefore, procedures have been developed to remove transglucosidase from preparations of amyloglucosidase. Such procedures include adsorption onto clay minerals[7], precipitation with chloroform[8], or inactivation by control of pH either in the presence of acidic polysaccharides[9] or of oxalic acid and specific cations[10].

The removal of transglucosidase without undue loss of amyloglucosidase activity is complicated by the similar substrate specificity of the enzymes[2]. Transglucosidase can be assayed on the basis of the panose produced on incubation of the enzyme with maltose[1]. P.c., t.l.c.[11], and h.p.l.c.[12] procedures have been employed; in one instance, a specific amyloglucosidase inhibitor, acarbose, was included[12]. Other approaches involved methyl α-D-glucopyranoside as a substrate which is hydrolysed by transglucosidase but not by amyloglucosidase[3,11], and the separation of transglucosidase and amyloglucosidase by ion-exchange chromatography before assay[13].

Transglucosidase acts on malto-oligosaccharides[1-6] but detailed kinetic studies have not been performed. Information on the properties of the enzyme and its effect in preparations of amyloglucosidase on the fermentability of worts (malt extracts) is also limited.

The aims of the work here reported were to (*a*) evaluate substrates for the assay of transglucosidase and to identify a simple assay which could be used for crude culture broths containing high levels of amyloglucosidase, (*b*) purify the transglucosidase and study its physical and kinetic properties, and (*c*) investigate the effect of the transglucosidase in preparations of amyloglucosidase on the production of highly fermentable worts and, thus, low-carbohydrate beer.

EXPERIMENTAL

Materials. — Commercial enzyme preparations of amyloglucosidase AMG 300 (Novo Industries, Denmark) and Amylo 300 (Biocon Biochemicals, Ireland) were used. Bentonite clay was obtained from Sigma (B3378) and the D-glucose oxidase–peroxidase reagent from Biocon (Australia) Pty Ltd. All other carbohydrates were commercial products.

Fermentation broth. — *Aspergillus niger* was cultured under conditions which induced maximum production of transglucosidase activity relative to that of amyloglucosidase[14]. The broth (150 L) was filtered through a Plate and Frame Filter (4.1 L/frame, 8 frames) with 6 kg of body-feed (Clarcel Flo, The British Ceca Company) and 1.5 kg of Clarcel Flow as a filter pre-coat. The broth was adjusted to a concentration of 300 U of amyloglucosidase/mL (assayed on maltose) and contained 0.3 U of transglucosidase/mL (assayed on phenyl α-D-glucopyranoside). After concentration by ultrafiltration, the broth contained 1.3 U of transglucosidase/mL and 1300 U of amyloglucosidase/mL.

Chromatography. — H.p.l.c. was performed with a Waters Sugar Analyser fitted with a Sugar Pak I column. The eluant was h.p.l.c.-grade water containing 20 p.p.m. of Ca EDTA, run at 90° and 0.5 mL/min. Elution patterns were monitored by changes in refractive index. T.l.c. was performed as previously described[15].

General methods. — Total carbohydrate was determined by the phenol–sulphuric acid method[16], protein by the Folin–Lowry procedure[17], reducing sugar by the Nelson–Somogyi procedure[18,19], and D-glucose with D-glucose oxidase–peroxidase.

Assay of transglucosidase. (*a*) *Phenyl α-D-glucopyranoside as substrate (standard assay).* A solution of phenyl α-D-glucopyranoside (0.5 mL, 10mM) in 0.1M sodium acetate buffer (pH 4.0) was pre-incubated for 5 min at 40°. Suitably diluted culture broth (0.5 mL) was added and incubation was continued for 10 min at 40°. Aqueous 1% sodium carbonate (2 mL), aqueous 1% 4-aminophenazone (1 mL), and aqueous 3% potassium ferricyanide (3 mL, 3% w/v) were added, the total volume was adjusted to 10 mL with water, and the absorbance at 545 nm was determined. In blank assays, distilled water replaced the enzyme solution. A standard curve was constructed using phenol at concentrations of 0–1.0 μmol/0.5 mL. Unless otherwise stated, 1 U of transglucosidase is defined as the amount of enzyme which releases 1 μmol of phenol per min at 40° and pH 4.0.

(*b*) p-*Nitrophenyl α-D-glucopyranoside as substrate.* A suitably diluted enzyme preparation (0.2 mL) was incubated with pre-equilibrated *p*-nitrophenyl α-D-glucopyranoside (0.2mL, 10mM) in 0.1M sodium acetate buffer (pH 4.0) for 5 min at 40°. The reaction was terminated by the addition of an aqueous solution of Trizma base (Sigma; 3.0 mL, 2% w/v), and the absorbance at 410 nm was measured. Enzyme activity is expressed in terms of μmol of *p*-nitrophenol released/min and calculated by reference to a standard curve.

(*c*) *Methyl α-D-glucopyranoside, oligosaccharides, and starch as substrates.* A suitably diluted enzyme preparation (0.2 mL) was incubated with an aqueous solution of starch or oligosaccharide (0.2 mL, 20 mg/mL) or methyl α-D-glucopyranoside (0.2 mL, 100mM) in 0.1M acetate buffer (pH 4.0) for 10 min at 40°. The reaction was terminated by heating for 1 min at ~100°. The glucose released was determined with the D-glucose oxidase–peroxidase reagent (3.0 mL) by incubation for 20 min at 40° and measurement of the absorbance at 510 nm. Activity is expressed as μmol of bonds cleaved/min. Thus, for the disaccharides maltose, nigerose, kojibiose, and isomaltose, the glucose values were halved.

In kinetic studies, concentrations of the above substrates ranged from 0.5–100mM.

Assay of amyloglucosidase. — The amyloglucosidase preparation (0.2 mL) was incubated with an aliquot (0.2 mL) of pre-equilibrated solutions of maltose (20 mg/mL) or starch (10 mg/mL) in 0.1M acetate buffer (pH 4.3), and the mixture was incubated for 5 min at 40°. The reaction was terminated by heating for 2 min at ~100°. The glucose released was determined with the D-glucose oxidase–peroxidase reagent. Unless otherwise stated, 1 U of amyloglucosidase is the amount of enzyme which cleaves 1 μmol of maltose/per min at 40° and pH 4.3.

Assay of alpha-amylase. — The assay involved[20] *p*-nitrophenyl maltoheptaoside as substrate in the presence of excess of amyloglucosidase and maltase ["Ceralpha, α-Amylase Assay Kit", Biocon (Australia) Pty Ltd.].

Transglucosidase. — (*a*) *Purification.* Filtered and concentrated culture broth (6 L) containing 1.3 U of transglucosidase/mL was treated with ammonium sulphate (500 g/L) overnight at 4°. The precipitate was collected by centrifugation (3,000*g*, 30 min), redissolved in the minimum volume of 10mM Tris/HCl (pH 7.5), and dialysed against ice-cold Tris/HCl (5 L, 10mM, pH 7.5) for 20 h. The dialysed solution (230 mL) was centrifuged (10,000*g*, 15 min), the pH was adjusted to 5.5 with sodium acetate buffer (20mM), and the solution was applied to a column (3.5 × 25 cm) of pre-equilibrated (20mM acetate buffer, pH 5.5) DEAE-Sepharose Fast Flow. The column was washed with 20mM acetate buffer (pH 5.5) and then eluted with a linear gradient (0→0.5M) of KCl in 20mM acetate buffer (pH 5.5; total vol., 3 L). Transglucosidase did not bind to the resin and was eluted at the solvent front. The active fraction was treated with ammonium sulphate (50 g/100 mL), the precipitate was recovered by centrifugation (10,000*g*, 15 min), dissolved in the minimum volume of water, and eluted from a column (2.5 × 80 cm) of Sephadex G-100 in 10mM citrate buffer (pH 3.5). The active fraction, which was eluted near the column void volume, was applied directly to a column (2.8 × 15 cm) of S-Sepharose Fast Flow pre-equilibrated with 10mM citrate buffer (pH 3.5) and eluted with a linear gradient (0→0.5M) of KCl in 10mM citrate (pH 3.5; total vol., 2 L). The transglucosidase was eluted at a salt concentration of 0.15–0.2M. The enzyme was dialysed against distilled water (5 L) for 48 h (two changes) and lyophilised.

(*b*) *Removal from amyloglucosidase preparations using bentonite.* Filtered fermentation broth (100 mL) containing 300 U of amyloglucosidase/mL was treated with 2M sodium acetate buffer (pH 5.5) to give a final buffer concentration of 10mM and a pH of 5.5. This mixture was applied to a column (2.5 × 20 cm) of pre-equilibrated DEAE-Sepharose Fast Flow. The column was washed with 10mM acetate buffer (300 mL, pH 5.5) then with a linear gradient (0→0.5M; total vol. of 1 L) of KCl in 10mM acetate buffer (pH 5.5). Fractions were assayed for activity on phenyl α-D-glucopyranoside, maltose, and starch. Two peaks (I and II) of amyloglucosidase were obtained. In a parallel experiment, culture broth (100 mL) was treated with acetate buffer as above, and then stirred with bentonite clay (2 g) for 20 h at 4°. The bentonite was removed by centrifugation (10,000*g*, 15 min), the supernatant solution was added to a column (2.5 × 20 cm) of DEAE-Sepharose Fast Flow, and the column was washed and eluted as for the non-bentonite-treated broth.

(*c*) *Properties.* The temperature stability was determined by incubating aliquots of enzyme in 0.1M acetate buffer (pH 4.5) for 15 min at 30–80°. The residual activity was assayed at 40° using *p*-nitrophenyl α-D-glucopyranoside. In order to determine the optimal temperature for activity, assays were performed for 5 min at 30–80° in 0.1M acetate buffer (pH 4.5), using *p*-nitrophenyl α-D-glucopyranoside. The optimal pH for enzyme activity was determined by incubating unbuffered enzyme (0.2 mL) with 10mM *p*-nitrophenyl α-D-glucopyranoside in 0.1M

citrate/0.1M phosphate buffer (pH 3.0–7.5) for 5 min at 40°. In order to identify the pH stability range, aliquots of transglucosidase in 0.1M citrate/0.1M phosphate buffer (pH 3.0–7.5) were incubated for 30 min and 20 h at 40°. Aliquots (50 μL) were then diluted 10-fold in 0.1M sodium acetate buffer (pH 4.0) and assayed for remaining activity with *p*-nitrophenyl α-D-glucopyranoside.

The total carbohydrate content of transglucosidase was measured[22] on aliquots (0.1 and 0.2 mL) of a solution (10 mg/10 mL) of purified enzyme in distilled water. The monosaccharide composition of purified transglucosidase (10 mg) was determined by the procedure of Blakeney *et al.*[21] except that hydrolysis was with 2M trifluoroacetic acid for 1 h at 120°. The acid was then evaporated and the residue was dissolved in M ammonium hydroxide before reduction and acetylation. G.l.c. was performed on a packed glass column (3% Silar 10C/80–100 WHP) at 210°; injector temperature, 250°; nitrogen flow-rate, 50 mL/min; 1-μL samples.

The susceptibility of the D-mannosyl residues in transglucosidase to hydrolysis by α- and β-D-mannosidase involved pre-digestion of the enzyme with protease. A solution of transglucosidase (4.0 mg/mL) was denatured at 100° (5 min) and treated with proteinase K (Merck 31073382) in 20mM sodium phosphate (pH 7.5). The hydrolysate was boiled for 1 min, adjusted to 50mM with sodium acetate (pH 4.5), and incubated with either α-D-mannosidase (Sigma M7257; 1.5 mU/mg of transglucosidase) or β-D-mannosidase[23] (1.5 mU/mg of transglucosidase) for 24 h at 40°. Enzyme reaction was stopped by incubation for 1 min at 100° and the increase in reducing sugar was determined[18,19].

Isoelectric focusing was performed with an LKB Multiphor with commercial LKB, PAG^R plates (pH 3.5–9.6)[24], and SDS-gel electrophoresis was modified from Laemmli[25] as described by Speirs and Brady[26], with a 5–18% polyacrylamide gradient gel. The Pharmacia high-molecular-weight-protein kit and purified *Aspergillus niger* β-D-glucosidase[27] were employed as standards.

(*d*) *Action on gluco-oligosaccharides.* Solutions of oligosaccharide (0.2 mL, 10 mg/mL) in 10mM acetate buffer (pH 4.0) were incubated with transglucosidase (0.05 mL; 0.025 U on phenyl α-D-glucopyranoside) at 40°. Reactions were stopped after 2, 5, 15, and 60 min by heating for 1 min at ~100°. The contents of each tube were diluted to 2.0 mL with distilled water, and the solutions were filtered (Millipore 0.45-micron durapore filter) and analysed by h.p.l.c.

Alternatively, solutions of maltose (20 mL, 20 mg/mL) in 10mM acetate buffer (pH 4.0) were incubated with transglucosidase (1 mL, 10 U/mL on phenyl α-D-glucopyranoside) at 40°. Aliquots (1 mL) were removed at 2, 5, 10, and 30 min and heated for 2 min at ~100° to terminate the reaction. Aliquots (10μL) were then analysed by t.l.c. on Kieselgel 60 (Merck), using 7:1:2 1-propanol–ethanol–water and detection by charring with sulphuric acid.

Amyloglucosidase purification. — Amyloglucosidases I and II recovered from DEAE-Sepharose chromatography (refer above) were treated separately with ammonium sulphate (50 g/100 mL) at 4° for 2 h. The suspensions were centrifuged (12,000*g*, 15 min), and a solution of each pellet in the minimum volume of water

was applied to a column (2.5 × 90 cm) of Sephadex G-100 and eluted with 10mM sodium citrate buffer (pH 3.5). The fractions containing amyloglucosidase were combined and added to a column (2.5 × 10 cm) of S-Sepharose Fast Flow and eluted with a linear gradient (0→0.5M; total vol., 600 mL) of KCl in 10mM citrate buffer (pH 3.5). The fractions containing amyloglucosidase were combined, dialysed against 10mM acetate buffer (pH 4.5), and stored frozen.

Effect of substrate concentration on pattern of reaction products. — Aliquots (0.2 mL) of a solution of maltose (5, 10, 20, or 40 mg/mL) in 10mM acetate buffer (pH 4.0) were incubated with transglucosidase (0.05 mL: 0.0125, 0.025, 0.05, or 0.10 U on phenyl α-D-glucopyranoside). Reactions were terminated after 2, 5, 15, or 60 min by heating for 2 min at 100°. The solutions were diluted to 1 mg/mL and filtered, and 200-μL samples were analysed by h.p.l.c.

Hydrolysis of maltose by pure amyloglucosidase variously contaminated with pure transglucosidase. — Solutions of maltose (1.0 mL, 20%) in 10mM acetate buffer (pH 5.5) were treated with purified amyloglucosidase II (0.5 mL, 5 U) in distilled water plus 0.5 mL of water and incubated at 60°. Reactions were terminated after 1, 2, and 4 h by heating for 5 min at ~100°. In a parallel experiment, the solutions of maltose were incubated with purified amyloglucosidase II (0.5 mL, 5 U) plus aliquots (0.5 mL) of either water or transglucosidase solution (3.5, 17.0, 35.0, or 140 mU on phenyl α-D-glucopyranoside). Reactions were terminated after 4 h by heating for 5 min at ~100°.

Each solution was diluted to 1 mg/mL carbohydrate, filtered (Millipore, 0.45-micron durapore filter), and analysed by h.p.l.c.

Effect of transglucosidase contamination on the conversion of wort sugars into glucose by amyloglucosidase. — Wort was prepared as follows. Malt flour (100 g) was mixed ("mashed-in") with water (300 mL) at 50°, the slurry was stirred for 10 min, the temperature was raised to 65°, and stirring was continued for 60 min. The temperature was raised to 72°, stirring was continued for 10 min, the temperature was raised to 76°, and the malt extract (wort) was recovered by filtration (Whatman 2V filter paper). The wort was boiled for 90 min and Whirlfloc (κ-carrageenan) (20–30 p.p.m.) was added. Boiling was continued for 5 min, the wort was cooled by storage overnight at 4° and filtered, and the specific gravity was adjusted to 1043–1046 ($g/cm^3 \times 10^3$) with water (~200 mL).

Aliquots of the wort (100 mL), containing ~10% of carbohydrate, were incubated for 4 h at 60° with Amylo 300 (1 mL, 100 U), which is essentially devoid of transglucosidase activity, and with purified transglucosidase (1 mL, 0.07–14.0 U). The solutions were then heated for 5 min to 95°. Samples of these worts (1 mL) were diluted 10-fold and analysed by h.p.l.c. The remainder of the solutions were adjusted to 30°, and inoculated with a suspension of yeast (0.1 g/100 mL; 10^6 yeast cells/mL of wort) and incubated for 4 days at 30°. The resulting beer was filtered (Whatman 2V papers) and aliquots were analysed for specific gravity (attenuation values) with an Anton-Parr DMA 55 Density Meter. Aliquots were also diluted 10-fold and analysed by h.p.l.c.

RESULTS AND DISCUSSION

Assay of transglucosidase. — Transglucosidase in crude fungal culture preparations usually occurs in the presence of at least a 100-fold greater concentration of amyloglucosidase activity, and a 10- to 100-fold greater concentration of alpha-amylase. The problems associated with the assay of transglucosidase or amyloglucosidase arise because the enzymes act on a very similar range of substrates, albeit at different relative rates (Tables I and II).

Amyloglucosidases from various sources hydrolyse starch, amylopectin, and malto-oligosaccharides of d.p. ≥4 at 4–5 times the rate for maltose[29]. However, maltose is commonly used to assay amyloglucosidase in fermentation broths because of its resistance to fungal alpha-amylase. If starch is used as substrate, the assay can be made relatively specific for amyloglucosidase by measuring the production of glucose. However, fungal alpha-amylase can also hydrolyse starch to glucose. The maximum rates of hydrolysis of maltose, maltitol, and methyl, phenyl, and *p*-nitrophenyl α-D-glucopyranosides by highly purified amyloglucosidases I and II (devoid of alpha-amylase and transglucosidase) from *A. niger* (Fig. 1) are shown in Table I. Phenyl α-D-glucopyranoside and, in particular, methyl α-D-glucopyranoside are resistant to hydrolysis. *p*-Nitrophenyl α-D-glucopyranoside is hydrolysed at 10-times the rate for phenyl α-D-glucopyranoside.

The resistance of methyl α-D-glucopyranoside to amyloglucosidase makes it a useful substrate for the specific assay of transglucosidase in the presence of amyloglucosidase, even though the rate of cleavage of this substrate by the former enzyme is quite low relative to that of maltose (~1.2% under standard assay conditions). However, methyl α-D-glucopyranoside cannot be used to measure transglucosidase in crude culture filtrates or even partially diafiltered concentrates because high levels of reducing substances (particularly glucose) in these preparations interfere with the assay.

TABLE I

RELATIVE RATES OF HYDROLYSIS OF SUBSTRATES BY AMYLOGLUCOSIDASES I AND II AND TRANSGLUCOSIDASE FROM *A. niger*

Substrate	*Relative rates of hydrolysis*[a]			*TG/AG ratio*
	Amyloglucosidase (AG)		*Transglucosidase (TG)*	
	I	*II*		
Maltose	100,000	100,000	100,000	1
p-Nitrophenyl α-D-glucopyranoside	500	600	2,800	~5
Phenyl α-D-glucopyranoside	50	40	3,400	~76
Methyl α-D-glucopyranoside	1	1	1,300	1,300
Maltitol	300	310	13,000	~43

[a]Standard assay conditions (see Experimental).

TABLE II

PROPERTIES OF PURIFIED *A. niger* TRANSGLUCOSIDASE

Property	A. niger *transglucosidase*
Molecular weight	116,000
pI	5.1
Carbohydrate (%)	27.6
pH Optimum (5 min, 40°)	4.5
pH Stability (22 h, 40°)	4.0–6.0
Temperature optimum	70°
Temperature stability	50°
Phenyl α-D-glucopyranoside	
V_{max} (U/mg at 40°)	2.5
K_m (mM)	2.4

Phenyl α-D-glucopyranoside is a less specific substrate for the assay of transglucosidase than is methyl α-D-glucopyranoside, but it can form the basis of a useful assay for transglucosidase in crude filtrates. It is hydrolysed by transglucosidase at ~3.5% the rate for maltose, whereas it is cleaved by amyloglucosidase at only 0.05% the rate for maltose, *i.e.*, a relative rate-ratio-difference of ~70. The phenol released can be determined with the 4-aminophenazone reagent (see Experimental) with only limited interference from other components in the culture filtrate.

Maltose is hydrolysed by transglucosidase at ~30 times the rate for phenyl α-D-glucopyranoside. Thus, when preparations of amyloglucosidase are treated to remove transglucosidase, the removal of every unit of transglucosidase (on phenyl α-D-glucopyranoside) will remove 30 units of activity on maltose, *i.e.*, an apparent loss of 30 units of amyloglucosidase activity (assayed on maltose), which, of course, is not the case. Also, because pure amyloglucosidase acts on phenyl α-D-glucopyranoside, albeit slowly, treated preparations of amyloglucosidase may appear to contain transglucosidase activity. In fact, culture broths which contain <0.05 unit of activity on phenyl α-D-glucopyranoside per 100 units of activity on maltose are actually devoid of transglucosidase (Table I).

Purification of transglucosidase. — Several chromatography procedures have been employed to purify transglucosidase[2,11,28]. Transglucosidase can also be removed specifically from crude culture filtrates by adsorption onto various clay minerals and metal oxides[7,11]. Clay minerals adsorb effectively transglucosidase and have little effect on other enzymes such as amyloglucosidase and alpha-amylase in the fermentation broth. This point is clearly demonstrated in Fig. 1. Fig. 1a shows the separation of starch-degrading enzymes in crude, untreated *A. niger* culture filtrate on DEAE-Sepharose Fast Flow. The chromatography patterns for the same original volume of a bentonite clay-treated broth are shown in Fig. 1b. The patterns for amyloglucosidase and alpha-amylase are similar, but the bentonite treatment effectively removes the transglucosidase (peak A).

Transglucosidase was purified by chromatography on DEAE-Sepharose Fast

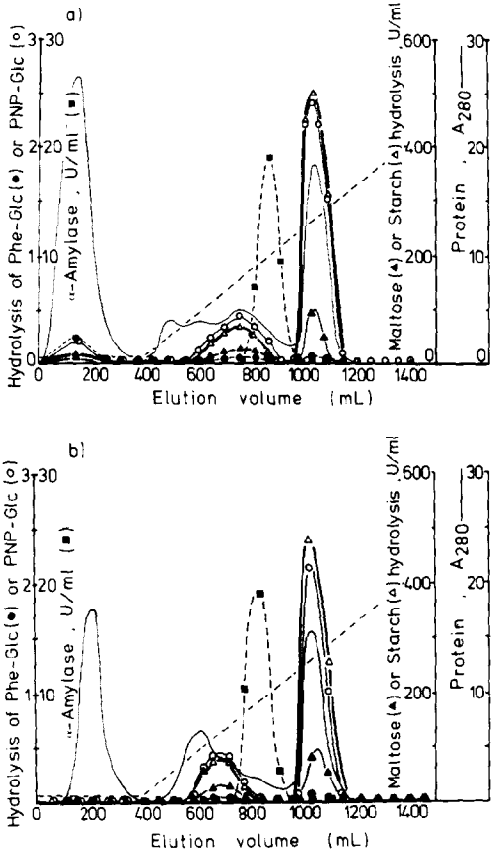

Fig. 1. Chromatography of crude *A. niger* culture filtrates (100 mL) on DEAE-Sepharose Fast Flow (see Experimental). Not treated (a), or treated (b) with bentonite clay before application to the column. TG, transglucosidase; I and II, the two forms of amyloglucosidase.

Flow (at pH 5.5), Sephadex G100, and S-Sepharose Fast Flow (at pH 3.5). The enzyme was obtained in 28% yield from the original culture broth (*i.e.*, 450 mg from 6 L) with an overall purification of 30-fold. It appeared as a single sharp protein band on SDS-gel electrophoresis (mol. wt. 116,000, Fig. 2) and as a major (~80%, pI 5.1) and a minor (~20%, pI 5.0) band on isoelectric focusing (Fig. 3). It is not known if both bands show enzymic activity. Some properties of the purified enzyme are shown in Table II. The enzyme is a glycoprotein and the carbohydrate moiety consists of mannose (79.1%), glucose (3.7%), galactose (9.1%), and unidentified sugars (8.1% of total carbohydrate; Fig. 4). On treatment of protease-degraded transglucosidase with α-D-mannosidase, ~80% of total carbohydrate was released. Transglucosidase shows optimal activity at 70° and is stable up to 50°. At 40°, it shows no loss in activity on storage for 20 h over the pH range 4.0–6.0, and shows maximum activity at pH 4.0.

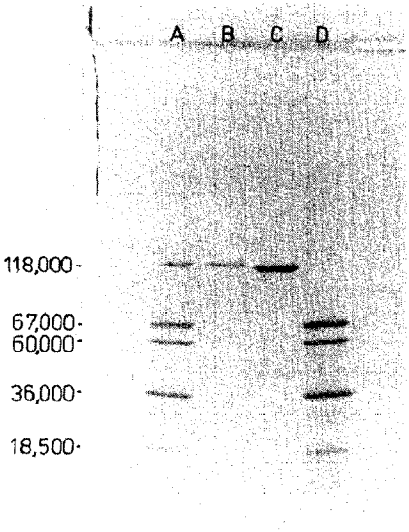

Fig. 2. SDS-gel electrophoresis of purified transglucosidase: A, standards plus *A. niger* β-D-glucosidase; B, *A. niger* β-D-glucosidase; C, transglucosidase; D, high-molecular-weight standards (Pharmacia).

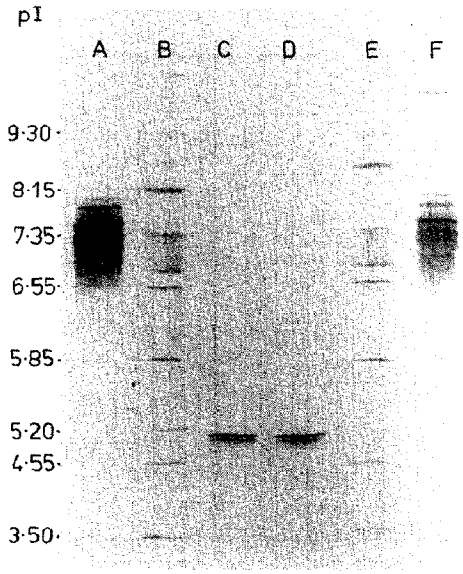

Fig. 3. Isoelectric focusing of purified transglucosidase: A and F, haemoglobin; B and E, isoelectric focusing standards (Pharmacia); C and D, purified transglucosidase. Samples A–C were applied towards the anode electrode side of the gel and D–F towards the cathode electrode side.

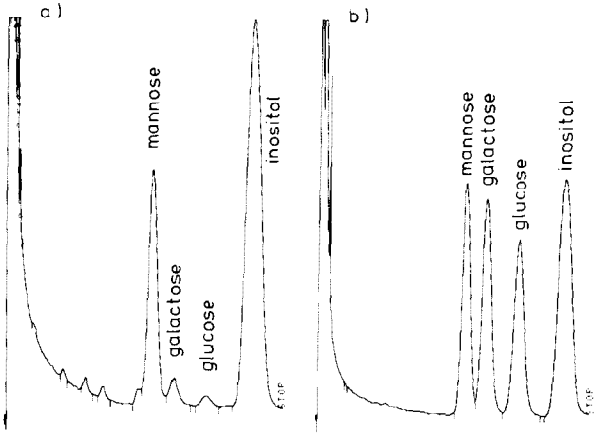

Fig. 4. G.l.c. of the alditol acetate derivatives of the sugars released on acid hydrolysis of transglucosidase: (a) transglucosidase hydrolysate; (b) sugar standards.

These results contrast with those of other workers. Iwano *et al.*[28] purified an *A. niger* transglucosidase to electrophoretic homogeneity, but this enzyme had a mol. wt. of 85,000. The transglucosidase purified by Pazur *et al.*[22] appeared as a rather diffuse band on isoelectric focusing with an apparent isoelectric point of 4.0 (*cf.* 5.1 for the enzyme reported here). Also, that enzyme had a carbohydrate content of 20% (*cf.* 27.6% for the enzyme reported here). In both enzymes, this carbohydrate material was found to consist of mannose, galactose, and glucose, but the relative proportions of each were not reported by Pazur *et al.*[22].

These reported differences indicate that the transglucosidase elaborated by different strains of *A. niger* may have different physicochemical properties. However, from the limited amount published on the substrate specificities and rates of transglucosylation, it appears that the enzymes have similar patterns of action.

Kinetic properties. — The kinetic properties of transglucosidase on natural and synthetic substrates are shown in Table III. The preferred substrates are maltose, maltotriose, and maltotetraose, which are cleaved at similar initial rates. The slightly higher value for maltotriose is because the maltose released on hydrolysis is a substrate for further reaction (releasing 2 mol of glucose per bond cleaved, thus inflating the rate of hydrolysis).

Phenyl, methyl, and *p*-nitrophenyl α-D-glucopyranosides are cleaved at <4% of the rate for maltose, and maltitol is hydrolysed at 13% of the rate for maltose. At saturating substrate concentrations, nigerose is cleaved at a rate similar to that for maltose, and isomaltose is cleaved at about half the rate. However, the K_m values for these saccharides are much greater than that for maltose, such that in a practical situation, as in the treatment of wort (malt-extract), these oligomers tend to persist due to the low rates of hydrolysis at the low concentrations present. Panose is cleaved more rapidly than isomaltose, even though the first bond hydro-

TABLE III

KINETIC PROPERTIES OF *A. niger* TRANSGLUCOSIDASE

Substrate	*α-Linkage Type*	V_{max} *(U/mg)*	K_m *(mM)*
Maltose	(1→4)	73.5	1.7
Maltotriose	(1→4)	88.6	1.5
Maltotetraose	(1→4)	62.8	1.2
Isomaltose	(1→6)	31.9	7.7
Panose	(1→6,4)	53.1	2.0
Nigerose	(1→3)	63.6	20.0
Kojibiose	(1→2)	22.1	3.6
p-Nitrophenyl α-D-glucopyranoside		2.1	0.7
Phenyl α-D-glucopyranoside		2.5	2.4
Methyl α-D-glucopyranoside		1.0	26.3
Maltitol	(1→4)	9.7	
Sucrose	(1→2)	0	

lysed in each substrate is α-(1→6). This situation is probably due to the greater susceptibility of the α-(1→4) linkage in panose and because two molecules of glucose are released when that bond is cleaved.

These results are in general agreement with those obtained in earlier studies by Pazur and Ando[2] and by Iwano *et al.*[3]. However, in those studies, K_m and V_{max} values were not reported.

The results shown in Table III demonstrate that transglucosidase is an α-D-glucosidase. Most glycosidases have some potential for transferring the monosaccharide released from the non-reducing end of the substrate to an acceptor molecule other than water. This phenomenon is usually more pronounced at high substrate concentrations. However, transglucosidase is unique in its ability to transglycosylate even at quite low concentrations of substrate, as shown in Figs. 5 and 6. In the initial stages of cleavage of maltose, the rates of accumulation of trisaccharide (mainly panose) and glucose were similar. However, as the reaction proceeded, panose was hydrolysed and the rate of depletion of disaccharide was reduced significantly. In the initial stages of reaction, the major transfer product was panose, but, as the reaction proceeded, the concentration of glucose increased, and this glucose acted as an alternative acceptor, with the synthesis and transient accumulation of mainly isomaltose (Fig. 7). Isomaltose is cleaved by transglucosidase, but only at a low rate, and this rate is dependent on the concentration of the isomaltose.

Transglucosidase also catalyses transglycosylation reactions in the cleavage of glucosaccharides other than maltose (Table IV). Appreciable quantities of oligosaccharides with d.p. one higher than that of the substrate were formed, but significantly lesser quantities of larger oligosaccharides were found (Figs. 5 and 7).

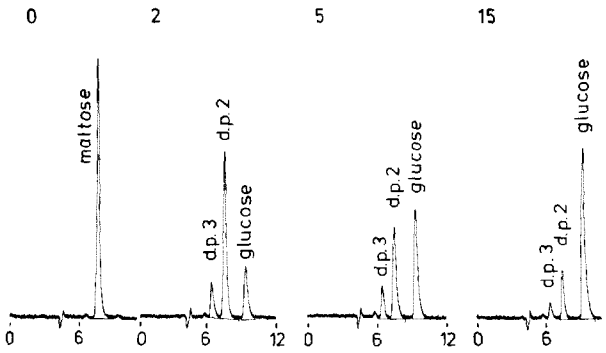

Fig. 5. H.p.l.c. of saccharides produced on incubation of maltose with transglucosidase. Aliquots (0.2 mL) of maltose solution (12.5 mg/mL) in 10mM acetate buffer (pH 4.0) were incubated with transglucosidase (50 μL, 25 mU) at 40°, and the reaction was terminated after 0, 2, 5, and 15 min by heating for 1 min at ~100°.

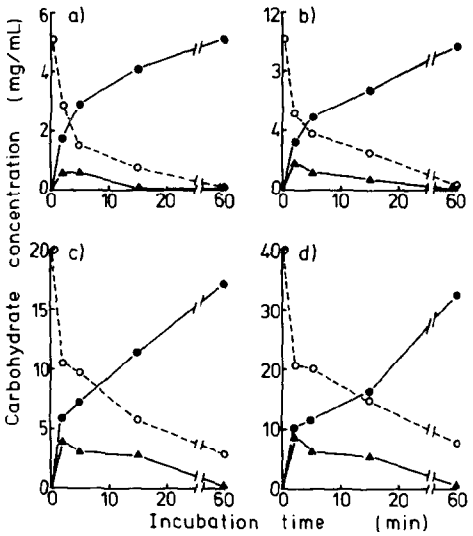

Fig. 6. Effect of the concentration of maltose on the pattern of saccharides produced on incubation with transglucosidase; initial concentrations of 5 (a), 10 (b), 20 (c), and 40 (d) mg/mL (see Experimental): ●, monosaccharide; ○, disaccharide; ▲, trisaccharide.

Effect of transglucosidase on hydrolysis of maltose and wort-saccharides by amyloglucosidase. — Treatment of aqueous 10% maltose (2 mL) with five units of pure *A. niger* amyloglucosidase II (assayed at pH 4.3) at pH 5.5 and 60° caused complete hydrolysis to glucose within 4 h (Fig. 8a–c). These conditions simulate those employed in the treatment of wort (containing ~10% of carbohydrate) with amyloglucosidase, except that, in the current experiment, twice as much amyloglucosidase was required for complete hydrolysis. This is probably because amylo-

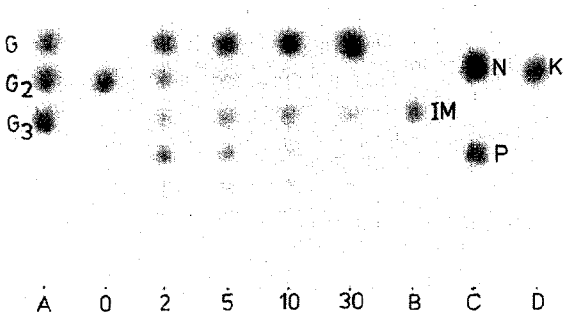

Fig. 7. T.l.c. of the saccharides produced on incubation of maltose (20 mL, 20 mg/mL) in 10mM acetate buffer (pH 4.0) with transglucosidase (1 mL, 10 U on phenyl α-D-glucopyranoside). Aliquots (2 mL) were removed at 2, 5, 10, and 30 min and heated at ~100° to terminate the reaction. Aliquots (10 μL) were then subjected to t.l.c. (see Experimental): A, glucose, maltose, and maltotriose; B, isomaltose; C, nigerose and panose; D, kojibiose.

TABLE IV

PRODUCTS FORMED ON INCUBATION OF GLUCO-OLIGOSACCHARIDES WITH TRANSGLUCOSIDASE[a]

Saccharide	*Time of incubation (min)*	*Concentration of reaction products (mg/mL)*				
		Glucose	*2*[b]	*3*	*4*	*5*
Maltose	0	0	8.224	0	0	
	5	2.183	5.509	1.193		
	21	4.283	3.314	1.049	tr	
	60	6.572	1.024	0.300	tr	
Maltotriose	0	0	0	8.403	0	
	5	1.807	1.814	4.309	0.155	
	21	3.546	2.323	2.831	0.300	
	60	6.288	2.271	0.948	0	
Maltotetraose	0	0	0	0	9.669	0
	5	1.210	0.610	1.341	5.689	1.100
	21	3.329	1.949	1.983	2.826	0.900
	60	5.645	2.074	0.871	0.400	0.200
Isomaltose	0	0	7.600	0	0	0
	5	2.121	5.346	0.890		
	21	3.563	4.094	0.400		
	60	5.902	1.943	0.200		
Panose	0	0	0	9.130	0	
	5	1.404	1.073	6.669	0.300	
	21	3.504	1.798	4.150	0.300	
	60	5.996	1.954	1.218	0.300	

[a]α-D-Gluco-oligosaccharide (0.2 mL, 10 mg/mL) in 10mM sodium acetate buffer (pH 4.0) was incubated with transglucosidase (0.05 mL, 25 mU on phenyl α-D-glucopyranoside) and the reaction was terminated after 5, 21, or 60 min by heating for 2 min at ~100°. Solutions were diluted 10-fold with distilled water and analysed by h.p.l.c. [b]D.p. of products.

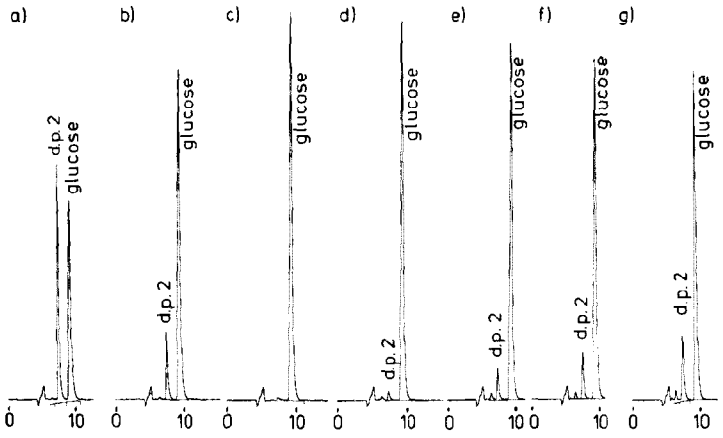

Fig. 8. Effect of transglucosidase (TG) on the conversion of maltose into glucose by amyloglucosidase (AG). Maltose (2 mL, 10%) in 10mM acetate buffer (pH 5.5) was incubated with AG (5 U) at 60°: (a) 1 h, (b) 2 h, (c) 4 h. Incubation with AG for 4 h plus TG (d) 3, (e) 20, (f) 40, and (g) 100 mU.

glucosidase II was employed, and this is the less effective of the two *A. niger* amyloglucosidases[29]. Fig. 8 (d–g) shows that contamination of the amyloglucosidase by transglucosidase reduces the recovery of glucose. Other saccharides of d.p. 2 and 3 accumulate, the amounts of which are directly proportional to the degree of contamination with transglucosidase. These saccharides are mainly isomaltose and panose.

A similar phenomenon occurs in the treatment of wort with amyloglucosidase to produce highly fermentable worts and thus low-carbohydrate beers. The effect of contamination of amyloglucosidase with transglucosidase on the oligosaccharide

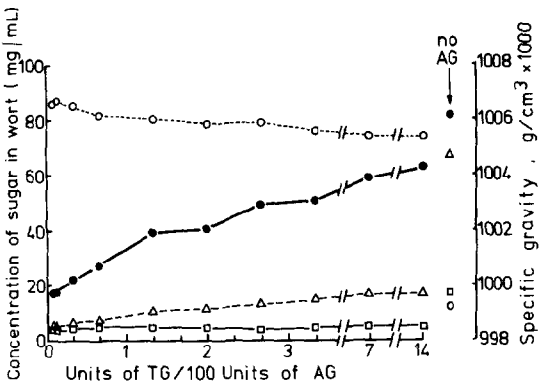

Fig. 9. Effect of transglucosidase on the conversion of wort sugars into glucose by a commercial preparation of amyloglucosidase and on the specific gravities of the beer produced following fermentation with yeast. (See Experimental): ●, specific gravity; ○, glucose; △, disaccharide; □, trisaccharide.

composition of wort and the specific gravity (attenuation value) for the final beer is shown in Fig. 9. Worts which were not treated with amyloglucosidase yielded beers with high specific gravities. This is due to the presence in the wort of α-limit dextrins of starch, which cannot be absorbed and fermented by yeast. Treatment with amyloglucosidase hydrolyses these saccharides and maltose to glucose. Thus, amyloglucosidase-treated worts contain negligible amounts of starch saccharides and, as a consequence, produce beers with low specific gravities. Contamination of the preparation of amyloglucosidase with transglucosidase leads to an accumulation of disaccharide (mainly isomaltose) in the wort which results in reduced fermentation and elevated specific gravity. Beers produced from such worts contain significant levels of non-fermented isomaltose.

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