STARCH-LIQUEFYING PROPERTIES OF CRYSTALLINE ALPHA-AMYLASES[1]

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ABSTRACT

The pH optima for liquefying action of crystalline amylases were 4.5, 6.8, and 6.0 for the enzymes from fungal, pancreatic, and bacterial sources, respectively. The enzymes exhibited a stability over the pH ranges of 4–8, 5–8, and 5–12, respectively. The temperatures at which inactivation started were 50°C. for Taka-Amylase, 45°C. for the enzyme of pancreatic origin, and above 60°C. for the enzyme of bacterial origin. An essentially linear plot was obtained for the relation between low levels of enzyme activity and viscosity drop. Both purified and commercial preparations of fungal and pancreatic amylases were completely inactivated by incubation with a number of quaternary ammonium compounds; the inactivation of the enzyme of bacterial origin was only partial. With the cereal amylases, the extent of inactivation varied with the concentration of the enzyme, the more purified enzyme being inactivated to a greater degree than the low-activity amylases.

The importance of starch-liquefying properties of alpha-amylases from different sources and the use of the amylograph in evaluating amylases from the standpoint of panary fermentation are well established. Properties of amylases from commercial preparations are known to vary from those of purified amylases. While a large amount of work has been reported on the dextrinogenic and saccharifying properties of crystalline amylases, little work has been done on the liquefying properties of purified enzymes. The authors have recently reported on the evaluation of amylases by the amylograph, employing pregelatinized

[1]Manuscript received January 8, 1963. Co-operative investigations between the Crops Research Division, Agricultural Research Service, U.S. Department of Agriculture, and the Department of Flour and Feed Milling Industries. Contribution No. 428, Kansas Agricultural Experiment Station, Kansas State University, Manhattan.

[2]Report taken, in part, from a thesis submitted by Miss Moro in partial fulfillment of requirements for the M. Sc. degree, Kansas State University, Manhattan.

starch as a substrate. Those reports deal with the action of amylases from commercial preparations under conditions of panary fermentation (10,11), with a procedure to evaluate starch-liquefying properties of amylase-supplements (7), and with inactivation studies of amylases from various sources (8,9). The present report deals with the properties of crystalline amylases on liquefaction of pregelatinized starch.

Materials and Methods

The crystalline Taka-Amylase A used in this study was a gift from Shoji Matsubara, Osaka University, Japan (6). The pancreatic, crystalline amylase was purchased from Nutritional Biochemicals Corporation, Cleveland, Ohio[3]; and the crystalline bacterial enzyme, from Worthington Biochemical Corporation, Freehold, N. J. Additionally, crude amylases from cereal, bacterial, and fungal origin were studied. Among the cereal amylases, two samples of wheat, two samples of barley, and a sample of sorghum amylase were tested. Of the amylases of microbiological origin, fungal and bacterial samples were tested. One of the wheat malt preparations and also the sorghum malt were partly purified. The other enzymes were concentrates furnished by three different commercial firms.

The quaternary ammonium compounds were of analytical grade purchased from Bios Laboratories, Inc., New York. The starch used as the substrate to measure the amylolytic action was Amaizo 721A — pregelatinized waxy maize starch, from American Maize-Products Company, New York.

Unless stated otherwise, alpha-amylase activity was measured by following the liquefying action of the enzyme through the following procedure: 33 g. of starch were added slowly to 320 ml. of buffer solution and mixed by means of a laboratory stirrer at 1,450 r.p.m. in a beaker. The starch suspension was mixed for 3 min. The apparently homogeneous dispersion was transferred with the aid of 120 ml. of additional buffer solution to an amylograph bowl. The Brabender Amylograph was employed to measure changes in starch viscosity as a result of the action of alpha-amylase. The instrument bowl was operated at 75 r.p.m.; the enzymatic action was measured at 37°C. After the starch was transferred to the amylograph bowl, the instrument was operated for 10 min. for temperature equilibration. Then 10 ml. of a 0.2% solution of calcium chloride, or 10 ml. of a 0.02*M* sodium chloride solution containing the enzyme, were added, and the viscosity was

[3]Mention in this publication of a trade product, equipment, or a commercial company does not imply its endorsement by the U.S. Department of Agriculture over similar products or companies not named.

recorded by the instrument during 40 min. Starch suspensions were 0.02*M* in phosphate buffer and, to determine pancreatic amylase, also 0.02*M* in sodium chloride. The effect of either pH or of inactivators on enzyme stability was studied by holding microgram quantities of crystalline enzyme (or 10 S.K.B. units of a noncrystallized preparation) suspended in 10 ml. of 0.2% calcium chloride, or in 0.02*M* sodium chloride in case of pancreatic amylase, and 10 ml. of 0.2*M* phosphate buffer in a stoppered Erlenmeyer flask for 18 hr. before the enzyme was placed in the amylograph bowl. The enzyme was then added to a starch suspension buffered to give optimum pH at the end of the starch-hydrolysis measurement.

Results and Discussion

Several factors of importance in the study of starch liquefaction by crystalline amylases were investigated.

Effect of pH on Enzyme Activity and Enzyme Stability. Optimum pH for amylase activity varied with the enzyme source (Figs. 1, 2, and 3). The three crystalline amylases exhibited maximum activity at (or about) 4.5, 6.8, and 6.0 for the fungal, pancreatic, and bacterial amylases, respectively. On testing the stability of the enzymes, it was found that no appreciable irreversible inactivation took place after incubation for 18 hr. at 25°C., between pH 4–8, 5–8, and 5–12 for the fungal, pancreatic, and bacterial amylases, respectively. In addition to exhibiting the widest range of pH optimum, the amylase from bacterial origin was most stable at low pH values, despite its relatively high pH optimum. This agrees with previously reported results on the high stability of a number of commercial amylases of bacterial origin (7).

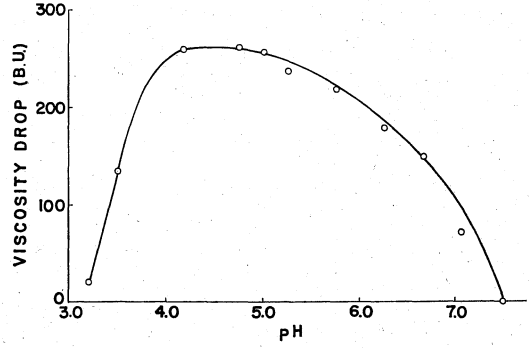

Fig. 1. Effect of pH on activity of Taka-Amylase.

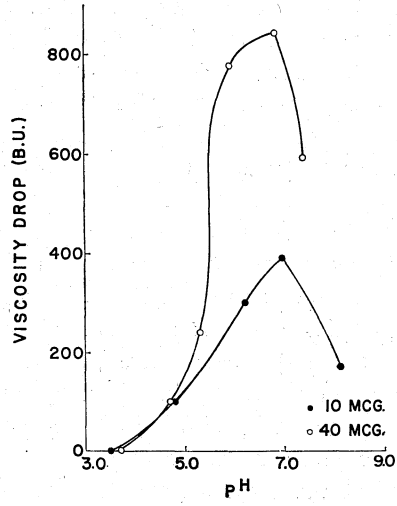

Fig. 2. Effect of pH on activity of pancreatic alpha-amylase.

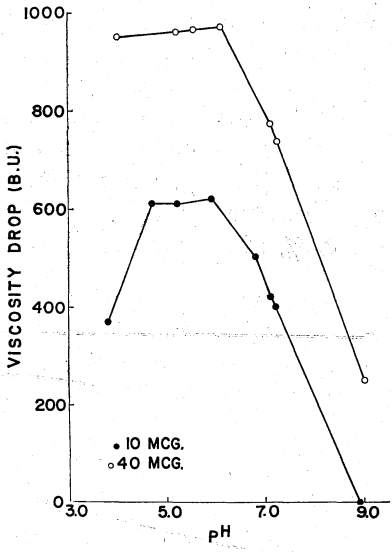

Fig. 3. Effect of pH on activity of bacterial alpha-amylase.

Effect of Temperature. The effect of temperature on amylase activity was studied. Table I shows the relation between log of velocity (viscosity drop, B.U./min. × 20) and temperature (reciprocal of absolute temp. × 10^4). Taka-Amylase showed enhanced activity up to a temperature of 50°C.; the increase in activity in case of pancreatic

amylase was up to 45°C.; bacterial-amylase activity decreased above 60°C. These results show that while amylases from different sources differ in their thermostability, the differences of crystalline preparations are much smaller than are those of enzymes from commercial sources (3). Adding calcium chloride had no effect on enzyme stability, apparently because the enzymes tested were purified in the presence of calcium ions. The amount necessary for amylase stabilization is bound very strongly and can be removed only by prolonged dialysis against ethylenediaminetetra-acetic acid (2).

Effect of Enzyme Concentration. Two desiderata for the relationship between the quantity of enzyme used and the changes resulting from enzymatic action are 1) a linear relationship between the quantity of enzyme and the measured product and 2) a linear time course. These desiderata may be achieved when the enzyme is saturated by the substrate used, or when measurements are made at points where only a small portion of the substrate has been converted.

In selecting the optimum substrate concentration, the authors were guided by the amount of pregelatinized starch which gave the maximum 1,000-B.U. reading, prior to beginning of enzymatic action. According to Kneen (5), the kinetics of liquefaction of starch by amylases differ somewhat from those operative in starch dextrinization or saccharification. No linear relationship between the quantity of enzyme and decrease in viscosity could be observed. The viscosity of gelatinized starch drops 50% when only 0.1% of the glucosidic linkages are opened, whereas no measurable change in viscosity takes place on cleavage of more than 0.5% of the linkages (1,4). It seems, therefore,

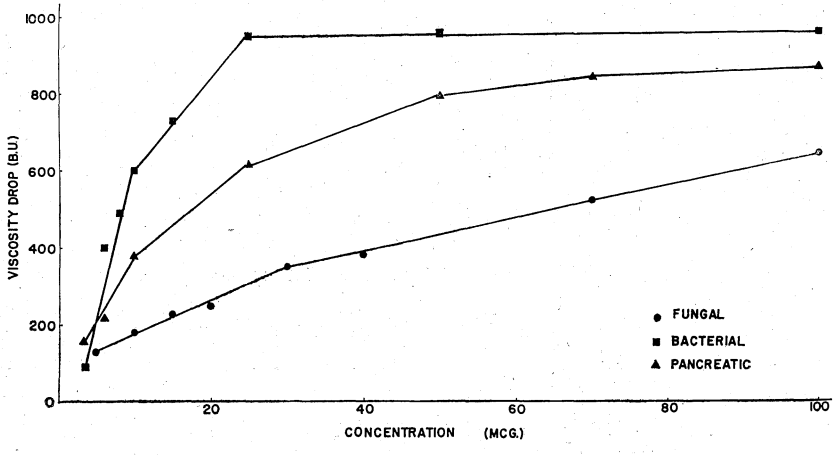

Fig. 4. Relation between concentration of amylase and starch liquefaction.

that to satisfy the requirements regarding substrate concentration, the results might follow a linear course over the low range of enzyme concentration. This has been previously shown to be true for commercial enzyme preparations and is the case with the three crystalline alpha-amylases reported here (Fig. 4). It should be noted, however, that in employing very low levels of alpha-amylases the results may be affected by denaturation of the enzyme.

Inactivation by Quaternary Ammonium Compounds. The effect of different amounts of cetyl pyridinium chloride on bacterial alpha-amylase is given in Table II. The results, summarized in Table III,

TABLE I

EFFECT OF TEMPERATURE ON AMYLASE ACTIVITY

TEMP.	$1/T \times 10^4$	Loc V				
		Taka-Amylase (100 γ)	Pancreatic Amylase (40 γ)		Bacterial Amylase (40 γ)	
			In Presence of NaCl	In Presence of $CaCl_2$	In Presence of NaCl	In Presence of $CaCl_2$
°C.						
25	33.5	1.954	2.556	2.556	2.462	2.447
30	33.0	2.114	2.613	2.699	2.544	2.532
35	32.4	2.146	2.699	2.716	2.562	2.586
40	31.9	2.204	2.935	2.748	2.695	2.686
45	31.4	2.260	2.929	2.763	2.724	2.708
50	30.9	2.301	2.929	2.748	2.744	2.712
55	30.4	1.605	2.623	2.690	2.763	2.720
60	30.0	....	....	....	2.690	2.724

TABLE II

EFFECT OF DIFFERENT AMOUNTS OF CETYL PYRIDINIUM CHLORIDE ON BACTERIAL ALPHA-AMYLASE

CETYL PYRIDINIUM CHLORIDE	VISCOSITY DROP AFTER ADDITION OF ALPHA-AMYLASE		
	20 γ	40 γ	80 γ
mg.	*B.U.*	*B.U.*	*B.U.*
0	600	870	960
2	550	840	940
5	540	740	940
10	400	640	940
20	340	550	910
40	250	490	840

show that employing a constant level of cetyl pyridinium chloride (40 mg. per 20 ml. of solution) and varying the amount of amylase between 5 and 100 γ made no substantial difference in the extent of bacterial amylase inactivation. The extent of inactivation was only slightly different, whether from cetyl ammonium bromide, cetyl pyridinium

TABLE III

EFFECT OF CETYL PYRIDINIUM CHLORIDE[a] ON LIQUEFACTION BY CRYSTALLINE BACTERIAL ALPHA-AMYLASE

ENZYME	INACTIVATION	ENZYME	INACTIVATION
γ	%	γ	%
5	87.0	25	84.0
10	86.0	40	86.3
15	83.3	50	76.0
20	85.0	100	83.0

[a] 40 mg. per 20 ml. solution.

TABLE IV

EFFECT OF 40 MG. OF VARIOUS QUATERNARY AMMONIUM SALTS ON STARCH LIQUEFACTION BY CRYSTALLINE BACTERIAL ALPHA-AMYLASE

ENZYME	VISCOSITY DROP			
	Control	With Cetyl Ammonium Bromide	With Cetyl Pyridinium Chloride	With Cetyl Trimethyl Ammonium Bromide
γ	*B.U.*	*B.U.*	*B.U.*	*B.U.*
25	860	640	740	760
50	960	925	940	960
100	975	975	970	970

chloride, or cetyl trimethyl ammonium bromide, as illustrated in Table IV. Whereas bacterial amylase was only partly inactivated by the action of quaternary ammonium compounds, the inactivation of pancreatic and fungal amylase after addition of 40 mg. of any of the tested quaternary ammonium compounds was practically complete. The results of testing commercial and partly purified amylases (Table V) confirm those obtained with crystalline enzymes. While

TABLE V

DEXTRINOGENIC ACTIVITY OF COMMERCIAL AMYLASE PREPARATIONS

ORIGIN	DEXTRINOGENIC ACTIVITY
	S.K.B. units
Wheat	19; 414
Barley	52; 284
Sorghum	840
Fungi	980; 4,590; 5,000; 7,900; 8,140
Bacteria	5,480; 7,590; 11,880

the enzymes of fungal origin were completely inactivated, a substantial residual activity was recorded in the bacterial amylases (Table VI). These results must, however, be interpreted with caution, as the nature of the impurities and their affinity for the quaternary ammonium compounds is unknown. That such impurities may exert a pro-

TABLE VI

EFFECT OF 40 MG. CETYL PYRIDINIUM CHLORIDE ON STARCH LIQUEFACTION BY NONCRYSTALLINE ALPHA-AMYLASE (10 S.K.B. UNITS)[a]

ENZYME SOURCE	VISCOSITY DROP	
	Control	With Cetyl Pyridinium Chloride
	B.U.	*B.U.*
Fungal	430	0
Fungal	330	0
Fungal	300	0
Fungal	360	0
Fungal	270	0
Bacterial	970	640
Bacterial	960	780
Bacterial	960	640
Wheat malt[b]	530	0
Sorghum malt[b]	420	0
Barley malt	740	470
Barley malt	890	610
Wheat malt	880	390

[a] See ref. 12.
[b] Partly purified.

nounced effect is shown by the two partially purified cereal amylases being completely inactivated by the detergent, while crude cereal preparations of low activity were inactivated only partially.

Acknowledgments

The authors gratefully acknowledge the financial support of the Rockefeller Foundation provided for the senior author. Acknowledgment is also made to American Maize-Products Company, New York, N.Y., for the sample of pregelatinized waxy corn starch, to P. Nordin for the sample of sorghum amylase, to J. Sullivan for a sample of partly purified wheat amylase, and to Miles Chemical Co., Elkhart, Ind., Rohm and Haas Co., Philadelphia, Pa., and Wallerstein Co., Staten Island 3, N.Y., for the samples of commercial amylase preparations.

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