[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, COLUMBIA UNIVERSITY]

A Study of the Purification and Properties of a Glucose-forming Amylase from *Rhizopus delemar*, Gluc Amylase[1]

BY LOUISE LANG PHILLIPS[2] AND M. L. CALDWELL

A glucose-forming amylase produced by the mold *Rhizopus delemar* and referred to here as gluc amylase has been prepared in highly purified form and freed from all detectable traces of alpha amylase which accompanies it in crude extracts. A study of the properties of purified gluc amylase reveals that it exerts maltase as well as amylase activity. All attempts to free gluc amylase from maltase activity have so far been of no avail. Any treatment that caused inactivation or removal of maltase activity resulted also in a corresponding loss of gluc amylase activity. The stability of gluc amylase has been studied under a number of conditions. Gluc amylase is less sensitive than the accompanying alpha amylase to exposure of its aqueous solutions to high hydrogen ion activities at relatively low temperatures, 5 to 10°. This property made possible the removal of traces of alpha amylase from the purified gluc amylase preparations. Calcium ions increase the inactivation of gluc amylase when its aqueous solutions are held at unfavorable temperatures. In measurements at 40°, gluc amylase exerts its optimal action on a number of different substrates at hydrogen ion activities that include *p*H 4.5. Glucose accounts for practically all of the reducing value of hydrolysates formed from starch by highly purified gluc amylase at least until approximately 90% of the theoretical glucose has been formed. The formation of glucose as the sole or almost the sole reducing product formed from starch by an amylase is of theoretical interest. Highly purified gluc amylase, freed from traces of other enzymes, offers a new tool for the study of amylase action and of the structure of its substrates.

Introduction

The work reported here deals with the purification and properties of a glucose-forming amylase produced by the mold, *Rhizopus delemar*.[3]

Experimental

Dry products[4] obtained by the commercial concentration of extracts of the mold were subjected to a number of procedures designed to concentrate the glucose-forming amylase, referred to here as gluc amylase, and separate it as completely as possible from other enzymes which accompanied it in the extracts. The success of each step was judged by activity measurements.

Activity Measurements and Analytical Procedures.—The total reducing values of the hydrolysates were determined by an iodometric method.[5] This gives a stoichiometric measure of the glucosidic linkages broken in the substrate. These total reducing values were calculated to their equivalents of glucose and are reported as such. Glucose was determined by a modification[6] of the method of Zerban and Sattler[7] and also occasionally by selective fermentation with yeast,[8] number 2019, as recommended by Schultz, *et al.*[9,10,11] Any difference between the total reducing value, calculated as glucose, and the reducing value actually due to glucose, represented the reducing value of products other than glucose and was taken as evidence of the presence of alpha amylase activity.

The amyloclastic or dextrinizing activity which is a measure of alpha amylase activity was determined by a modification[12] of the Wohlgemuth[13] method.

Preliminary experiments with gluc amylase at different stages in its purification showed that optimal amylase activity and also optimal maltase activity was obtained in hydrolyses at 40° when the substrates, starch or maltose, were adjusted to *p*H 4.3 to *p*H 4.7 in the presence of 0.01 *M* acetate. Therefore, all hydrolyses reported here were carried out at *p*H 4.5, 0.01 *M* acetate and 40°.

Comparisons of the activities of different enzyme solutions were based upon measurements of activity units as follows.

A unit of amyloclastic or of alpha amylase activity represents the weight of starch hydrolyzed per unit weight of enzyme preparation in 30 minutes at 40° to products that give a clear red color with iodine when the enzyme reacts under specified conditions[12] with 1% Lintner soluble potato starch adjusted to *p*H 4.5 and 0.01 *M* acetate.

A unit of gluc amylase activity represents the weight of glucose produced per unit weight of enzyme preparation in 30 minutes at 40° from 1% Lintner soluble potato starch adjusted to *p*H 4.5 and 0.01 *M* acetate and when the enzyme concentration is sufficient to form approximately 15% of the theoretical glucose, determined as glucose.[6]

A unit of maltase activity represents the weight of glucose produced per unit weight of enzyme preparation in 2 hours at 40° from 1% maltose adjusted to *p*H 4.5 and 0.01 *M* acetate and when the enzyme concentration is sufficient to form approximately 15% of the theoretical glucose.

Purification Procedure.—The most successful procedure so far developed for the purification and concentration of gluc amylase is based upon repeated fractionation of its solutions with ammonium sulfate with intervening dialyses of the fractions and, after constant maximum gluc amylase activity is attained, by repeated fractionation with ethanol.

A typical procedure, starting with commercial concentrates[4] of extracts of the mold is: (1) Two hundred grams of concentrate are triturated in a mortar with a small volume of distilled water and diluted to 2000 ml. The suspension is then tumbled for 5 hours at room temperature and allowed to remain overnight in the refrigerator (5–10°).

(2) The mixture is centrifuged in a refrigerated centrifuge[14] at approximately 5° for one hour and the precipitate discarded. The volume of the supernatant solution is measured and solid ammonium sulfate added to give a concentration of 40% (40 g. ammonium sulfate per 100 ml. of solution).

(3) The mixture is centrifuged and the precipitate taken up in a minimum volume of distilled water. This fraction is then dialyzed for 6 to 8 hours or until no test for sulfate is given with dilute barium chloride. This dialysis is carried out at room temperature in bags made of cellophane tubing[15] against distilled water which is changed frequently. Because cellulase, present in the crude enzyme preparation destroys the dialysis bags, it is necessary to change the bags frequently, every 10 or 15 minutes. Dialyses at room temperature were much more efficient for the removal of dark colored material and other impurities than dialyses carried out at lower temperatures.

(1) The authors wish to thank the Takamine Laboratory, Inc., for generous grants-in-aid of this investigation.

(2) The data reported here were taken from a dissertation submitted by Louise Lang Phillips in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Chemistry under the Faculty of Pure Science of Columbia University.

(3) J. Corman and A. F. Langlykke, *Cereal Chem.*, **25**, 190 (1948).

(4) The authors wish to thank the Takamine Laboratory, Inc., for generous gifts of these concentrates.

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(8) The authors wish to thank Dr. A. S. Schultz and the Fleischmann Laboratories for generous gifts of the yeast and for helpful suggestions.

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(10) M. Somogyi, *J. Biol. Chem.*, **119**, 741 (1937).

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(12) H. C. Sherman and A. W. Thomas, THIS JOURNAL, **37**, 623 (1915), and unpublished work.

(13) J. Wohlgemuth, *Biochem. Z.*, **9**, 1 (1908).

(14) Manufactured by the International Equipment Company, Boston.

(15) Purchased from the Visking Corporation, Chicago, Illinois.

(4) The supernatant solution from step 3 which contains 40% ammonium sulfate is brought to 55% ammonium sulfate and centrifuged. The supernatant solution is discarded and the precipitate taken up in a minimum of water and dialyzed. During this dialysis, a flocculent precipitate is formed. This precipitate is not active and is discarded.

(5) The dialyzed solution obtained from the 40% ammonium sulfate precipitate (step 3 above) is refractionated with ammonium sulfate to 40% and the precipitate discarded. The supernatant solution is then brought to 55% ammonium sulfate. The resulting precipitate is dissolved, the solution dialyzed, and added to the first 40–55% fraction (step 4).

(6) Steps 1 to 5 are carried out several times and the products combined to give quantities large enough to work with efficiently. The dialyzed solutions obtained from the 40–55% fractions (steps 4 and 5) are then refractionated with ammonium sulfate to 45% and the precipitate discarded. The supernatant solution is brought to 60% ammonium sulfate. The precipitate is dissolved and the solution dialyzed. The precipitate obtained during dialysis is again discarded.

(7) The dialyzed solution obtained from the 45–60% ammonium sulfate precipitate (step 6) is cooled to 5° and subjected to an alcohol fractionation at 25, 50, 60 and 70% ethanol by volume at 5°. The precipitates are dissolved in cold water and measured for activities. The 25% and 70% fractions are usually very low in activity and are discarded. Activities are determined on the 50% and on the 60% fractions because the results of this fractionation are somewhat unpredictable.

(8) The fraction (step 7) with the lowest ratio of alpha amylase to gluc amylase activities (A/G) is diluted, if necessary to give a dry weight of approximately 20 mg./ml., cooled to 5°, and refractionated at 25, 50, 60 and 70% ethanol. The fraction with the highest gluc amylase activity and the lowest A/G ratio is usually obtained in the 60% alcohol fraction.

Starting with a number of dry products that averaged a gluc amylase activity of 2.6, an alpha amylase activity of 16 and a maltase activity of 2.6, the purified products had gluc amylase activities of 510 to 550, maltase activities of 551 to 571 and alpha amylase activities of 25 or less. These data show that the gluc amylase and the maltase activities of the final products had been increased more than 200-fold while the relative concentration of alpha amylase activity to gluc amylase activity and to maltase activity had been greatly decreased. Subsequent work made it possible to free the highly active gluc amylase preparations from all detectable alpha amylase activity but not from maltase activity.

Maltase Activity of Gluc Amylase.—The data summarized in Table I show that there was very little change in the ratio of maltase activity to gluc amylase activity of amylase solutions obtained at different stages in the purification of gluc amylase. These data are typical of the results obtained not only during purification procedures but throughout this investigation. The ratios of maltase activity to gluc amylase activity of gluc amylase solutions have in all cases remained close to unity even after adsorption studies and after attempts at selective inactivation at different temperatures and at different hydrogen ion activities. Granting that measurements of gluc amylase activity and of maltase activity are not sufficiently precise to make agreement in these ratios entirely conclusive, the evidence indicates that gluc amylase itself exerts significant maltase activity. Although the ratios of gluc amylase activities to the maltase activities discussed here give values close to unity, they actually represent much higher gluc amylase activity than maltase activity because of the units arbitrarily selected for the comparisons. The gluc amylase activities were determined after 30 minutes of hydrolysis of 1% starch at 40° whereas the maltase activities were determined after 120 minutes of hydrolysis of 1% maltose.

TABLE I

RATIOS OF ALPHA AMYLASE AND OF MALTASE ACTIVITIES TO GLUC AMYLASE ACTIVITIES OF GLUC AMYLASE SOLUTIONS AT DIFFERENT STAGES IN ITS PURIFICATION

Enzyme solution	Ratios of enzyme activities: Maltase to gluc amylase M/G	Alpha amylase to gluc amylase A/G
Initial soln. (av. values)	1.0	6
After fractionation with different concentrations of ammonium sulfate	1.05	7
	0.84	4
	.92	7
	.88	4
	1.08	6
	0.92	2
	.90	0.9
	.85	1.2
	.92	0.8
	.98	.6
	.87	.6
After alcohol fractionations	1.12	<0.10
	1.07	< .05
	1.10	< .05
	0.98	< .05
	1.05	< .05

The data given in Table II summarize results obtained in inactivation studies. They extend the evidence that the maltase activity exerted by purified gluc amylase preparations is largely if not entirely due to the gluc amylase itself. No evidence of selective inactivation of gluc amylase activity or of maltase activity was obtained when solutions of purified gluc amylase were subjected to unfavorable temperatures or to unfavorable hydrogen ion activities. Treatments that caused inactivation of gluc amylase also caused closely corresponding losses of maltase activity. Although it is possible that two enzymes with similar stabilities are

TABLE II

RESULTS OF ATTEMPTS TO CAUSE SELECTIVE INACTIVATION OF GLUC AMYLASE AND OF MALTASE ACTIVITIES OF PURIFIED GLUC AMYLASE

Treatment of amylase solution[a]: Temp., °C.	Time, minutes	pH	Gluc amylase activity[b]: Units G	Original remaining, %	Maltase activity[c]: Units M	Original remaining, %	Ratios of activities maltase to gluc amylase M/G
5	30	6.3	501	100	551	100	1.1
5	30	2.4	467	93	511	93	1.09
5	30	7.9	522	104	511	93	0.98
5	1440	6.3	489	98	463	84	0.95
5	1440	2.4	445	88	481	87	1.09
5	1440	7.9	514	102	463	84	0.91
55	15	6.3	392	78	413	75	1.05
55	15	2.4	0	0	0	0	
55	15	7.9	0	0	0	0	

[a] Amylase preparation with gluc amylase activity of 501; maltase activity of 551; low alpha amylase activity, A/G = < 0.05. Solution adjusted to 0.142 mg. per ml.; 0.10 *M* acetate; *p*H values as indicated. [b] Weight of glucose[6] formed from starch per unit weight of enzyme preparation in 30 minutes at 40°; Lintner soluble potato starch; 1%; 0.01 *M* acetate; *p*H 4.5. [c] Weight of glucose[6] formed from maltose per unit weight of enzyme preparation in 120 minutes at 40°; maltose 1%; 0.01 *M* acetate; *p*H 4.5; maltose $[\alpha]^{25}D$ 131.3°.

involved, it appears more probable that gluc amylase hydrolyzes both starch and maltose. Studies of the affinities of gluc amylase for these two substrates are reported elsewhere.[16]

Removal of Traces of Alpha Amylase from Highly Purified Gluc Amylase.—In contrast to the results with maltase activities, the ratios given in Table I for alpha amylase to gluc amylase activities show that the original enzyme solutions used here contained a separate alpha amylase and that this alpha amylase was almost entirely eliminated during the purification process.

The data summarized in Table III are typical of the results obtained in the hydrolysis of Lintner soluble potato starch by highly purified preparations of gluc amylase. Taken by themselves, the data given in section A of Table III would suggest that no significant traces of alpha amylase remained in the purified preparations of gluc amylase. The hydrolysate from 1% Lintner soluble potato starch gave a blue color with iodine after 30 minutes of hydrolysis when 26% of the theoretical glucose had been formed and a red-violet color with iodine even after 24 hours of hydrolysis, when 87% of the theoretical glucose had been produced. However, the data given in sections B and C of Table III indicate that sufficient alpha amylase may remain with the purified gluc amylase to influence its action when it is used in relatively high concentrations or for prolonged periods of time. Thus 10- and 25-fold increases in the concentration of the purified gluc amylase resulted in the somewhat more rapid disappearance from the hydrolysates of products that give color with iodine. Perhaps more significant is the observation that, with these higher concentrations of gluc amylase, there was evidence of the formation and subsequent removal of reducing products other than glucose, presumably by the joint action of gluc amylase and of traces of alpha amylase. Attempts to free preparations of purified gluc amylase from these last traces of alpha amylase activity were carried out in a number of different ways and finally were successful.

Neither the alpha amylase nor the gluc amylase was adsorbed to any significant extent on bentonite, acid-washed vegetable charcoal, or upon potato starch, from gluc amylase solutions containing measurable alpha amylase activity and adjusted to *p*H 4.3, 5.1, 6.3 or 7.9. Therefore, attempts to separate these amylases by selective adsorption were abandoned.

Attempts to cause the selective inactivation of alpha amylase in gluc amylase solutions at different stages of its purification by exposure of these aqueous solutions to unfavorable temperatures also were not successful. Whenever the exposure caused a significant loss of alpha amylase activity, it also caused marked inactivation of the gluc amylase activity. Under the conditions of these experiments, both the gluc amylase and the alpha amylase activities of partially and of highly purified gluc amylase preparations were relatively unstable when their aqueous solutions were held at 60° or at 70° for short periods of time. The inactivation at 50° was much less for both amylases.

(16) L. L. Phillips and M. L. Caldwell, in press.

TABLE III

HYDROLYSIS OF LINTNER SOLUBLE POTATO STARCH BY INCREASING CONCENTRATIONS OF PURIFIED GLUC AMYLASE

Hydrolysis of starch[a] time, minutes	Total reducing values as glucose equivalents	Glucose produced	Reducing products other than glucose	Color with iodine
	Expressed as per cent. theoretical glucose			
A. Gluc amylase,[b] relative concentration, 1				
30	26	27		Blue
60	46	43	3	Blue
120	60	60	0	Violet-blue
180	70	69	1	Blue-violet
240	73			Blue-violet
300	77			Blue-violet
1440	87	89		Red-violet
B. Gluc amylase,[b] relative concentration, 10				
5	35	28	7	Blue
10	54	39	15	Blue-violet
15	64	49	15	Blue-violet
30	74	69	5	Blue-violet
60	81	81	0	Blue-violet
120	82	84		Blue-violet
180	89	89	0	Violet
240	89			Violet-red
300	87			Violet-red
1440	101	101	0	Yellow
C. Gluc amylase,[b] relative concentration, 25				
5	56	45	11	Violet-blue
10	73	66	7	Blue-violet
15	79	70	9	Blue-violet
30	85	83	2	Blue-violet
60	86	86		Violet
120	86	87		Red-violet
180	93	92	1	Violet-red
240	92			Red-orange
300	97			Yellow

[a] Lintner soluble potato starch 1%; 0.01 *M* acetate; *p*H 4.5; 40°. [b] Gluc amylase activity, 516; concentration: A, 0.057 mg. amylase preparation per 100 mg. substrate; B, 0.57 mg. per 100 mg. substrate; C, 1.43 mg. per 100 mg. substrate.

The data summarized in Table IV are typical of the results obtained when solutions of gluc amylase at different stages in its purification were adjusted to different hydrogen ion activities and held for known periods of time at 5 to 10°. The results suggested that the alpha amylase activity was less stable than the gluc amylase activity when the amylase solutions studied here were adjusted to low *p*H values at 5 to 10°. Subsequent work confirmed and extended this finding. Solutions of highly purified gluc amylase held at *p*H 2.4 and 5 to 10° for increasing lengths of time showed decreasing evidence of alpha amylase activity. After 9 days, no traces of alpha amylase activity were detectable even after 24 hours hydrolysis of starch with relatively high concentrations of treated gluc amylase preparations representing 25 times that used in activity measurements of 30 minutes. During this acid treatment of the purified amylase, approximately 20% of the gluc amylase activity also was lost. Typical data for the hydrolysis of Lintner soluble starch by such treated gluc amylase solutions are summarized in Table V. These data af-

TABLE IV

INFLUENCE OF UNFAVORABLE HYDROGEN ION ACTIVITIES UPON GLUC AMYLASE AND ALPHA AMYLASE ACTIVITIES OF PARTIALLY PURIFIED GLUC AMYLASE

Treatment of amylase solution[a]			Gluc amylase activity		Alpha amylase activity	
Temp., °C.	Time, minutes	pH	Units	Original remaining, %	Units	Original remaining, %
5	30	6.3	342	100	1386	100
5	1440	6.3	342	100	1386	100
5	1440	3.0	337	99	1240	90
5	4320	3.0	332	97	1046	76
5	1440	4.5	342	100	1386	100
5	1440	7.2	323	95	1386	100

[a] Amylase preparation with gluc amylase activity of 342; alpha amylase activity of 1386; ratio A/G = 4.0; 0.184 mg. per ml.; 0.10 *M* acetate; adjusted to *p*H values indicated.

ford good evidence that all significant traces of alpha amylase are removed from purified preparations of gluc amylase, such as those used here, by exposure of their aqueous solutions to *p*H 2.4 at 5 to 10° for 9 days. In contrast to the results summarized in Table III for highly purified but untreated gluc amylase, the data given in Table V show that hydrolysates obtained from Lintner soluble potato starch by the action of even relatively high, 25-fold, concentrations of treated gluc amylase retained products that gave color with iodine after 24 hours hydrolysis and when 88% of the theoretical glucose had been formed. Moreover, there was no longer the tendency for reducing products other than glucose to be formed in small concentrations in the earlier stages of the hydrolyses and then to disappear. This observation indicates that traces of alpha amylase were no longer available to cause the formation of reducing products that were susceptible to the action of gluc amylase.

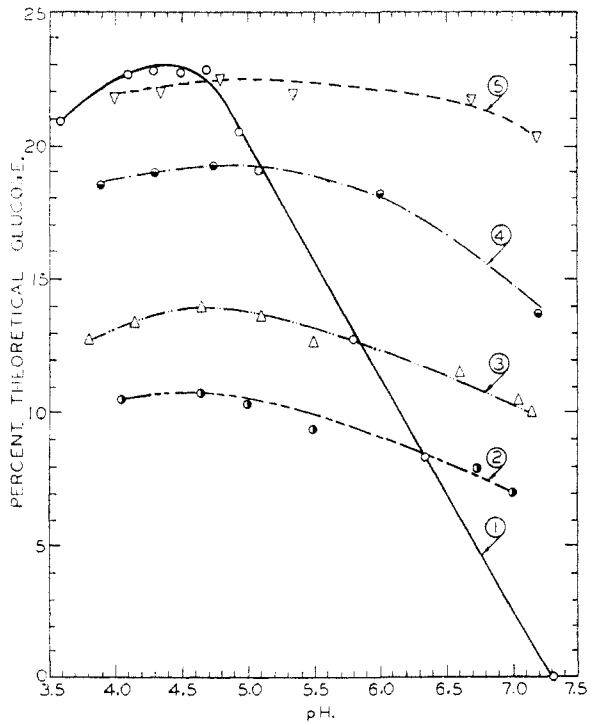

Fig. 1.—Influence of the hydrogen ion activities of its substrates upon the action of purified gluc amylase: curve 1, maltose; curve 2, linear substrate; curve 3, glycogen; curve 4, Lintners soluble potato starch; curve 5, branched fraction from corn starch; substrates 1%; 0.01 *M* acetate; 0.05 *M* potassium chloride. Hydrolyses at 40°; maltose 2 hours; other substrates 30 minutes; purified gluc amylase, 0.04 mg. per 100 mg. substrate. These data do not necessarily represent the relative rates of hydrolysis of the different substrates. Glucose determined by copper acetate method.[6]

TABLE V

EVIDENCE THAT PURIFIED GLUC AMYLASE PREPARATIONS ARE FREED FROM ALL SIGNIFICANT TRACES OF ALPHA AMYLASE ACTIVITY BY SPECIAL TREATMENT[a]

Hydrolysis of starch[b] time, minutes	Total reducing values as glucose, equivalents	Glucose produced	Reducing products other than glucose	Color with iodine
	Expressed as per cent. theoretical glucose			
5	55	47	8	Violet-blue
10	70	62	8	Violet-blue
15	76	73	3	Blue-violet
30	80	73	7	Blue-violet
60	82	78	4	Blue-violet
120	83	76	7	Blue-violet
180	85	80	5	Blue-violet
240	85			Blue-violet
300	82			Blue-violet
1440	88	81	7	Violet

[a] Purified gluc amylase preparation; gluc amylase activity 516; ratio A/G < 0.05; adjusted to *p*H 2.4 and 2.84 mg. per ml. and held at 5° for 9 days. [b] Lintner soluble potato starch 1%; 0.05 *M* acetate; *p*H 4.5; 40°; 1.4 mg. treated amylase preparation per 100 mg. starch; relative concentration, 25 times that used for usual activity measurements.

It is interesting to note in passing that the differences in stability in acid solution observed here for the gluc amylase and the alpha amylase obtained from *Rhizopus delemar* resemble the differences in the stabilities at high hydrogen ion activities of the beta amylase and the alpha amylase from barley and from malted barley. These differences were used by Ohlsson[17] to cause the inactivation of the alpha amylase in beta amylase solutions from barley and from malted barley, a procedure widely adopted.

Influence of Calcium Ions on Stability of Gluc Amylase.—The data summarized in Table VI show that calcium ions do not protect purified gluc amylase in aqueous solutions from inactivation at unfavorable temperatures. In fact, the calcium

TABLE VI

INFLUENCE OF CALCIUM IONS ON STABILITY OF GLUC AMYLASE IN AQUEOUS SOLUTION

Treatment of amylase solution[a]				Gluc amylase activity	
Temp., °C.	Time, minutes	pH	Calcium chloride, *M*	Units G	Original remaining, %
5	10	4.5	0	230	100
5	10	4.5	0.02	229	100
55	10	4.5	0	210	91
55	10	4.5	0.02	177	77
60	10	4.5	0	39	17
60	10	4.5	0.02	22	10

[a] Gluc amylase preparation; 0.034 mg. per ml.; 0.01 *M* acetate *p*H 4.5 with or without 0.02 *M* calcium acetate.

(17) E. Ohlsson, *Z. physiol. Chem.*, **189**, 17 (1930).

ions appear to have an unfavorable influence. In this respect again gluc amylase resembles beta rather than alpha amylases.[18,19]

Influence of Hydrogen Ion Activities of Its Substrates upon the Action of Gluc Amylase.—The data summarized in Fig. 1 show the influence of hydrogen ion activities of its substrates upon the action of highly purified gluc amylase in hydrolyses at 40° of 120 minutes for maltose and of 30 minutes for a number of other substrates. The substrates included Lintner soluble potato starch; a linear fraction[20] from corn starch; a branched fraction[20] from corn starch, glycogen[21]; maltose.

For this work each substrate except maltose was dissolved in molar potassium hydroxide, diluted with water, neutralized with hydrochloric acid and all were adjusted to a final concentration of 1% substrate, 0.01 *M* acetate and 0.05 *M* chloride.

The data given in Fig. 1 show that the optimal action of gluc amylase under the conditions used here was obtained with each of the substrates at hydrogen ion activities that included *p*H 4.5. Similar results were obtained in hydrolyses of 24 hours; reaction mixtures adjusted to *p*H 4.5 again favored the action of the amylase. Therefore, it is recommended that the action of gluc amylase under the other conditions used here be studied at *p*H 4.5.

The data given in Fig. 1 compare the influence of hydrogen ion activity upon the hydrolysis of the different substrates by gluc amylase. They do not necessarily represent the relative rates of the hydrolysis of the different substrates. Such comparisons are discussed elsewhere.[16]

In addition to establishing the conditions which favor the action of gluc amylase at 40°, the data given in Fig. 1 show that gluc amylase produces glucose from starch, from its components, and from glycogen under conditions that prevent or inhibit the formation of glucose from maltose. Therefore, these data offer additional strong evidence that gluc amylase forms glucose directly from starch or its components without the preliminary formation of maltose.

In the course of these experiments it was found that concentrations of potassium chloride up to 0.1 *M* and of acetate up to 0.05 *M* did not change appreciably either the hydrogen ion activities that favor the action of gluc amylase or the activity of the amylase under the other conditions used here. These findings are of interest because the use of potassium hydroxide to dissolve starches and their components and glycogen sometimes results in the presence of different concentrations of potassium chloride in the final reaction mixtures.

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(19) R. B. Alfin and M. L. Caldwell, THIS JOURNAL, **70**, 2534 (1948).

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, COLUMBIA UNIVERSITY]

A Study of the Action of Gluc Amylase, a Glucose-producing Amylase, Formed by the Mold, *Rhizopus delemar*[1]

BY LOUISE LANG PHILLIPS[2] AND M. L. CALDWELL

The action of highly purified gluc amylase, treated to free it from traces of alpha amylase, has been investigated with a number of different substrates. Given sufficient time for the hydrolyses and sufficiently high concentrations of amylase, such preparations of gluc amylase produce 95 to 96% of the theoretical glucose from the linear and from the branched fractions from corn starch and from defatted waxy maize starch; 92% from glycogen; 89% from residual beta dextrins, 100% from maltose. Glucose is the sole reducing product formed in these hydrolysates at least until approximately 90% of the theoretical glucose has been produced. Gluc amylase appears to hydrolyze glucose from the non-aldehydic ends of the glucosidic chains of its substrates in a manner similar to the hydrolysis of maltose from its substrates by beta amylase, but with gluc amylase, there is no evidence of the formation of high molecular weight non-reducing residual dextrins. Neither highly purified gluc amylase nor the alpha amylase that accompanies it in the crude extracts has any detectable influence upon the alpha or the beta Schardinger dextrins, upon a dextran, a polymer of α-D-glucopyranose, having predominantly 1,6-α-D-glucosidic linkages; or upon isomaltose (brachiose). The extensive hydrolysis of the branched substrates shows that gluc amylase either hydrolyzes the 1,6-α-D-glucosidic linkages of these substrates or passes by them in some manner. However, the failure to reach 100% hydrolysis with the branched substrates such as is attained with maltose and the failure of the gluc amylase to hydrolyze the dextran or isomaltose suggest that gluc amylase does not hydrolyze the 1,6-α-D-glucosidic linkages of its substrates. This point has not been established conclusively and is being investigated further. In the hydrolysis of the linear substrate by gluc amylase, a straight line relationship is obtained between the blue values and the glucose values of the hydrolysates. A similar relationship between the blue values of a linear hydrolysate and its maltose values has been reported previously for beta amylase. Gluc amylase appears to resemble beta amylases rather than alpha amylases in its action and in certain of its properties. The Michaelis or affinity constants of gluc amylase for linear and for branched fractions from corn starch and for maltose have been determined and are discussed.

Introduction

A glucose-producing amylase formed by the mold *Rhizopus delemar*[3] has been prepared in highly purified form[4] and freed from all detectable traces of alpha amylase activity.[4] The work reported here deals with a study of the action of such highly purified preparations of gluc amylase upon a number of substrates.

Experimental

Substrates.—The substrates investigated included: a

(1) The authors wish to thank the Takamine Laboratory, Inc., for generous grants in aid of this investigation.

(2) The data reported here are taken from a dissertation submitted by Louise Lang Phillips in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Chemistry under the Faculty of Pure Science of Columbia University.

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