[CONTRIBUTION FROM THE CHEMICAL LABORATORIES OF COLUMBIA UNIVERSITY, NO. 317.]

INFLUENCE OF HYDROGEN-ION CONCENTRATION UPON ENZYMIC ACTIVITY OF THREE TYPICAL AMYLASES.[1]

BY H. C. SHERMAN, A. W. THOMAS AND M. E. BALDWIN.

Received November 9, 1918.

Since enzymes are known by their actions and as yet the best guide to progress and indication of success in attempts to isolate, concentrate or purify a natural enzyme is afforded by properly controlled quantitative determinations of its activity, we have devoted much attention to the problem of defining the conditions under which each of the three amylases with which we have chiefly worked may exert its full activity as an enzyme.

Pancreatic and malt amylases and that of *Aspergillus oryzae* (prepared from taka-diastase) have been selected as representative of the starch-splitting enzymes of the higher animals, higher plants, and fungi, respectively.

In connection with purification experiments previously described we have noted the conditions employed to secure a suitable environment for the activity of each of these enzymes and have established, by means of an extended series of experiments[2] with strong and weak acids and acid salts, the fact that the optimum activity of malt amylase, as shown in tests of 30 minutes' duration at 40°, is obtained at a hydrogen-ion concentration of P_H 4.4 to 4.5 (Sorensen notation) or $C_{H^+} = 1 \times 10^{-4.4}$ to $C_{H^+} = 1 \times 10^{-4.5}$.

The purpose of the experiments described in this paper was to determine as definitely as possible the hydrogen-ion concentration which induces optimum activity of the pancreatic and the fungus amylase and to establish for each of the three amylases the limits of hydrogen-ion concentration within which any enzymic activity is shown and the form of the curve representing the activities at all concentrations of hydrogen ion between these limits. In all of the experiments upon the influence of hydrogen ion herein described the intention has been to supply sufficient electrolyte to enable the enzyme in each case to show as high an activity as the given concentration of hydrogen ion permits.

The experimental methods employed in the present research were essentially those used in our earlier study of the effect of acids and salts upon malt amylase,[3] except that in working with the enzymes increased precautions were taken to prevent any action of light, and in measuring

[1] Eighteenth paper on amylases and related enzymes. For earlier papers see THIS JOURNAL, 1910–1918.

[2] Sherman and Thomas, *Ibid.*, 37, 623 (1915).

[3] Sherman and Thomas, *Loc. cit.*

hydrogen-ion concentrations by the electrometric method, the use of a current of hydrogen was replaced by the Clark cell and rocking electrode.

The amount of copper reduced by the solution upon which the enzyme had acted was corrected in all cases for the reducing power of the original substrate and, in the few cases in which it was necessary, for the deterioration in activity of the enzyme occurring during the time occupied in the completion of the series of measurements required for the construction of the curve representing the activity of each enzyme at the various hydrogen-ion concentrations from P_H 2.0 to P_H 10.0 (Sorensen notation), as shown in Fig. 1.

Pancreatic Amylase.

The measurements of activity of pancreatic amylase were made in solutions containing always sodium chloride together with such amounts of primary or secondary phosphate or of phosphate and phosphoric acid or phosphate and carbonate as would give the desired concentration of hydrogen ion. In determining the hydrogen-ion concentrations the electrometric method with Clark cell and rocking electrode was used. The determinations recorded were those actually measured at room temperature of approximately 25°. The action of the enzyme upon the substrate took place at a temperature of 40°, accurately maintained by means of a large water-bath thermostat. The activity of the enzyme is expressed in terms of diastatic power on the scale used in this laboratory since 1910.[1] If expressed on the Lintner scale the values would be about 50% higher.

The results of the chief series of experiments upon pancreatic amylase are shown in Tables I and II.

The approximate optimum concentration of chloride and phosphate had been established by earlier work in this laboratory. As a further

TABLE I.—INFLUENCE OF SODIUM CHLORIDE UPON ACTIVITY OF PANCREATIC AMYLASE IN PRESENCE OF SECONDARY SODIUM PHOSPHATE.

Final concentration of Na_2HPO_4 = 0.001 moles per liter.

Final concentration of NaCl. Moles per liter.	Activity. Preparation 60.
0.0001	450
0.01	2690
0.02	2715
0.03	2750
0.04	2765
0.05	2900
0.06	2875
0.07	2830
0.08	2800
0.09	2870
0.10	2830
0.11	2830

[1] Sherman, Kendall and Clark, THIS JOURNAL, 32, 1082 (1910).

check upon the choice of concentration of chloride, a set of 12 measurements of activity was made in which the concentration of phosphate was kept uniform and that of chloride varied with the results shown in Table I.

This confirms the use of a final concentration of 0.05 *M* sodium chloride, in agreement with our earlier work. This concentration of chloride was uniformly maintained in the two series of determinations with varying concentrations of hydrogen ion shown in Table II.

TABLE II.—INFLUENCE OF HYDROGEN-ION CONCENTRATION UPON THE ACTIVITY OF PANCREATIC AMYLASE.

Final concentration. Moles per liter (NaCl = 0.05 *M*).					Activity.	
H_3PO_4.	NaH_2PO_4.	Na_2HPO_4.	Na_2CO_3.	P_H.	Preparation 60.	Preparation N 14 B.
0.00067	0.06	...	..	4.02	0	0
...	0.03	...	..	4.75	570	..
...	0.025	...	..	4.77	..	531
...	0.005	...	..	5.07	884	905
...	0.003	...	..	5.22	1045	..
...	0.001	...	..	5.63	1783	..
...	...	...	..	5.81	2170	2427
...	...	0.00015	..	6.20	..	2825
...	...	0.00050	..	6.72	2930	3020
...	...	0.0010	..	7.09	2905	3005
...	...	0.0025	..	7.47	2615	..
...	...	0.003	..	7.55	2545	2611
...	...	0.005	..	7.72	2355	..
...	...	0.008	..	7.94	..	1975
...	...	0.010	..	8.00	1790	..
...	...	0.015	..	8.10	1580	..
...	...	0.05	..	8.35	985	..
...	...	0.05	0.0002	8.46	..	825
...	...	0.05	0.0004	8.80	450	..
...	...	0.05	0.0010	9.15	..	285
...	...	0.05	0.0025	9.51	100	..
...	...	0.05	0.008	10.11	0	0

In the interpretation of P_H values it should be kept in mind that the specially purified distilled water and the starch solutions which have been made neutral to rosolic acid, the indicator commonly used in such work show values not of 7.0 but of 5.8.

Amylases of Malt and of Aspergillus oryzae.

The general plan of the experiments upon these enzymes was analogous to that just described upon pancreatic amylase. The final concentration of electrolytes and the hydrogen-ion concentrations are shown together with the corresponding measurements of enzyme activities in Table III.

TABLE III.—INFLUENCE OF HYDROGEN-ION CONCENTRATION UPON THE ACTIVITY OF MALT AMYLASE AND THE AMYLASE OF *Aspergillus Oryzae*.

Final concentration. Moles per liter.					Activity.[1]	
H_3PO_4.	NaH_2PO_4.	Na_2HPO_4.	Na_2CO_3.	P_H.	Malt amylase. Preparation 155.	Aspergillus amylase. Preparation 22.
$0.0166^2/_3$	0.06	...	..	2.34	0	0
$0.0056^2/_3$	0.06	...	..	2.70	99	...
$0.0046^2/_3$	0.06	...	..	2.80	175	48
$0.0033^1/_3$	0.06	...	..	3.13	447	120
$0.0023^2/_3$	0.06	...	..	3.40	671	191
$0.0016^2/_3$	0.06	...	..	3.64	805	250
$0.0006^2/_3$	0.06	...	..	4.06	1000	366
...	$0.0666^2/_3$	...	..	4.47	1101	...
...	0.06	...	..	4.48	..	484
...	0.0660	$0.0006^2/_3$	..	4.80	1100	516
...	0.0600	$0.0066^2/_3$	..	5.69	1059	465
...	$0.0333^1/_3$	$0.0333^1/_3$	..	6.65	969	...
...	...	0.0002	..	6.82	..	359
...	...	0.0010	..	7.38	..	185
...	$0.0066^2/_3$	0.0600	..	7.53	628	...
...	...	0.004	..	7.78	..	57
...	...	0.008	..	8.15	..	9
...	...	0.02	..	8.25	240	...
...	...	0.05	0.0004	8.80	64	...
...	...	0.05	0.0025	9.33	0	0

The curves in Fig. 1 summarize the experimental data for each of the three enzymes and show its activities throughout the range of hydrogen-ion concentration within which any activity could be found.

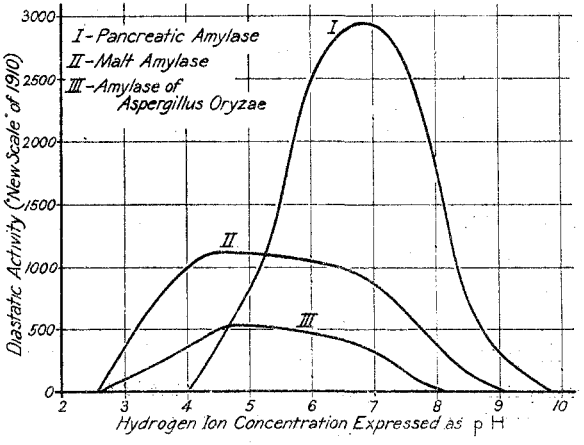

Fig. 1.

[1] Expressed as "diastatic power" on the "new scale" used in this laboratory since 1910.

Summary.

Pancreatic and malt amylase and that of *Aspergillus oryzae* were selected as representative of the starch-splitting enzymes of the higher animals, higher plants, and of the fungi, respectively.

By the use of the Clark cell and rocking electrode the findings of Sherman and Thomas on the optimum hydrogen-ion concentration for malt amylase were confirmed and the optimum for pancreatic amylase was much more sharply defined than had been possible in previous work.

The optimum hydrogen-ion concentration for the amylase of *Aspergillus oryzae* resembles that of malt rather than that of pancreatic amylase.

Pancreatic amylase was active between the limits of P_H 4 to 10 with optimum activity at about 7, the solutions commonly considered neutral showing under similar conditions a P_H value of 5.8.[1]

Malt amylase was active between P_H 2.5 and P_H 9 with optimum activity at 4.4 to 4.5.

The amylase of *Aspergillus oryzae* showed activity from P_H 2.6 to 8 with optimum at about P_H 4.8.

The activities of the three amylases throughout the range of hydrogen-ion concentration in which activity was found are summarized by means of curves (Fig. 1).

The influence of concentration of electrolyte, as distinguished from concentration of hydrogen ion alone, appeared greatest in the case of pancreatic amylase and least in the case of the amylase of *Aspergillus oryzae*.

We are greatly indebted to the Carnegie Institution of Washington for grants in aid of this investigation.

NEW YORK.

THE NITRATION OF SUCROSE: SUCROSE OCTANITRATE.[2]

BY E. J. HOFFMAN AND V. P. HAWSE.

Received November 16, 1918.

The product of the action of nitric-sulfuric acid on cane sugar has hitherto proved of little practical value as an explosive on account of its extreme sensitiveness and liability to spontaneous decomposition. Among the explosive mixtures[3] containing this substance which have been proposed, may be mentioned "glukodine," an intimate mixture of nitroglycerin and "nitrosaccharose" prepared by the nitration of a saturated solution of cane sugar in glycerin, and Bjorkmann's explosive consisting of glukodine mixed with sugar, sodium nitrate and nitrocellulose or carbon. As a constituent of low-freezing dynamites it has likewise received some attention.

[1] Compare Fales and Nelson, THIS JOURNAL, 37, 2769 (1915).

[2] Published by permission of the Director, U. S. Bureau of Mines.

[3] L. Gody, "Matière Explosives," 1907, p. 398.