Hydrolytic Proteins of Sugarcane: Amylase

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INTRODUCTION

HISTORICAL

The amylase of wheat was probably the first enzyme discovered (*22*).[2] Kirchoff, in the year 1814, demonstrated that starch was converted to sugar by extracts of wheat as well as by dilute acid. However, so inextricably bound was this discovery to the evolving history of enzymology, that nearly three-quarters of a century was yet to pass before an inkling of the nature and distribution of amylase was understood.[3]

By the second half of the eighteenth century investigators such as Reaumur and Thenard realized that certain biological reactions proceeded far more rapidly than could be explained by reaction potentials observed in the chemical laboratory. Thus, Lavoisier and Gay-Lussac believed that the conversion of glucose to ethyl alcohol and carbon dioxide was caused by a "yeast ferment".[4] In 1833 a starch-degrading "ferment" (diastase, an amylase) was precipitated from malt extract by alcohol, but neither its mode of action nor its relationship to other biological catalysts was clearly recognized.

In 1836 Berzelius proposed his "catalysis theory," claiming that certain reactions were subject to an unknown chemical force which he termed "catalysis" (Greek *katalysis* = dissolution). The catalytic power was attributed to "substances" which, by their presence alone, were able to set into activity "affinities" which otherwise would be "dormant" at a particular temperature. Berzelius was vague, and his theory is only partly verified by modern concepts of enzyme catalysts, but nevertheless it was too much for many contemporaries to accept. In the words of Liebig, "the

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[2] Italic numbers in parentheses refer to Literature Cited, pp. 323–4.

[3] The literature of amylase is voluminous. The reader is referred to Sumner and Somers (*22*, p. 116) and to Fruton and Simmonds (*14*, pp. 211–14) for historical aspects, and to the papers by Hopkins (*17*), Geddes (*15*), and Myrbäck and Neumüller (*19*).

[4] It is known today that alcoholic fermentation requires the combined action of about 12 distinct enzymes. Both Lavoisier and Gay-Lussac were engrossed in clarifying the quantitative relationships between initial substrate and final products, and were less concerned with the nature of "yeast ferment." Nevertheless, they were likely ahead of their times in recognizing that a catalytic agent was necessary.

assumption of this new force is detrimental to the progress of science, since it appears to satisfy the human spirit, and thus provides a limit to further research" (*14*, p. 210).

By 1860, a starch-degrading factor with properties similar to those found in wheat extracts half a century earlier was also recognized in malt and saliva. Other reactions which today are attributed to invertase, pepsin, and trypsin had likewise been discovered, but the "enzyme" concept as it is known today still eluded the best minds of the period. Pasteur stated " . . . If I am asked in what consists the chemical act of the decomposition of sugar, and what is its real cause, I admit that I am completely ignorant of it" (*14*, p. 213). The key difficulty at this time was the failure of scientists to recognize organic catalysts as separate entities, distinct from the life processes which they catalyzed. Thus there arose the misleading concepts of "formed" and "unformed" ferments. "Organized ferments" (formed), of which the living yeast cell was an example, were distinguished from "unorganized ferments" (unformed), which included those that could be extracted from cells. Diastase and invertase were classified as unorganized ferments.

The confusion generated by the double use of "ferment" prompted Kühne, in 1878, to suggest the term *en zyme* (Greek = in yeast) to describe all unformed ferments. The last basis for distinguishing between organized and unorganized ferments was removed in 1897, when it was shown that cell-free extracts of yeast would cause the fermentation of glucose to ethanol and carbon dioxide.

ACTION, DISTRIBUTION, AND SIGNIFICANCE OF AMYLASE IN CANE

In 1876 O'Sullivan discovered that the primary end-product of amylase is maltose (*21*), and within 2 years it was recognized that maltose was produced as a result of at least two types of amylases acting upon starch. Since that time the amylases have been grouped into two broad classes (α and β) which are based upon the portion of the starch molecule being attacked (*17*).

The designation α or β does not refer to the configuration of the glycosidic bond that is hydrolyzed, since both types of enzymes hydrolyze α (1 $\rightarrow$ 4)-glucosidic linkages. B amylases hydrolyze the amylose fraction of starch by attacking the polysaccharide from the nonreducing end of the chain, cleaving alternate α (1 $\rightarrow$ 4)-glycosidic bonds, and forming the dissacharide β-maltose.[5] Hydrolysis will approach 100 percent when straight-chain amylose is substrate. With the branched-chain amylopectin, activity is

[5] The appearance of β-maltose by action of β-amylase is not clearly understood, since starch contains only alpha linkages. Sumner and Somers (*22*, p. 117) proposed that a Walden inversion might occur.

stopped at the points of branching, *i.e.*, at the (1 → 6)-glycosidic linkages. In contrast to the β-amylases, α-amylases produce maltose very slowly, while rapidly decreasing the capacity of amylose to yield a blue color with iodine. A-amylases attack the glycosidic linkages in the interior of the polysaccharide chain, yielding oligosaccharides (*14*, p. 434), and causing a decline in viscosity of the starch solution. Hence, α-amylases are sometimes termed "dextrinogenic" or "liquifying" amylases.

Amylases have been found in micro-organisms, molds, higher plants, and animals. They are especially abundant in molds and in the salivary glands of certain animals. Ungerminated cereal grains contain large quantities of amylase (*4*), and the enzyme is also known in the sweetpotato (*8,12*) and in sugarcane (*16,1*).

Both starch and the enzymes responsible for its degradation have been generally ignored in sugarcane. One exception is the variety Uba (*24*), the excessive tendencies toward starch accumulation of which have created difficulties for cane processors by retarding the filtration and crystallization rates of cane juices (*7,13*). Enzyme-catalyzed hydrolysis has been studied as a means of removing excess starch from the juices in the factory (*10*). In 1936, Hartt (*16*) reported that amylase was less active in the meristematic tissue of the Uba variety than in other varieties tested. Initial investigations at this laboratory have shown strong amylase activity in caneleaf extracts (*1*), and subsequent studies of both amylase and starch phosphorylase have been completed here (*2,3*).

The presence of an amylase-starch phosphorylase complex in sugarcane must have considerable bearing on the sucrose status. On the one hand, starch phosphorylase would compete directly with sucrose-forming mechanisms for glucose-1-phosphate, and, in turn, its equilibrium constant would be affected by the rate of the amylase-catalyzed degradation of starch. On the other hand, the degradation of amylase products such as maltose and oligosaccharides to free glucose, would give the glucose units another opportunity to become phosphorylated and to enter pools of the glucose-1-phosphate precursor needed for sucrose formation. It follows that a thorough knowledge of sugarcane amylase is necessary in order that cane producers understand the starch-sucrose balance in sugarcane, and know how to alter this relationship in favor of increased sucrose. This paper summarizes the properties of sugarcane amylase extracted from immature leaves.

EXPERIMENTAL PROCEDURE

Plant materials were prepared and extracted according to procedures outlined previously for invertase (*5*), with the exceptions that only 10-

month-old plants were used, and that it was unnecessary to subject samples to sonic disintegration to obtain maximum amylase yields.

Amylase was assayed by measuring the amount of reducing sugar formed when substrate was acted upon under standardized conditions. The method was a modification of that suggested by Bernfeld (*9*), in which amylopectin was used as substrate and reducing sugar was determined by the dinitrosalicylic acid technique. Other methods commonly employed for amylases include measuring the blue-value decline and viscosity decrease of a standard starch solution. Reducing-power determination was employed during the present study because of the speed and reliability of the technique, and because it is applicable for both α- and β-amylases.

The standard amylase digest was composed of 0.5 ml. of acetate buffer

TABLE 1.—*Specific activity of amylase extracted from different tissues of 10-month-old sugarcane*[1]

Substrate	Data for amylase source indicated—						
	Meristem	Leaves +1 and 0	Leaves +2 and +3	Leaves +5 and +6	Sheaths +5 and +6	Nodes 8 to 10	Internodes 8 to 10
Amylopectin	60.5	133.0	40.8	33.3	53.3	122.9	47.4
Amylose	51.2	97.0	34.5	16.7	30.0	91.7	41.0

[1] Each reaction mixture was composed of 0.5 ml. of succinate buffer (pH 6.5), 0.5 ml. of 1-percent substrate solution, and 0.25 ml. of distilled water or enzyme preparation. All trials were run for 20 minutes at 30° C.

(pH 5.5), 0.5 ml. of substrate solution sufficient to make 3.5 mg. of amylopectin per milliliter of digest, 0.25 ml. of water or additive solution, and 0.25 ml. of undialyzed enzyme preparation. The reaction was allowed to proceed for 20 minutes at 30°C., at which time 2 ml. of dinitro reagent were added and the reaction vessels were transferred to boiling water. After completing color development, optical density was recorded at 525 mμ and the amount of maltose present was determined by reference to a standard curve representing 0.10 to 2.0 mg. of maltose.[6]

Amylase activity is expressed in terms of specific activity—units per milligram of protein. The activity unit was arbitrarily defined as the amount of enzyme catalyzing the formation of 0.10 mg. of maltose under the specified conditions of the assay. Protein content of the enzyme preparations was determined by the microtechnique of Sutherland *et al.* (*23*).

[6] In the instance of purely α-amylase action, the products are dextrins rather than maltose.

RESULTS AND DISCUSSION

LOCALIZATION OF AMYLASE

The best single source of enzyme protein from sugarcane is the tissue comprising leaves +1 to +6, so long as general surveys only are being conducted. During the present study we desired the richest possible source of amylase, and to that end, a series of tissues was tested, including meristem, leaves, sheaths, nodes, and internodes. Enzyme preparations consisted of undialyzed protein precipitated from clarified water extracts by ammonium sulfate between 0 and 95 percent saturation. Results are summarized in table 1.

Employing both amylose and amylopectin as substrate, amylase specific

TABLE 2.—*Specific activity of sugarcane amylase preparation acting upon different polysaccharides at acidic, neutral, and alkaline pH values*[1]

Buffer	Data for polysaccharide substrate indicated—				
	Inulin[1]	Corn starch	Potato starch	Amylopectin[1]	Amylose[1]
Acetate (pH 4.0)	82	90	328	305	123
Phosphate (pH 7.0)	84	91	333	307	137
Tris (pH 8.5)	74	78	143	109	117

[1] Each reaction mixture was composed of 0.5 ml. of buffer, 0.5 ml. of 1-percent substrate solution, and 0.25 ml. of distilled water or enzyme solution. Enzyme preparation consisted of undialyzed protein precipitated from leaf extracts with ammonium sulfate between 35- and 65-percent saturation. All reactions were run for 20 minutes at 30° C.

activity was found to predominate in immature leaves (−1 and 0) and nodes. Protein content of leaf samples was 2 to 3 times that of the nodes, and immature leaves were, therefore, employed as enzyme source throughout the remainder of the study.

SUBSTRATE SPECIFICITY AND pH DEPENDENCE

Although Bernfeld (*9*) recommended amylopectin as substrate for α- and β-amylase, the cane amylase preparation was also tested against potato starch, corn starch, amylose, and inulin.[7] Acetate, phosphate, and tris

[7] Substrate solutions were prepared as follows: Exactly 1 gm. of A.C.S.-grade substrate was placed in a 200-ml. pyrex culture tube. The material was wetted with 5 ml. of ethanol and taken up in 50 ml. of 0.5 *N* NaOH. Complete solution was accomplished by placing the tube in boiling water and allowing it to stand for 3 minutes. The solution was cooled, adjusted to pH 7 with 0.5 *N* H_2SO_2 , and brought to 100 ml. with distilled water.

buffers were employed to obtain pH values of 4.0, 7.0, and 8.5, respectively. Potato starch and amylopectin were both readily attacked at pH 4 and 7 (table 2). The potato-starch preparations were found to be less stable than amylopectin, and the latter was used thereafter in all amylase experiments. Little information was gained from the varying pH values, other than that amylase was more active on the acidic side of pH 7 than the alkaline side.

SALT FRACTIONATION

Preliminary precipitation tests with ammonium sulfate revealed that very little amylase was removed below 40-percent or above 80-percent

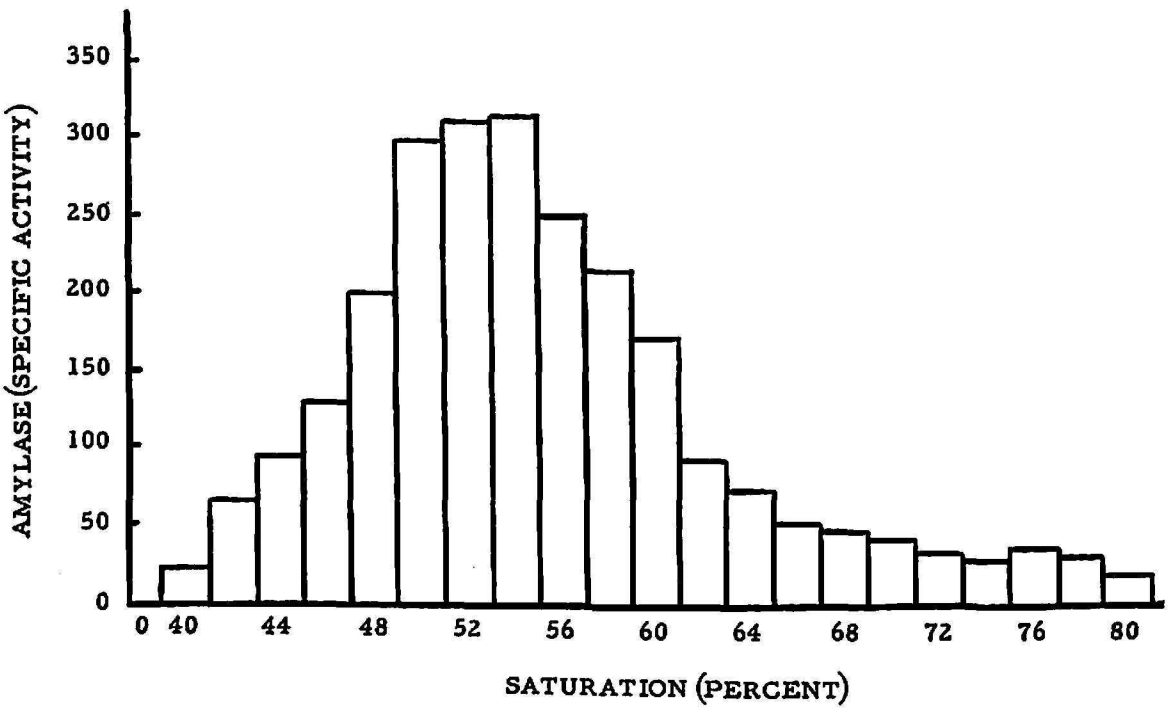

FIG. 1.—Amylase activity among protein increments precipitated by ammonium sulfate from extracts of immature sugarcane leaves.

saturation. The 40- to 80-percent fraction thus gave higher specific activity values than could obtained with the 0 to 95-percent preparation originally employed. Protein increments of 2-percent saturation were collected and assayed individually. Whereas some amylase activity was obtained as low as 40- and as high as 80-percent saturation, the highest specific activity values were obtained between 48- and 60-percent saturation (fig. 1). This fraction accounted for slightly more than 75 percent of the total amylase activity of the extract and was used as enzyme source throughout the remainder of the study.

The amylase preparation described above did not in any sense represent a homogeneous protein solution. In particular, an α-amylase encountered

previously while studying the Q enzyme (*4*) was absent. The 48- to 60-percent fraction contained acid phosphatase and invertase, although the latter two enzymes are more predominant in meristem tissues than in immature leaves. Nevertheless, the amylase preparation obtained from immature leaves was undoubtedly characteristic of the great bulk of amylase active in sugarcane.

OPTIMUM pH

Amylase preparations were tested at pH values ranging from 3 to 9, employing 1-percent solutions of amylopectin and amylose. All reactions

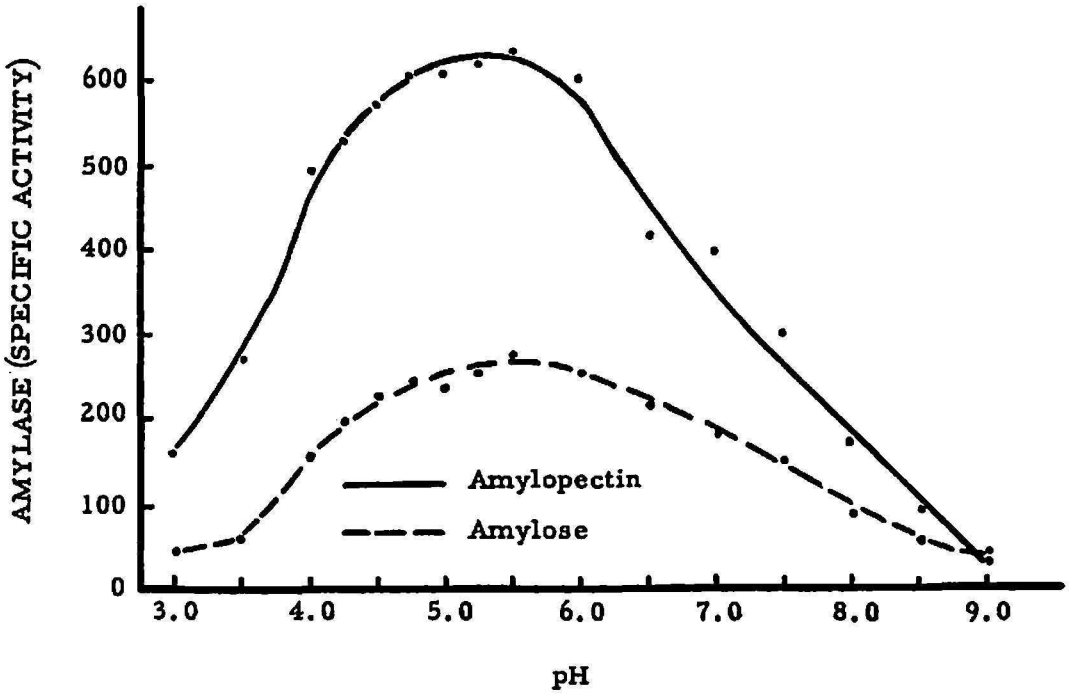

FIG. 2.—Effects of variable pH upon amylase from immature leaves of sugarcane. Amylopectin and amylose were employed as substrates at 3.5 mg./ml. of digest.

were run for 20 minutes at 30°C. Typical results are illustrated in figure 2.

Maximum activity against both substrates was recorded at pH 5.5. Amylase was highly active against amylopectin from pH 4.5 to 6.0, with activity declining rapidly above pH 6. The amylase-amylopectin curve presented in figure 2 is almost identical with that used by Myrbäck and Neumüller ((*19*), p. 677) to illustrate the pH optima of barley β-amylase.

VELOCITY *vs.* SUBSTRATE CONCENTRATION AND TIME

Amylopectin concentrations ranging from 0.10 to 10 mg./ml. of digest were employed to determine maximum velocity of sugarcane amylase. All tests were run for 20 minutes at 30° C. and pH 5.5. Maximum velocity was achieved at about 8 mg., although a linear response was achieved only to about 2 mg./ml. of digest (fig. 3). The curve plotted in figure 3 is fairly

typical of the effects of substrate on the velocity of any enzyme. The diphasic character of such curves led early workers to believe that the enzyme formed an intermediate complex with its substrate, and that the reaction could not take place without the formation of this complex (*20*, p. 66). This interpretation proved correct.

An interesting feature of sugarcane amylase is the length of time during which the enzyme will act independently of substrate concentration (fig. 4). According to the equation of Michaelis and Menton (*18*),

$$v = \frac{V[S]}{K_m + [S]} = \frac{V}{1 + (K_m/[S])}$$

where v = actual (or "initial") velocity, K_m = the Michaelis constant,

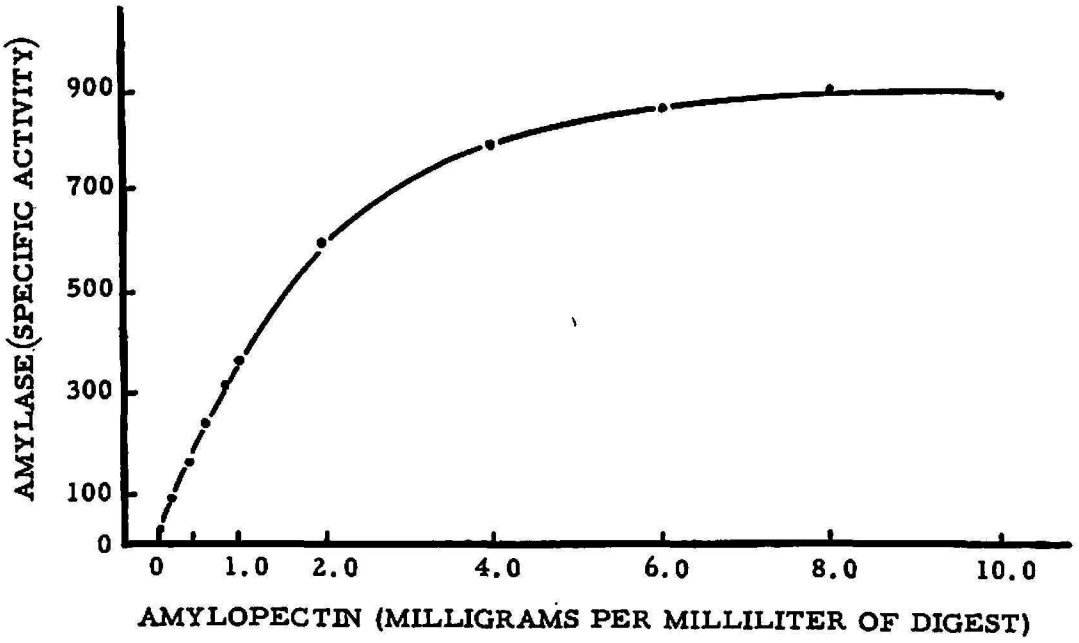

FIG. 3.—Response of sugarcane amylase to increasing substrate concentrations.

i.e., substrate concentration at half maximum velocity, V = maximum velocity, and S = substrate concentration. The actual velocity is independent of substrate only when the latter is very large. In the instance where S is very large in relation to K_m, the equation becomes

$$v = \frac{V[S]}{[S]},$$

maximum velocity is achieved, it is independent of S, and is, therefore, of zero order (*20*, p. 71).

The amylase reaction illustrated in figure 4[8] employed a substrate con-

[8] The velocity *vs.* time experiment was accomplished by setting up a standard reaction of 40 ml. volume. Aliquots of 1.5 ml. volume were withdrawn at appropriate time intervals and the reaction was terminated with dinitro reagent. The experiment was conducted at pH 5.5 and 30° C.

centration of 3.5 mg. of amylopectin per milliliter of digest, and attests to the very large number of α (1 → 4)-glucosidic linkages subject to hydrolysis in this quantity of polysaccharide. Thus, after an initial lag of about 5 minutes, the reaction proceeded rapidly and continued unabated for 3 hours, at which time the experiment was ended.

RESPONSE TO INCREASING TEMPERATURE

Hopkins (*17*) has pointed out that the type and purity of a given amylase greatly affects its stability to heat. Other writers minimize the effect of

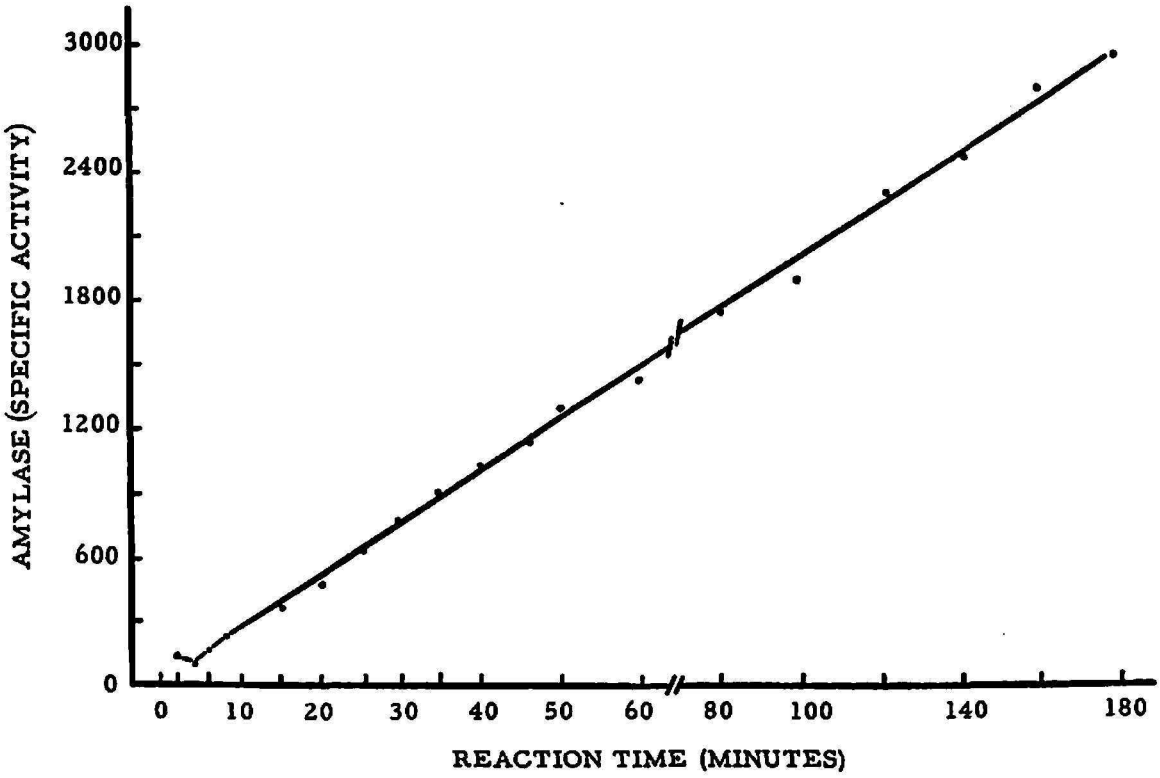

FIG. 4.—Amylase hydrolysis of amylopectin (3.5 mg./ml. of digest) over a prolonged period of time.

reaction conditions in general (*11*, p. 23), while Myrbäck and Neumüller discount the usual significance of optimum temperature when dealing with amylases (*19*, p. 676). Nevertheless, it was of interest to learn the behavior of sugarcane amylase when subjected to variable temperatures.

Amylase was measured at temperatures ranging from 16 to 52° C. (fig. 5). Activity increased rapidly to 46° C. and thereafter became somewhat unstable. Maximum activity was recorded at 50° C., exemplifying a seventeenfold increase over that recorded at 16° C.

When considering the inactivity of cane amylase at relatively low temperature, one must bear in mind the possible effects of other starch-regulat-

ing enzymes, especially when dealing with only partly purified preparations such as those employed here. Thus, in one of the few studies of its kind, Arreguin and Bonner (*6*) showed that potato tubers retain their starch when stored above 10° C., and rapid starch decline occurs between 0° and 10° C. Starch decline was accompanied by sucrose increases. These investigators concluded that, at low temperatures, starch breakdown is induced by the disappearance of a starch phosphorylase inhibitor, and increased activity of systems such as phosphohexose isomerase and sucrose phosphorylase.

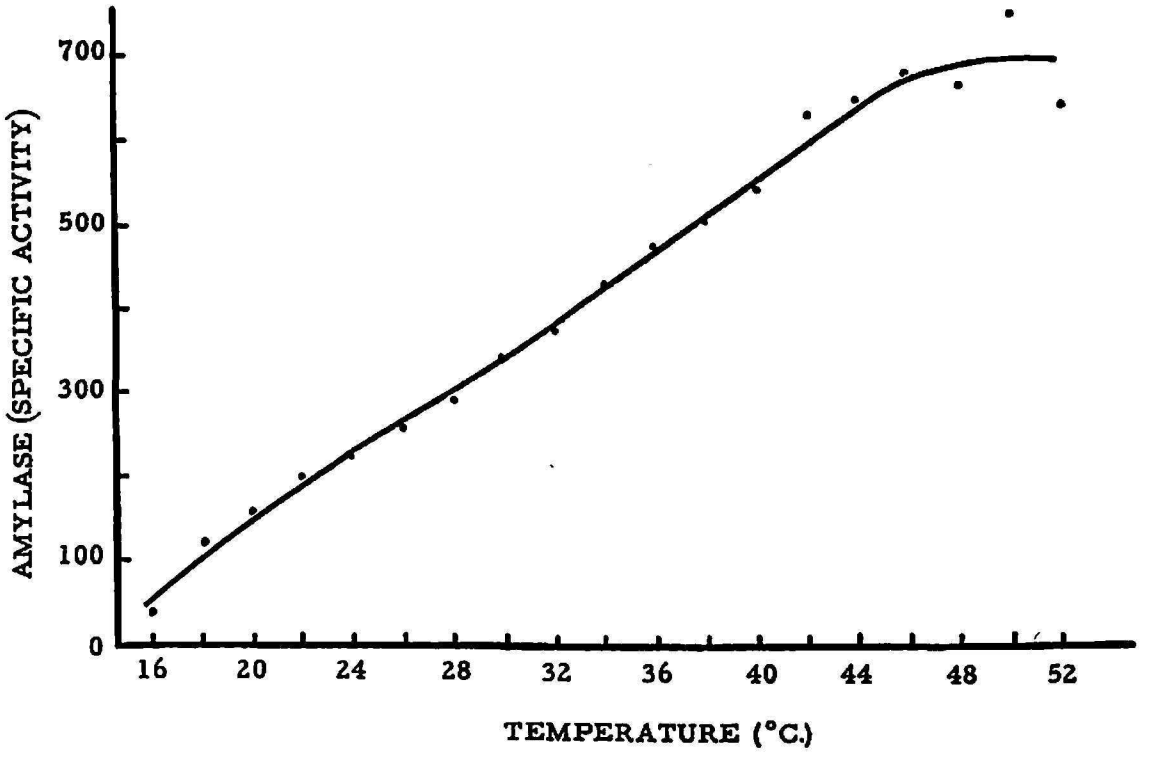

FIG. 5.—Effects of temperature upon amylase from immature leaves of sugarcane.

INHIBITORS

As was true of invertase during a previous study (*5*), a primary objective of these investigations was to discover inhibitors which would effectively suppress amylase in minute quantities. To this end a large number of elements were tested upon sugarcane amylase at the concentration of 0.1 μmole/ml. of digest (fig. 6).

Amylase was completely inhibited by mercury (Hg) and iodide (I), table 3. Magnesium (Mg) suppressed amylase to about a third of control values, while manganese (Mn) and lead (Pb) appeared to cause slight activation. No appreciable differences were obtained with bromide, boron, calcium, copper, fluoride, molybdenum, cyanide, zinc, tungsten, arsenic,

or chloride. Subsequent tests showed that Hg and I inhibition was readily measurable at concentrations of 3×10^{-6} and 2×10^{-5} μmoles/ml. of digest, respectively. Both elements caused complete inhibition of amylase at 1×10^{-4} μmoles/ml. of digest.

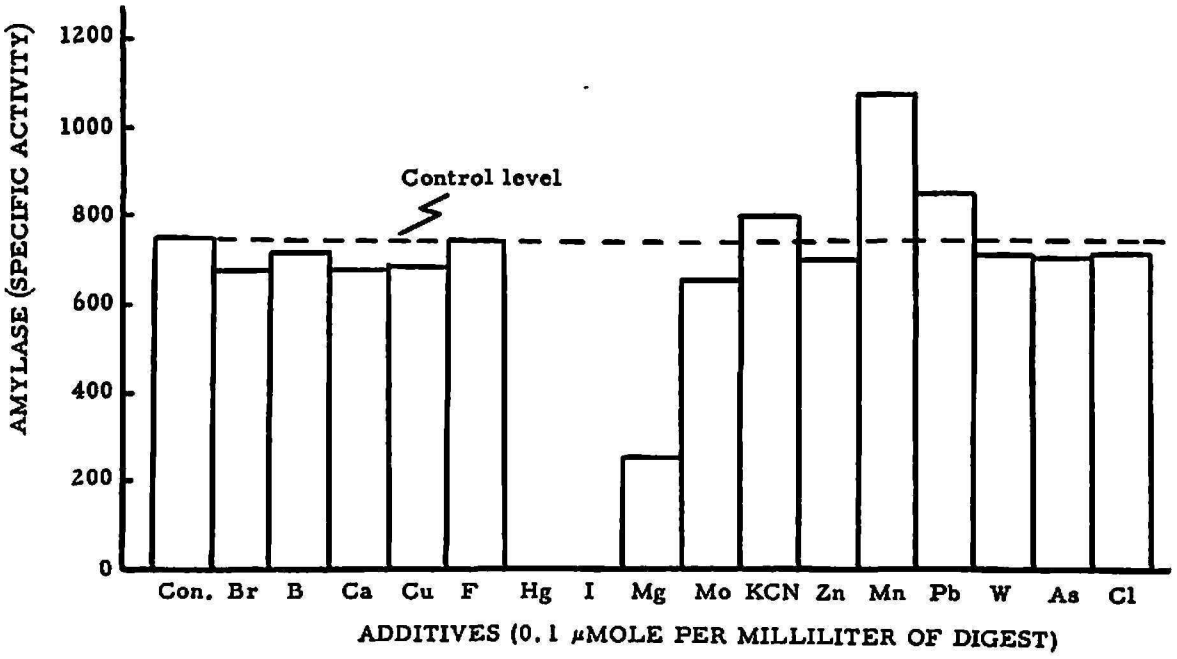

FIG. 6.—Effects of additives upon amylase from immature leaves of sugarcane.

TABLE 3.—*Inhibitory effects of iodide and mercury on the specific activity of amylase from immature leaves of sugarcane*[1]

Inhibitor	*Data for iodide concentration (μmoles/ml. of digest) at—*								
	0	1×10^{-5}	2×10^{-5}	4×10^{-5}	6×10^{-5}	8×10^{-5}	1×10^{-4}	3×10^{-4}	6×10^{-4}
Iodide	304	288	225	96	28	16	0	0	0

	Data for mercury concentration (μmoles/ml. of digest) at—										
	0	1×10^{-5}	3×10^{-5}	6×10^{-5}	1×10^{-5}	3×10^{-5}	6×10^{-5}	1×10^{-4}	3×10^{-4}	6×10^{-4}	1×10^{-3}
Mercury	464	452	382	372	308	42	28	0	0	0	0

[1] Each reaction mixture was composed of 0.5 ml. of acetate buffer (pH 5.5), 0.5 ml. of substrate solution (sufficient to make 3.5 mg. of amylopectin per milliliter of digest, 0.25 ml. of water or inhibitor solution, and 0.25 ml. of enzyme preparation. All reactions were run for 20 minutes at 30° C.

Mercury and I inhibition (0.01 μmole/ml.) was measured with substrate concentrations ranging up to 12 mg./ml. of digest in order to gain a clearer picture of the type of inhibition involved. Employing an amylopectin level of 4 mg. for the standard inhibition reaction, the suppressing effects of I

were removed completely by raising the substrate level to 8 mg./ml. of digest (fig. 7). Mercury inhibition was alleviated very slowly, and even at 12 mg. of substrate per milliliter the element still exerted a 60-percent inhibition. One may surmise from the slope of the Hg curve in figure 7 that complete reversal of the Hg suppression would eventually be gained, at a substrate concentration between 30 and 32 mg./ml. of digest, or about 3 percent. It is unlikely that equivalent starch concentrations occur in cane tissues. Nevertheless, both Hg and I should be regarded as competitive (reversible) inhibitors of amylase. As noted previously (*5*), Hg also inhibits

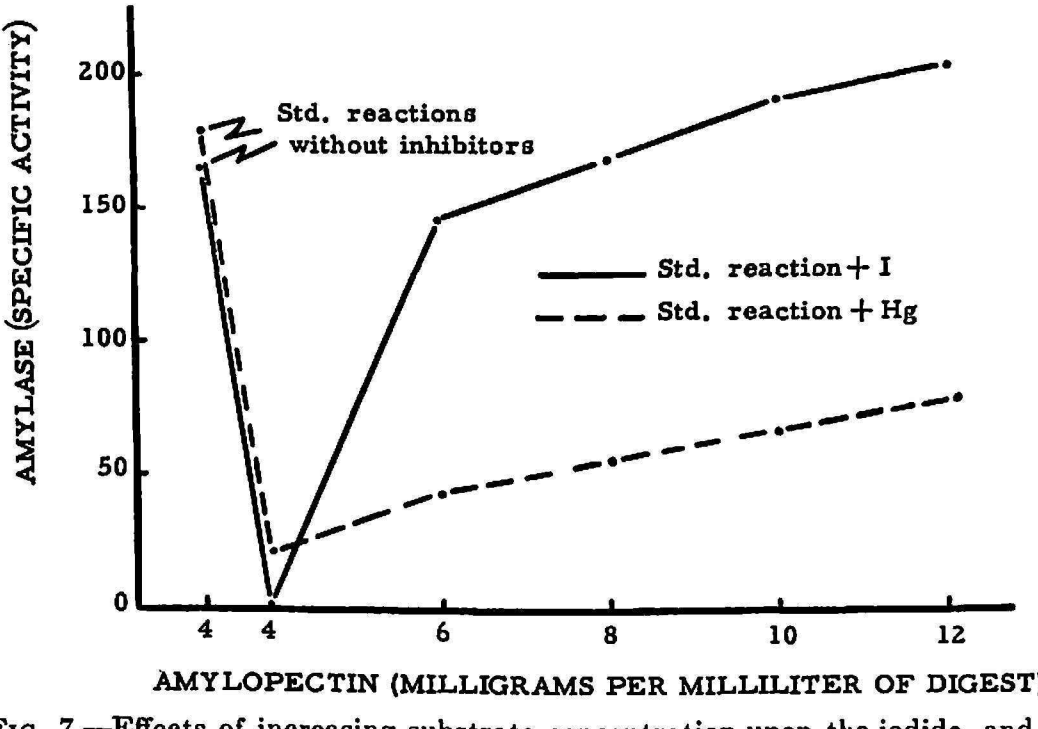

FIG. 7.—Effects of increasing substrate concentration upon the iodide- and mercury-inhibited activity of amylase.

cane invertase, but the effect is not appreciably reversed by increasing concentrations of sucrose.

ACTIVATION BY MANGANESE AND SUGAR ADDITIVES

Following initial observations that Mn and Pb exerted slight stimulating effects upon amylase, the two elements were tested further as possible activators. Lead caused only slight activation and this effect was confined to the Pb range of 0.03 to 0.3 μmole/ml. of digest. On the other hand, Mn stimulated amylase at concentrations as low as 0.003 μmole/ml., and continued to do so up to 0.6 μmole/ml. of digest (fig. 8).

The response of amylase to Mn was almost identical with that of invertase. It is, in fact, apparent that several characteristics of cane amylase

corresponded closely with its sucrose-hydrolyzing counterpart, particularly with regard to saturation values at which it is precipitated from extracts, pH and temperature effects, and response to Hg. One striking difference between the two catalysts was the inhibition of invertase by Pb, while amylase was slightly activated by it.

The similar properties of the two systems tend to support a contention expressed earlier (*5*) that one of the invertases present in cane, α-glucosidase, may act upon maltose and related products of starch hydrolysis. The

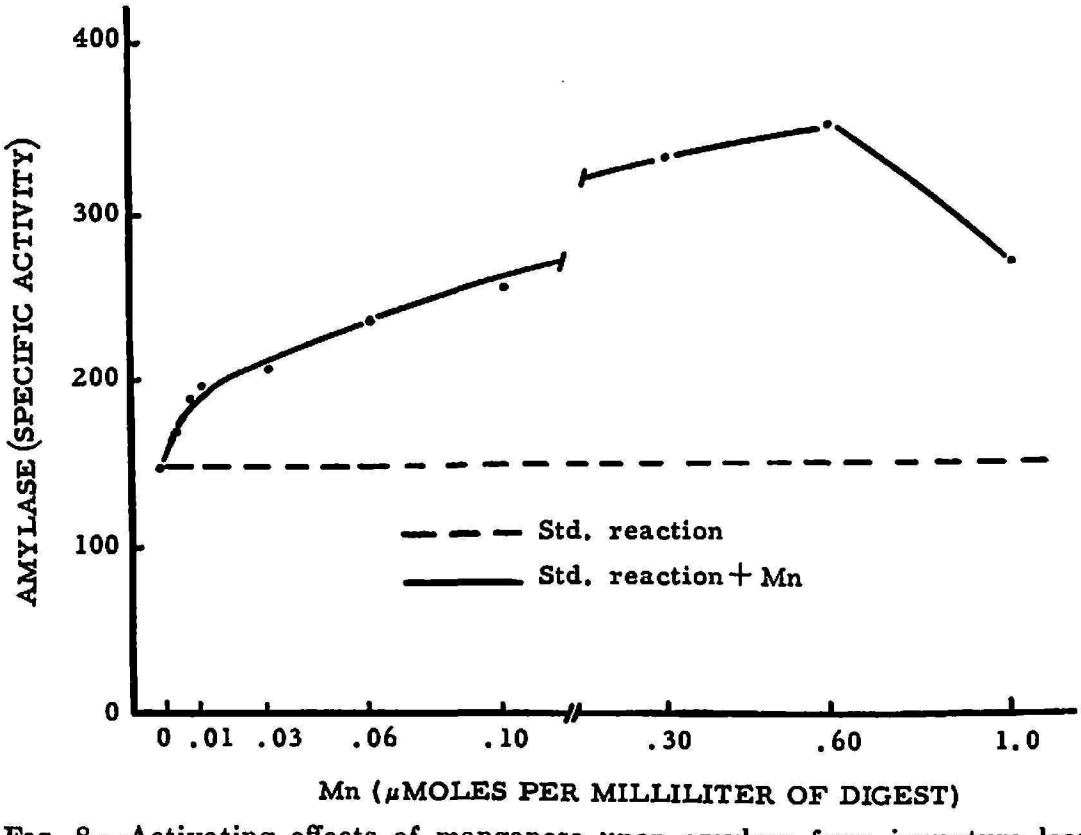

FIG. 8.—Activating effects of manganese upon amylase from immature leaves of sugarcane.

partly purified enzyme preparation employed in the present study is presumed to contain both α- and β-amylases, and, in all probability, it contains complexes of enzymes capable of degrading the polysaccharide completely to free glucose. Should this be the case, then the dinitrosalicylic acid test employed herein would measure the glucose along with maltose and any other reducing products of the amylase action. Our present thinking, therefore, is that the invertase attacking the glucose end of sucrose, and the amylase attacking the glucosidic linkages of starch, are not so completely unrelated as their names imply. Rather, a complex of hydrolytic enzymes must exist in cane with a common affinity for the α-glucosidic linkage in whatever saccharide it might appear.

It was observed while studying invertase that the preparation lost nearly all activity during prolonged dialysis, and that the activity returned in the presence of Mn and dilute sugar solutions. Amylase was somewhat retarded by dialysis against distilled water, but repeated attempts of up to 48 hours dialysis did not remove more than 45 to 50 percent of control (undialyzed) activity. Manganese stimulated the dialyzed preparations equally well as those undialyzed.

A series of dialyzed protein concentrates was diluted with sugar solutions, as was done with invertase preparations,[9] in an effort to determine whether amylase required the presence of sugars for maximum activity. Comparison of protein-water and protein-sugar solutions with undialyzed preparations showed that only about 35 percent of the activity was lost during dialysis (table 4). Nevertheless, activity of enzyme protein diluted with sugar

TABLE 4.—*Effects of dialysis upon sugarcane amylase, and activating effects of dilute sugar solutions*[1]

Sugar concentration of enzyme solution (μmoles/ml.)	Specific activity of amylase prepared as follows—						
	Undialyzed + H_2O	Dialyzed + H_2O	Dialyzed + sucrose	Dialyzed + maltose	Dialyzed + galactose	Dialyzed + glucose	Dialyzed + fructose
2	223	147	205	296	300	264	277
4	228	148	223	323	314	293	293

[1] Each reaction mixture was composed of 0.5 ml. of acetate buffer (pH 5.5), 0.5 ml. of substrate solution (sufficient to make 3.5 mg. of amylopectin per milliliter of digest), and 0.25 ml. of enzyme preparation made up in distilled water or dilute sugar solution. All reactions proceeded for 20 minutes at 30° C.

solutions exceeded both dialyzed and undialyzed protein dissolved in distilled water. As was true of the invertase studies, only sucrose failed to increase activity of dialyzed preparations beyond that of the undialyzed enzyme.

These results again emphasize the similarity between sugarcane amylase and invertase. Both enzymes are stimulated by Mn and dilute sugar solutions, but amylase, in contrast to invertase, apparently does not require either additive to achieve high activity. Possibly some components of the

[9] The following procedure was adopted for preparing protein-sugar solutions: Protein concentrate was dialyzed against distilled water for 40 hours at 2° C. Just prior to running the amylase assays, 6 equal samples were withdrawn from the concentrate, and 1 sample was diluted to the standard concentration with water. The remaining 5 samples were diluted with sugar solutions of sufficient strength to yield a final concentration of 2 or 4 μmoles/ml. of protein solution. Sugar-protein solutions were made up with sucrose, maltose, galactose, glucose, and fructose.

sugarcane amylases are also true invertases and are subject to suppression by dialysis, thus accounting for the 35 to 50-percent loss of "amylase" activity. Conversely, the 4 to 6 percent of invertase activity which could not be removed by dialysis may have represented true amylase acting as "invertase".

SUMMARY

Amylase was extracted from lyophilized tissues of the meristem, leaf, sheath, node, and internode areas of 10-month-old sugarcane. Immature leaves (−1 and 0) were the richest source, while strong activity was also obtained with 8- to 10-node preparations. About 70 percent of the amylase of water extracts was precipitated with ammonium sulfate between 48- and 60-percent saturation.

Both potato starch and amylopectin were readily hydrolyzed, although amylose, corn starch, and inulin were also acted upon. Maximum velocity was obtained with 8 mg. of amylopectin per milliliter of digest. Optimum pH was 5.5. Amylase was highly active between pH 4.5 and 6.0, and declined rapidly above pH 6. Amylase was almost inactive at 16° C., but increased seventeenfold to a maximum at 50° C.

Both mercury and iodide completely inhibited amylase at 1×10^{-4} μmoles/ml. of digest. The effects of iodide, and to a lesser extent of mercury, were reversed by increasing substrate concentrations. Magnesium partly inhibited the enzyme at 0.1 μmole/ml. Manganese stimulated amylase over the range of 0.003 to 0.60 μmole/ml. of digest.

Thirty-six- to forty-eight-hour dialysis of amylase preparation against distilled water caused moderate activity decreases of 35 to 50 percent. The activity was readily restored with added manganese and traces of sugars. Sucrose was least effective, but maltose, galactose, glucose, and fructose all stimulated amylase activity above that of control, *i.e.* undialyzed, preparations.

Several similarities were observed between amylase and invertase, including identical saturation ranges during salt fractionation, identical pH optima, similar responses to increasing temperature, inhibition by mercury, and activation by manganese and sugars. It is suggested that the amylases and α-glucosidase of sugarcane are very closely related, if not identical, and that a common property of the two types of enzymes is an affinity for α-glucosidic bonds.

RESUMEN

A caña de azúcar de 10 meses de edad se le extrajo amilasa de los tejidos liofilizados del meristemo, de la hoja, de la yagua, de los nudos y de distintas áreas de los entrenudos. Las hojas inmaduras (−1 y 0) fueron

la más rica fuente de esta substancia, mientras que se observó fuerte actividad con las preparaciones que se hicieron de los nudos del 8 al 10. Alrededor del 70 por ciento de la amilasa presente en los extractos de agua se precipitó con sulfato de amonio a una saturación de 48 a 60 por ciento.

Tanto el almidón de papa como la amilopectina se hidrolizaron con rapidez, aunque la hidrólisis actuó también sobre la amilosa, el almidón de maíz y la inulina. Obtúvose una velocidad máxima con 8 mg. de amilopectina por mililitro de digesto. El pH óptimo fue de 5.5. La amilasa fue muy activa a un nivel de pH que fluctuó entre 4.5 y 6.0, y declinó rápidamente a un pH en exceso de 6. La amilasa se inactivó virtualmente a una temperatura de 16° C, pero aumentó 17 veces más hasta alcanzar un máximo a 50° C.

El mercurio y el yoduro inhibieron por completo la amilasa a 1×10^{-4} μmoles/ml. de digesto. Los efectos del yoduro, y en menor grado los del mercurio, se revirtieron al aumentarse las concentraciones del substrato. El magnesio inhibió parcialmente la enzima al nivel de 0.1 de μmol/ml. Por el contrario, el manganeso estimuló la amilasa al nivel de entre 0.003 a 0.60 de μmol/ml. de digesto.

La acción dializadora por un período de 36 a 48 horas sobre una preparación de amilasa en presencia de agua destilada, causó una reducción moderada de 35 a 50 por ciento en la actividad.

La actividad se restauró prestamente al añadirse manganeso y cantidades mínimas de azúcares. La sacarosa fue la menos efectiva, pero la maltosa, galactosa, glucosa y fructosa estimularon todas la actividad de la amilasa a un nivel superior al que se obtuvo con las preparaciones-testigos (sin dializar).

Se observaron varias similaridades entre la amilasa y la invertasa, inclusive idénticas saturaciones durante la fraccionación química de la sal; un pH óptimo igual; reacciones similares al aumentar la temperatura; inhibición por la acción del mercurio; y activación en presencia de manganeso y azúcares. Parece existir una estrecha relación entre la amilasa y la α-glucosidasa de la caña de azúcar, si es que no son idénticas y a la vez, común a ambos tipos de enzimas una afinidad química para establecer enlaces α-glucosídicos.

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