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Purification and Some Properties of an Extracellular Maltase from *Bacillus subtilis*[1]

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Bacillus subtilis P-11, capable of producing extracellular maltase, was isolated from soil. Maximum enzyme production was obtained on a medium containing 2.0% methyl-α-D-glucoside, 0.5% phytone, and 0.2% yeast extract. After the removal of cells, extracellular maltase was precipitated by ammonium sulfate (85% saturation). The enzyme was purified by using the following procedures: Sephadex G-200 column chromatography, diethylaminoethyl-Sephadex A-50 ion-exchange column chromatography, and a second Sephadex G-200 column chromatography. A highly purified maltase without amylase or proteinase activities was obtained. Some properties of the extracellular maltase were determined: optimum pH, 6.0; optimum temperature, 45 C, when the incubation time was 30 min; pH stability, within 5.5 to 6.5; heat stability, stable up to 45 C; isoelectric point, pH 6.0 (by gel-isoelectric focusing); molecular weight, 33,000 (by gel filtration with Sephadex G-200); substrate specificity: the relative rates of hydrolysis of maltose, maltotriose, isomaltose, and maltotetraose were 100:15:14:4, respectively, and there was no activity toward alkyl or aryl-α-D-glucosides, amylose, or other higher polymers. Transglucosylase activity was present. Glucose and tris(hydroxymethyl)aminomethane were competitive inhibitors, with K_i values of 4.54 and 75.08 mM, respectively; cysteine was a noncompetitive inhibitor. Michaelis constants were 5 mM for maltose, 1 mM for maltotriose, and 10 mM for isomaltose. A plot of pK_m ($-\log K_m$) versus pH revealed two deflection points, one each at 5.5 and 6.5; these probably corresponded to an imidazole group of a histidine residue in or near the active center; this assumption was supported by the strong inhibition of enzyme activity by rose bengal.

The existence of maltases was first demonstrated in 1880. Developments since then have been reviewed by Gottschalk (7). Maltases extracted from mammalian tissues, yeasts, and molds have received the most attention, and a summary of their properties has been prepared (L.-H. Wang, Ph.D. thesis, Iowa State Univ., Ames, 1975).

Although a number of studies have been published on bacterial maltases (2, 5, 9, 17, 22), only two bacterial maltases have been discovered; both were clostridial enzymes (9, 17). Only one bacterial extracellular maltase, also clostridial, has been partly characterized (6).

Because of the potential usefulness of maltases in basic research, clinical laboratories, and industry, we searched for extracellular maltase-producing bacteria. Of seven positive isolates from soil, strain P-11 produced the most maltase; this isolate was identified as a strain of *Bacillus subtilis*. The purpose of this report is to present data on the purification and some properties of the new maltase.

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MATERIALS AND METHODS

Extracellular maltase activity was determined by adding 1 ml of enzyme preparation to 9 ml of 3.3 mM maltose solution in 0.067 M phosphate buffer, pH 6.5. After incubation at 40 C for 30 min, the enzyme was inactivated by immersing the tube containing the reaction mixture in boiling water for 5 min. The amount of glucose produced in the reaction mixture was determined by the use of PGO reagent (Sigma Tech. Bull. 510, 1969, Sigma Chemical Co., St. Louis, Mo.). PGO reagent is a mixture of peroxidase and glucose oxidase; it contains *o*-dianisidine as a chromogen. One unit of maltase activity was defined as the amount of enzyme that produced 1 μmol of glucose from maltose under the conditions used; appropriate blanks were run to account for glucose present in the maltose and maltase preparations.

Amylase activity was determined by a blue-value method, which measures the disappearance of iodine-starch blue color due to amylase action (16).

The semiquantitative analysis of proteinase activ-

ity was determined by a disk-plate method. Clear zones due to proteinase action were formed around paper disks on milk agar plates (11).

The qualitative assay of transglucosylase activity was conducted by a paper chromatographic method (22). Chromatograms were developed according to the procedure of Robyt and French (14).

Protein concentrations during most stages of enzyme purification were determined by the Folin-phenol method (12). Bovine serum albumin was used as the standard. The protein concentration of each fraction during column chromatography was estimated by observing the absorbance at 280 nm.

RESULTS

Enzyme production. Maltase preparations normally were produced by shaking strain P-11 at 180 rpm in Fernbach flasks containing 1.0 liter of medium. A medium composed of 2.0% methyl-α-D-glucoside, 0.5% phytone, and 0.2% yeast extract was used, and the fermentation beer was collected by removing the cells by centrifugation after incubation at 37 C. Other details of media development and enzyme production can be found elsewhere (Wang, Ph.D. thesis). Typical growth, pH, and maltase production data are shown in Fig. 1. Both viable counts and absorbance values are plotted in Fig. 1, because there was not a close correlation between these two growth parameters. Cultures were harvested after about 18 h of incubation, when maltase activity had reached maximum levels (Fig. 1). Under the cultural conditions used, almost all the maltase activity produced was extracellular (data not shown).

Enzyme purification. Procedures developed for purification of the extracellular maltase are shown in Fig. 2. More than 50% of the activity was lost when the enzyme was concentrated by acetone or ethanol precipitation at −15 C. More than 50% of the activity also was lost when enzyme concentration was affected by an XM-50 ultrafiltration membrane (Amicon Corp., Lexington, Mass.); very slow release of pressure to prevent foaming after concentration failed to reduce inactivaton. Ammonium sulfate precipitation, therefore, was the best method to obtain maltase concentrates. Most maltase activity was precipitated at between 50 and 85% saturation with ammonium sulfate (Fig. 2); the precipitate was dissolved in a small amount of phosphate buffer (25 mM, pH 6.5).

Sephadex G-200 was used to further purify the maltase (Fig. 3). Maltase was not retained by the column and appeared in the early fractions, associated with the 170-ml void volume. Proteinase activity could be detected in fractions 35 to 70 (data not shown); therefore, the maltase was still contaminated with protein-

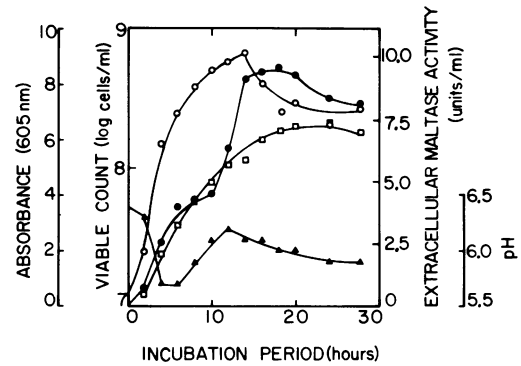

FIG. 1. *Relationship between extracellular maltase activity* (●), *viable cell counts* (○), *absorbance* (□), *and pH* (△) *during cultivation of B. subtilis P-11, as described in the text.*

ase(s) and probably other proteins. Amylase activity was well separated from the maltase, appearing in fractions 78 to 100.

Diethylaminoethyl (DEAE)-Sephadex A-50 column chromatography was used for the next step of the maltase purification process (Fig. 2). Proteinase activity was not adsorbed at pH 6.5 and was eluted before the NaCl gradient was applied (data not shown). The maltase activity was eluted at an NaCl concentration of approximately 0.10 to 0.15 M (Fig. 4). The maltase was now devoid of detectable amylase and proteinase activities; the identity of the second peak shown in Fig. 4 is not known (see Discussion).

Fractions 45 to 65, obtained from the DEAE-Sephadex column (Fig. 4), were collected, concentrated by precipitation with ammonium sulfate (Fig. 2), and passed through a Sephadex G-200 column (2.5 by 40 cm). There was a single maltase-containing peak, as shown in Fig. 5; only the significant fractions collected are shown in the figure. The maltase-containing peak was shifted from the void volume (Fig. 5) to near the position originally occupied by amylase in the first Sephadex G-200 fractionation (Fig. 3). This phenomenon will be discussed later.

Table 1 shows the specific activities and other parameters at each stage of purification. The final preparation was about 900 times more pure than that of the original fermentation beer; the yield, however, was less than 20%.

Enzyme characterization. The pH optimum for activity of the purified enzyme was 6.0 (Fig. 6); this was 0.5 unit lower than that of the crude preparation (data not shown). The temperature optimum for enzyme activity (Fig. 7) was about 45 C when the incubation time was 30 min. At temperatures above 45 C, the enzyme was inactivated rapidly.

Cell-free filtrate ..... (I)

add solid $(NH_4)_2SO_4$ to 50% saturation;
centrifuge (10,000 rpm, 30 min, with GSA rotor)

Precipitate (discard)

Supernatant ..... (II)

add solid $(NH_4)_2SO_4$ to 85% saturation;
centrifuge (15,000 rpm, continuous, with SS-34 rotor)

Supernatant (discard)

Precipitate

dissolve in 0.067 M phosphate buffer, pH 6.5;
centrifuge (15,000 rpm, 30 min, with SS-34 rotor)

Precipitate (discard)

Supernatant ..... (III)

Sephadex G-200 column chromatography (Pharmacia)

Maltase fractions ..... (IV)

DEAE-Sephadex A-50 ion-exchange chromatography (Pharmacia)

Maltase fractions ..... (V)

concentration by $(NH_4)_2SO_4$ precipitation (85% saturation) and redissolve in a small amount of 0.067 M phosphate buffer, pH 6.5;

Sephadex G-200 column chromatography

Pure maltase ..... (VI)

FIG. 2. *Procedures developed for purification of B. subtilis P-11 maltase. A Sorvall RC-2B superspeed centrifuge (10 C) was used in (I) and (II).*

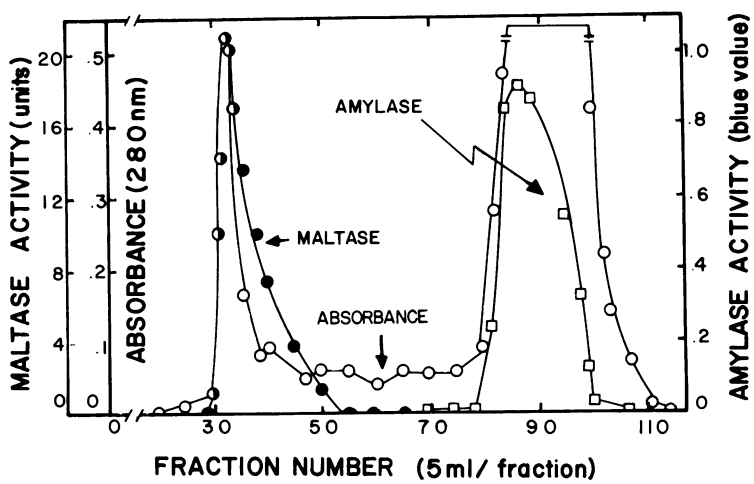

FIG. 3. *Elution pattern of the initial Sephadex G-200 chromatograph of B. subtilis P-11 maltase. Sephadex G-200 was hydrated, packed in a 2.5- by 90-cm column, and equilibrated with 25 mM phosphate buffer, pH 6.5. For each chromatographic run, 10 ml of the concentrated crude preparation (Fig. 2), containing about 90 mg of protein, was applied to the column. Elution was carried out at a flow rate of 10 to 15 ml/h with the same buffer used for equilibration, and 5-ml fractions were collected.*

To determine the pH stability, aliquots of the enzyme preparation were held at 45 C for 90 min in 0.15 M buffers of various pH values; then the pH was adjusted to 6.0, and residual activities were determined (Fig. 8). The purified maltase was relatively stable at pH values of 5.5 to 6.5. Only 10% of the enzyme activity was lost after incubation for 90 min at 45 C and

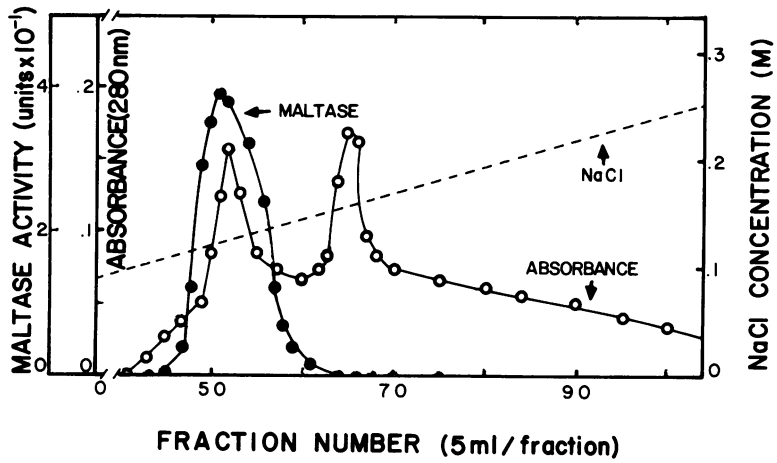

FIG. 4. *Elution pattern of B. subtilis P-11 maltase from a DEAE-Sephadex A-50 ion-exchange column. DEAE-Sephadex A-50 was hydrated, packed in a 2.5- by 35-cm column, and equilibrated with 25 mM phosphate buffer, pH 6.5. The maltase-containing fractions (fractions 30 to 50), which had been collected from the Sephadex G-200 column (Fig. 3), were applied to the DEAE-Sephadex column. The elution flow rate was 15 to 20 ml/h, and 5-ml fractions were collected.*

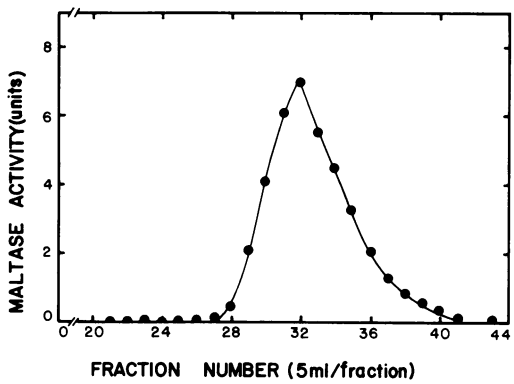

FIG. 5. *Elution pattern of the second Sephadex G-200 column chromatograph of B. subtilis P-11 maltase. The flow rate was 10 to 15 ml/h, fraction volume was 5 ml, and void volume was 75 ml.*

pH 6.0, but the enzyme was unstable at pH values of 7.0 and above.

About 20% of the activity of the purified enzyme was lost when the enzyme was held at 45 C for 2 h in 0.15 M phosphate buffer, pH 6.0 (Fig. 9). At 50 C, almost half the activity was lost after 2 h of incubation. Since more than 90% of the activity in the crude preparation was lost at 50 C (data not shown), the purified maltase was more stable than the crude preparation. Inactivation of both crude and purified enzyme preparations was rapid at 55 C (Fig. 9).

The molecular weight of the maltase, estimated by using Sephadex G-200 gel filtration (1), was approximately 33,000 (Fig. 10). The isoelectric point of the enzyme, determined by using a gel-isoelectric focusing technique, was approximately pH 6.0 (data not shown). The absorption spectrum of the extracellular maltase was taken by using a Beckman model DB spectrophotometer with a recorder; the enzyme exhibited maximum absorption at 275 nm, and the ratio of absorption at 280/260 was 1.39 (data not shown).

Table 2 shows the substrate specificity of the maltase. The rate of hydrolysis decreased as the degree of polymerization of the substrate increased; maltotriose was attacked at about one-fifth the rate of maltose, and maltotetraose at one-fourth the rate of maltotriose. The enzyme could not hydrolyze large polysaccharides, such as amylose, amylopectin, and glycogen, bearing α-1,4-glucosidic bonds. There was a trace of activity towards soluble starch (Table 2); this probably was because the starch was contaminated with small oligosaccharides. The enzyme could not hydrolyze aryl or alkyl glucosides. The enzyme possessed α-1,6-glucosidase activity because isomaltose was hydrolyzed.

The effects of certain organic compounds on maltase activity were measured (Table 3). The compounds were preincubated with enzyme for 10 min at room temperature before residual activities were determined. Rose bengal was a strong inhibitor, and diphenylamine and 4-chloromercuribenzoic acid were weak inhibitors. Cysteine inhibited the enzyme activity noncompetitively. Tris(hydroxymethyl)aminomethane and glucose were competitive inhibitors. Mannose and galactose, which are glucose epimers with respect to carbon atoms 2 and 4, respec-

TABLE 1. *Parameters observed during the purification of the extracellular maltase of B. subtilis P-11*

Purification step (see Fig. 2)	Vol (ml)	Maltase activity (units/ml)	Total units ($\times 10^3$)	Protein (mg/ml)	Sp act (units/mg)	Yield (%)	Purification
I	11,500	3.8	43.7	2.1	1.8	100.0	
II	13,000	3.3	42.9	1.8	1.8	98.2	1.0
III	131	233.3	30.6	9.0	25.9	69.9	14.4
IV	710	25.1	17.8	0.3	83.7	40.8	46.5
V	110	76.4	8.4	0.1	764.0	19.2	424.4
VI	103	80.6	8.3	0.05	1,612.0	19.0	895.6

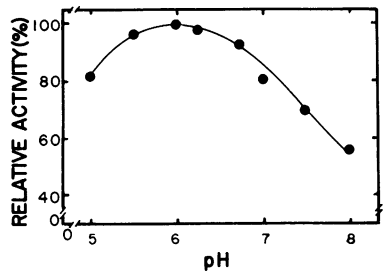

FIG. 6. *pH activity profile of purified B. subtilis P-11 maltase. The enzyme activity at pH 6.0 was taken as 100%.*

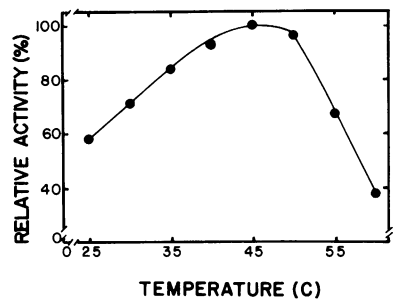

FIG. 7. *Temperature activity profile of purified B. subtilis P-11 maltase. The enzyme activity at 45 C was taken as 100%.*

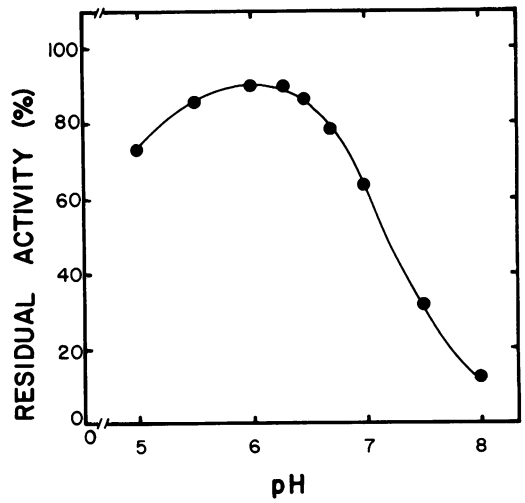

FIG. 8. *pH stability of purified B. subtilis P-11 maltase. Experimental conditions are given in the text.*

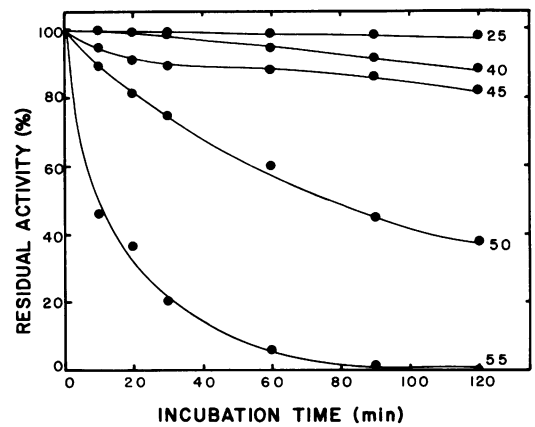

FIG. 9. *Temperature stability of purified B. subtilis P-11 maltase. Experimental conditions are given in the text.*

tively, did not inhibit the maltase. Xylose, structurally similar to glucose except for carbon atom 5, also did not inhibit the enzyme. Turanose, an inhibitor of the α-glucosidases from *Bacillus cereus* (22), did not inhibit the *B. subtilis* maltase. The chelating agent, ethylenediaminetetraacetic acid, had no effect on maltase activity; this indicated that either the enzyme did not require a metal ion for activity or the metal was tightly bound. Polyols and certain other selected compounds (Table 3) also were without effect.

The *B. subtilis* maltase possessed transglucosylase activity. To determine this, 9 ml of substrate (50 mg) and 1 ml of enzyme solution were incubated at 45 C. At various time intervals, 25-μl samples of the hydrolysates were applied to Whatman no. 3 filter paper; three ascents were made (22), and then the chromatogram was developed (14). Hydrolysates of maltose and isomaltose are shown in Fig. 11a and b,

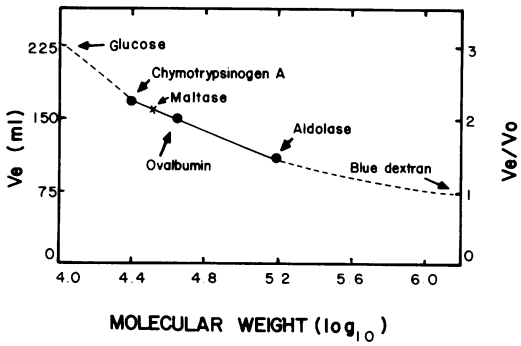

FIG. 10. *Estimated molecular weight of purified B. subtilis P-11 maltase. V_e, Elution volume (milliliters); V_o, void volume (measured with blue dextran 2000). Experimental conditions were the same as for Fig. 5.*

TABLE 2. *Substrate specificity of the extracellular maltase from B. subtilis P-11*

Substrate[a]	Relative rate of hydrolysis (%)
Maltose	100
Maltotriose	16
Isomaltose	15
Maltotetraose	4
Soluble starch[b]	4
Others[c]	0

[a] Concentration: 3 mM; see footnote *b*.

[b] Concentration: 1 mg/ml.

[c] Others: Amylopectin (1 mg/ml), amylose (1 mg/ml), cellobiose, glycogen (rabbit liver) (1 mg/ml), lactose, maltitol, melezitose, melibiose, methyl-α-D-galactoside, methyl-α-D-glucoside, methyl-α-D-mannoside, *p*-nitrophenyl-glucoside, phenyl-α-D-glucoside, raffinose, salicin, sucrose, trehalose, and turanose. Concentrations were 3 mM unless otherwise specified. The amylose was prepared according to Robyt and French (15).

TABLE 3. *Effects of various organic compounds on the extracellular maltase of B. subtilis P-11*

Effector[a]	Concn (mM)	Relative residual activity (%)
δ-Gluconolactone	0.5	100
	1.0	100
	10.0	86
4-Chloromercuribenzoic acid	0.5	100
	1.0	95
Diphenylamine	0.5	92
	1.0	89
Cysteine	1.0	99
	10.0	32
Tris	5.0	94
	25.0	76
Glucose	0.5	60
	1.0	52
Rose bengal	0.5 (mg/ml)	0
	1.0 (mg/ml)	0
None		100
Others[b]		100

[a] The effector was preincubated with enzyme for 10 min at room temperature before enzyme assays were performed. Tris, Tris(hydroxymethyl)aminomethane.

[b] Others: Galactose, mannose, ribose, and xylose, each at 1 and 5 mM concentrations; erythritol, fructose, glucosamine-hydrochloride, inositol, maltitol, phenyl-α-D-glucoside, sorbitol, sucrose, and turanose, each at 0.5 and 1 mM concentrations; ethylenediaminetetraacetic acid, histidine, and methionine, each at 5 and 25 mM concentrations.

respectively. Maltose (Fig. 11a) was hydrolyzed to produce glucose rapidly. Transglucosylation products (trisaccharides, tetrasaccharides, etc.) also were produced, and their concentrations increased as incubation time increased. This transglucosylase activity may limit usefulness of the maltase in applications for which maximum yields of glucose are desired. When isomaltose (Fig. 11b) and maltotetraose (data not shown) were substrates, the quantities of glucose and transglucosylation products increased as the incubation time was extended; low levels of maltose and maltotriose were additional products of maltotetraose hydrolysis. Maltotriose (data not shown) was transglucosylated to form maltotetraose, maltopentaose, and higher oligosaccharides.

The rates of hydrolysis of maltose, maltotriose, maltotetraose, and isomaltose by the extracellular maltase were determined. Reaction mixtures containing 1 ml of enzyme and 9 ml of 3.3 mM substrate were incubated at 45 C. Toluene was used as a preservative, and the tubes were covered to retard evaporation. The percentages of hydrolysis of the substrates (quantities of glucose produced) were measured at various time intervals (Fig. 12). After 24 h of incubation, half of the initial quantity of maltose, and 22, 13, and 11% of the initial quantities of maltotriose, maltotetraose, and isomaltose, respectively, were hydrolyzed.

Lineweaver-Burk plots of maltase action (Fig. 13) were used to calculate K_m values of 5 mM for maltose, 1 mM for maltotriose, and 10 mM for isomaltose. In addition, inhibitor constants (K_i values; see reference 5) for tris(hydroxymethyl)aminomethane and glucose were 75.08 and 4.38, respectively. Cysteine was

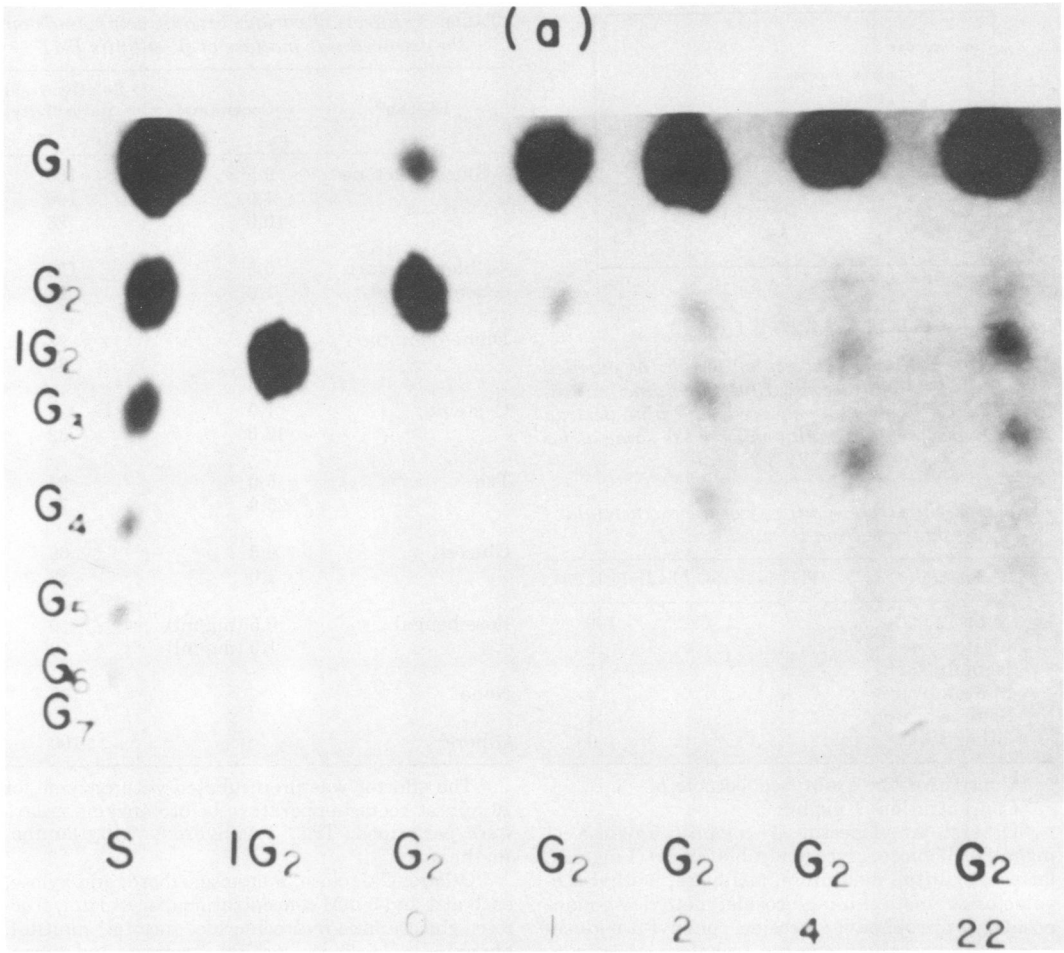

FIG. 11. *Chromatographic analyses of purified B. subtilis P-11 maltase action on maltose (a) and isomaltose (b). G_x, Oligosaccharides with x glucose units; IG_2, isomaltose; S, standard reference mixture, kindly supplied by John Robyt; 0, O-h sample; other numbers refer to sampling times.*

a noncompetitive inhibitor (Fig. 13). Figure 14 shows the effects of pH on K_m, V_{max}, and V_0 of the maltase when maltose was used as the substrate. Within the pH range of 5.5 to 6.5, the K_m and V_{max} values, respectively, were almost the same. Outside this range, the K_m values increased, whereas the V_{max} values decreased.

DISCUSSION

Some anomalous properties of the maltase were observed during preliminary experiments with Sephadex G-200 and DEAE-Sephadex A-50 columns. The procedure outlined in Fig. 2 resulted in complete separation of maltase and amylase activities. In this procedure, the enzyme preparation was passed through a Sephadex G-200 column before it was passed through a DEAE-Sephadex column. Passage through the DEAE-Sephadex column altered the size, shape, or another property of the molecule containing maltase activity so that passage was retarded in the Sephadex G-200 column (cf. Fig. 3 and 5). If the crude enzyme (ammonium sulfate precipitate of cell-free culture fluid) was passed through a DEAE-Sephadex column before passage through a Sephadex G-200 column, the maltase and amylase peaks overlapped, and separation of the two enzymes was not possible. These results indicate that the molecular weight of the maltase in the initial, cell-free culture fluid may be 200,000 or greater or that the maltase was associated with some other material. This association or complex could have been disrupted during DEAE-Sephadex chromatography. The second peak shown in Fig. 4 could be this inert "fragment" or "car-

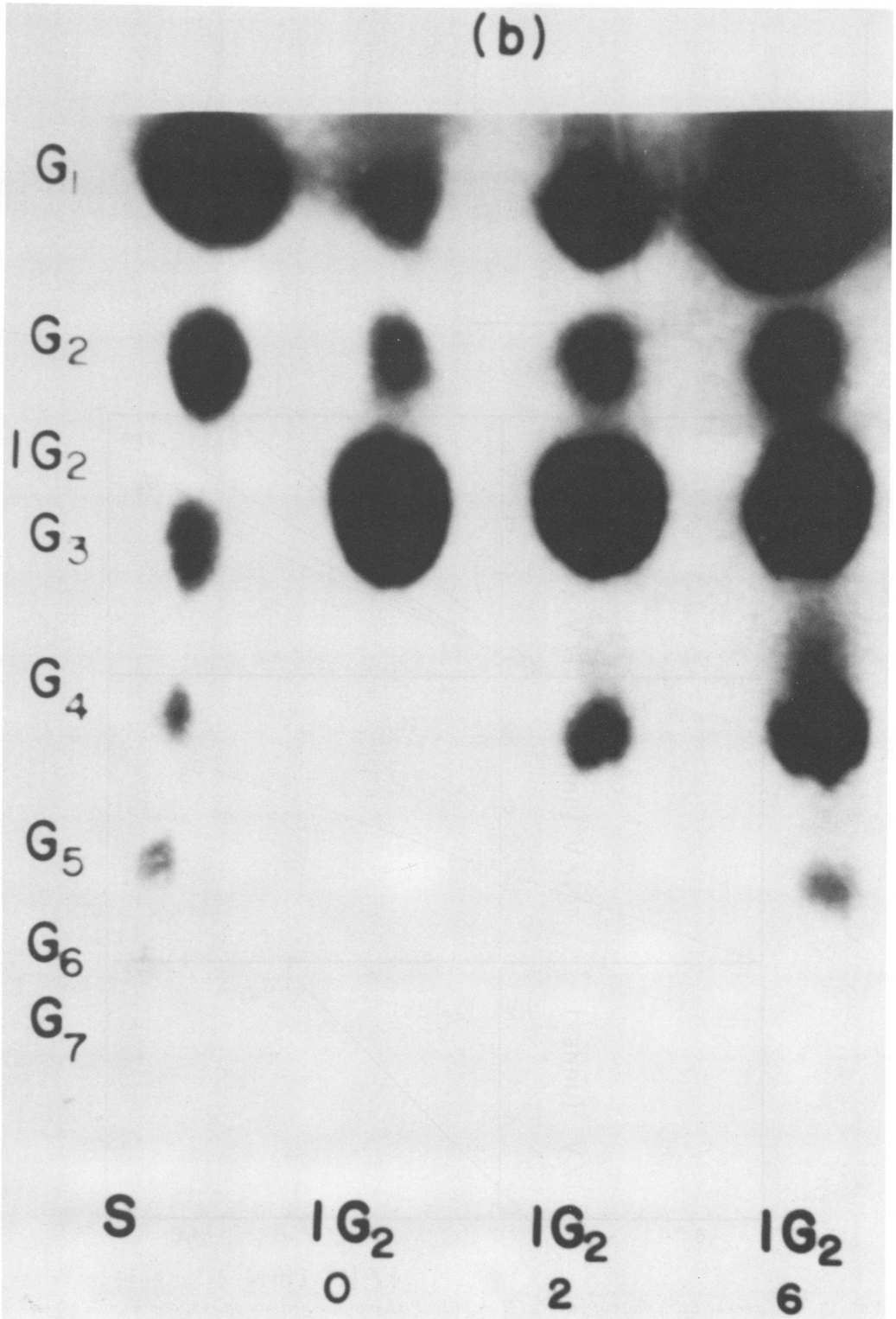

FIG. 11b

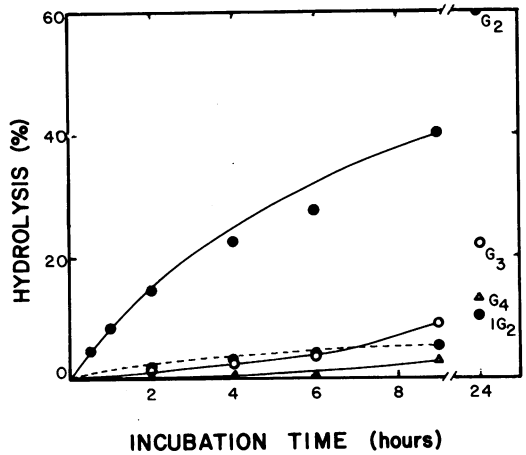

FIG. 12. *Comparison of the rates of hydrolysis of maltose (G_2), maltotriose (G_3), maltotetraose (G_4), and isomaltose (IG_2) by purified B. subtilis P-11 maltase.*

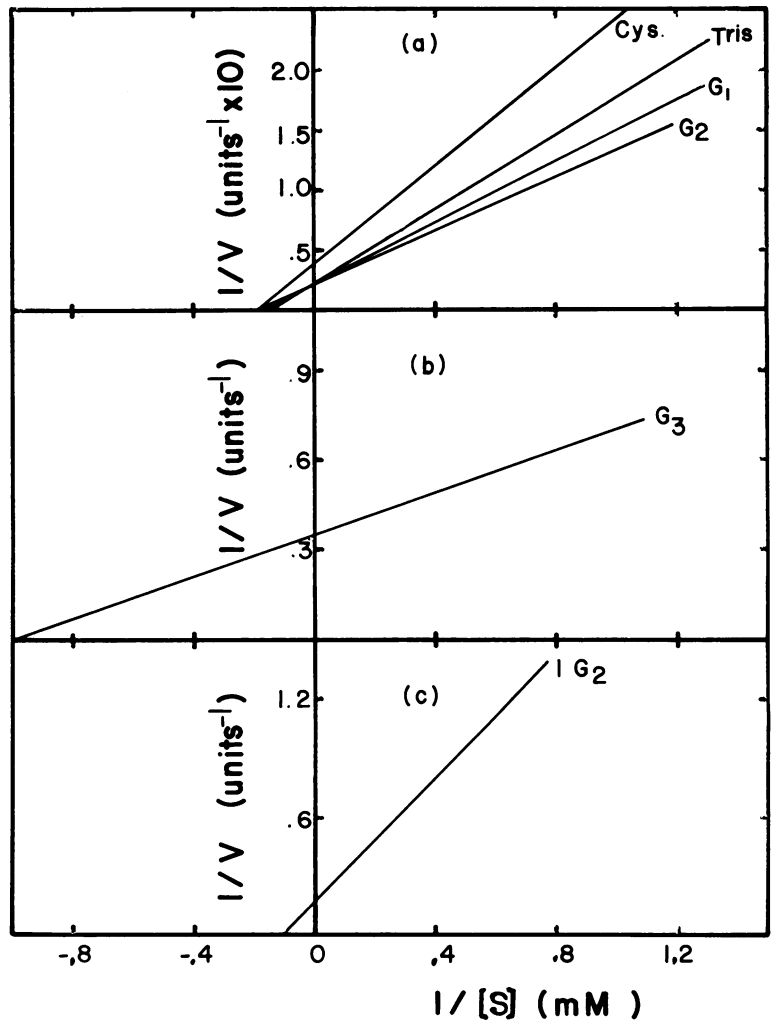

FIG. 13. *Lineweaver-Burk plots of purified B. subtilis P-11 maltase activity on maltose (a), maltotriose (b), and isomaltose (c). Incubation was for 30 min at 45 C in 0.067 M phosphate buffer, pH 6.0. (a) Cys, Cysteine, Tris, tris(hydroxymethyl)aminomethane buffer; G_1, glucose; G_2, maltose.*

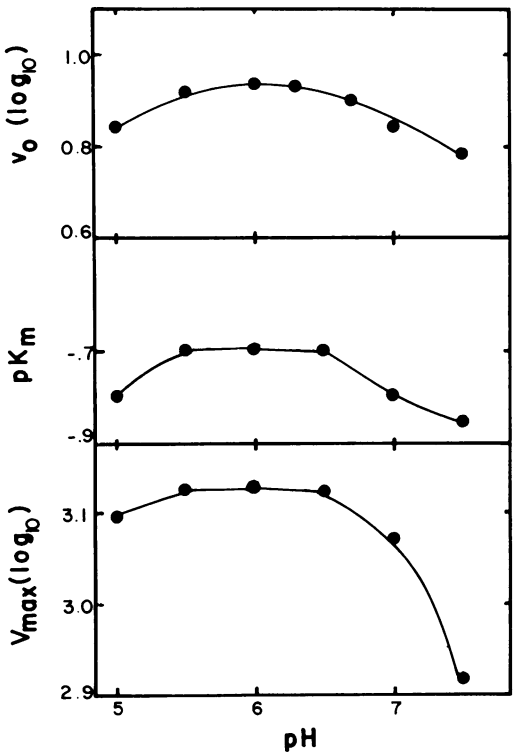

FIG. 14. *Effects of pH on K_m, V_{max}, and V_o of purified B. subtilis P-11 maltase. Maltose (3.3 mM) was the substrate.*

rier." The shift in position of the maltase peak during serial column chromatography seems not to have been caused by a change from tetramer to dimer or monomer because Robyt and Ackerman (13) discovered, in their studies on multiple forms of *B. subtilis* α-amylase, that the specific activity of the tetramer was greater than that of the trimer, which was more than that of the dimer.

The specific activity of the *B. subtilis* P-11 maltase was increased about nine times by DEAE-Sephadex ion-exchange chromatography (Table 1). Therefore, by taking advantage of association of the maltase to another substance during an initial passage through a Sephadex G-200 column, dissociation of enzyme from the inert substance by ion-exchange chromatography, and a second passage through Sephadex G-200 (Fig. 5), the extracellular maltase could be freed of contaminating amylase and proteinase activities.

A glycosidase has at least two types of functional groupings, a binding site and a catalytic site (10). The binding site determines the carbohydrates that will be hydrolyzed, the specificity being determined by the glucon in the substrate molecule. The catalytic site of the enzyme determines the nature of the bond that will be hydrolyzed, which, for a glycosidase, is determined by the configuration of the bond binding the glycon to the aglycon. A strong competitive inhibitor of glycosidase activity, therefore, should fit both the specific and catalytic sites, preventing the hydrolysis of any one α- or β-glycoside. Inasmuch as glucose is a competitive inhibitor of maltase activity, with a K_i value of 4.38 mM (Fig. 12a), but ribose and the glucose epimers mannose and galactose are not inhibitors of the maltase, carbon atoms 2, 3, and 4 of the glucose molecule must be necessary for the substrate-enzyme complex. The pentose, xylose, whose structure is similar to glucose except for the absence of the primary alcohol group, is not an inhibitor. Hence, carbon atom 6 is required also for maltase action. When the aldehyde group of the glucon moiety was changed, either to an alcohol (as in sorbitol) or to a lactone group (as in δ-gluconolactone), the inhibitory properties of glucose were largely lost. The entire glucose molecule, therefore, seems to be required to fit specific binding sites. An intact aglucon moiety also is required for this binding; this is the reason phenyl-α-D-glucoside and maltitol were neither the substrates for, nor inhibitors of, the maltase. Bulky α-1,4-linked molecules, such as amylose, also seem not to fit the specific site. The specificity of the catalytic site may not be critical, however, because both α-1,4 and α-1,6 bonds were cleaved by our maltase preparations; α-1,4 linkages were hydrolyzed much more rapidly than α-1,6 linkages. We believe that α-1,6 activity is an inherent property of the maltase and not of a contaminating enzyme, but this conclusion is based only on apparent homogeneity of the final preparation as determined by electrophoresis, electrofocusing, and ultracentrifugation techniques.

The binding of the substrate to the enzyme molecule and the hydrolysis of the substrate were pH dependent (Fig. 14). From the plot of pK_m ($-\log K_m$) versus pH, there were two ionic groups with pK_a values of 5.5 and 6.5, respectively, involved in or near the active center of the enzyme. These pK_a values correspond closely to the imidazole group of histidine. That histidine may reside at the catalytic site is substantiated by the finding that rose bengal, a reagent that modifies histidine residues, is a strong inhibitor of the maltase (Table 3), whereas 4-chloromercuribenzoic acid, specific for cysteine residues, is a very weak inhibitor.

To compare the properties of the extracellular maltase of *B. subtilis* P-11 with those of microbial maltases previously reported, a summary table was made (Table 4). As shown in the table, cysteine was a stimulator for the α-glucos-

TABLE 4. *Comparison of the properties of the extracellular maltase of B. subtilis P-11 to those of maltases previously reported*[a]

Source (and reference)	Properties
Bacillus subtilis P-11	Opt. pH, 6.0; opt. temp., 45 C (30 min); stable within pH 6.0 to 6.3 and up to 45 C; pI, pH 6.0 (by gel-isoelectric focusing); mol. wt., 33,000 (Sephadex G-200); rose bengal, strong inhibitor; glucose and Tris, competitive inhibitors; cysteine, noncompetitive inhibitor; K_m, 5.0 mM (maltose); histidine residue involved in active center.
Bacillus cereus (22)	Opt. pH, 6.8 to 7.3; opt. temp. 40 C (15 min); stable within pH 6.8 to 7.3 and up to 35 C; pI, pH 4.5; mol. wt., 12,000 (SDS-polyacrylamide electrophoresis); Tris and turanose moderate inhibitors; K_m, 5.55 mM (maltose); histidine and cysteine involved in active center.
Escherichia coli (18, 19)	Opt. pH, 6.9; mol. wt., 124,000 (ultracentrifugation); methyl-α-D-glucoside and glucose, competitive inhibitors.
Mucor javanicus (20, 21)	Opt. pH, 4.6; opt. temp., 55 C (15 min); stable within pH 4.0 to 7.0 and up to 45 C; pI, pH 8.6; Tris and turanose, moderate inhibitors; K_m, 0.68 mM (maltose); histidine involved in active center.
Saccharomyces italicus (8)	Opt. pH, 6.6 to 6.8 (PNPG); stable within pH 6.0 to 7.8; mol. wt., 85,000 (ultracentrifugation); PCMB, a strong inhibitor; cysteine, glutathione, mercaptoethanol, stimulators; K_m, 0.28 mM (PNPG).
Saccharomyces logos (3, 4)	Opt. pH, 4.6 to 5.0; opt. temp., 40 C (30 min); stable within pH 3.6 to 6.6 and up to 40 C; mol. wt., 270,000 (Sephadex G-200); K_m, 7.7 mM (maltose).

[a] Abbreviations: opt., optimal; mol. wt., molecular weight; Tris, tris(hydroxymethyl)aminomethane; SDS, sodium dodecyl sulfate; PNPG, *p*-nitrophenyl-β-D-glucoside; PCMB, *p*-chloromercuribenzoate.

idase of *Saccharomyces italicus*, but it was an inhibitor of the maltase of *B. subtilis* P-11. The amino acid residues involved in the active centers were histidine for the maltases of *B. subtilis* P-11 and *Mucor javanicus* and both cysteine and histidine for the maltase of *Bacillus cereus*. Similarities and differences in several other properties, such as in pH optima, are summarized in Table 4.

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