The author wishes to take this opportunity to express his sincere thanks to Dr. E. C. Franklin, at whose suggestion and under whose careful and kindly direction this investigation was carried out.

Summary

Any compound which is derived from ammonia by substituting negative groups for one or two of the three hydrogen atoms is an ammono acid.

The strength of the ammono acids is dependent upon the negative character of the components of the molecule; thus, the acidity of pyrrole, imidazole, triazole and tetrazole increases in the order given as shown by the conductivity measurements.

A number of the metallic salts of the ammono acids lophine, triazole and tetrazole have been prepared.

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ESTABLISHMENT OF THE OPTIMAL HYDROGEN-ION ACTIVITIES FOR THE ENZYMIC HYDROLYSIS OF STARCH BY PANCREATIC AND MALT AMYLASES UNDER VARIED CONDITIONS OF TIME AND TEMPERATURE[1]

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The development of our investigation of the amylases and related enzymes makes it important now to know, and to be able experimentally to establish and maintain, the reaction most favorable to the activity of each enzyme under investigation throughout a wider range of time and temperature than is involved in the determination of the so-called diastatic powers, for which conditions only had optimal hydrogen-ion activities been established in the course of the earlier work of this Laboratory.

Experiments were therefore undertaken to establish the optimal hydrogen-ion activities for malt and pancreatic amylases when the temperature and time of enzymic hydrolysis were varied. The data obtained are discussed briefly below.

Experiments and Discussion

The experimental methods of measuring amylase activity have been described in detail in previous papers from this Laboratory.[2]

The slight reducing action of the substrate without enzyme was measured for each of the times, temperatures and hydrogen-ion activities

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

[2] Sherman and co-workers, THIS JOURNAL, **41**, 231 (1919); **32**, 1073 (1910); **43**, 2461 (1921); **37**, 623 (1915).

studied, and used as a correction in reporting the enzymic activities. The hydrogen-ion activities reported in this paper were determined electrometrically in portions of the actual substrate systems used.

Malt Amylase in the Presence of Phosphates.—The experiments with malt amylase were carried out both with extracts of barley malt prepared in a uniform manner and with a purified preparation which had been obtained according to the method of Sherman and Schlesinger and these two forms of the enzyme gave essentially concordant results. In the earlier series of these experiments, the enzyme was allowed to act upon starch solutions containing 0.06 *M* dihydrogen phosphate and adjusted to a systematic series of Sørensen values at close intervals from P_H 4.0 to P_H 6.0. The time was varied from one-half hour to two hours and the temperature from 30 to 70°. These experiments showed that as the temperature of the hydrolysis is increased, malt amylase becomes more sensitive to changes in the hydrogen-ion activity of its environment. Also the enzyme exerted its optimal activity in less acid solutions as the temperature of the hydrolysis was increased. Thus malt amylase exerted its optimal activity under the conditions of these experiments in solutions adjusted at room temperature to Sørensen values of P_H 4.4 to 5.0 at 30 and 40°; of 4.6 to 5.0 at 50°; of 5.2 to 5.5 at 60° and of 5.3 to 5.8 at 70°. With the purified preparation the zone of optimal activity was slightly narrower, P_H 4.6 to 4.8 at 40° and 5.3 to 5.4 at 60°.

At any given temperature, however, malt amylase exerts its activity under practically the same optimal conditions when the period of hydrolysis is varied from one-half to two hours.

Between the completion of this part of our experimental work and its preparation for publication, Olsen and Fine[3] published the results of experiments with a mixture of wheat and malted barley flours in which also it was found that the enzyme exerts its optimal activity at different hydrogen-ion activities according to the temperature, and noted a greater influence of small changes of hydrogen-ion activity at the higher temperatures.

Electrometric Measurements at Temperatures of Enzyme Experiments.—The electrometric measurements of the solutions so far considered were made at room temperature and calculated with the usual temperature corrections to Sørensen or P_H values.[4] As shown above, these values were found to differ markedly for those solutions which enable malt amylase to exert its optimal activity at the different temperatures. The question therefore arose as to whether the hydrogen-ion activities of the starch solutions measured at room temperature are also the hydrogen-

[3] Olsen and Fine, *Cereal Chemistry*, **1**, 215 (1924).

[4] Clark, "The Determination of Hydrogen Ions," Williams and Wilkins, Baltimore, **1923**, 2nd ed., pp. 457, 459.

ion activities of the solutions during hydrolysis. To answer this question e.m.f. measurements were made at the higher temperatures as well as at room temperature upon starch solutions (substrate systems) made up exactly as were those which had been found to afford the most favorable conditions for the amylase activity at each temperature. The P_H values were calculated according to the equation e.m.f. (saturated calomel) − e.m.f. (obs.) = $0.000198T \log(C_{H^+}/10^{-4.72})$, where $0.000198T \log(C_{H^+}/10^{-4.72})$ is the electrode potential for hydrogen at atmospheric pressure[5] and where 0.5266 is[6] the e.m.f. of the saturated calomel cell at 25°. The calomel cell was of the type described by Wilson and Kern[7] and could not conveniently be immersed in the thermostat. There would, therefore, be a slight Peltier effect which would cause some error in the above calculations but which would not be significant in the changes in which we are interested here. The e.m.f. observed was corrected for barometric pressure by use of the equation,[4] $E_{bar.} = (0.000198T/2) \log(760/x)$, where x is the barometric pressure minus the vapor pressure of water at the temperature under consideration. This correction was added to the observed e.m.f. in the above equation.

The results are given in Table I and show that the e.m.f. and Sørensen values obtained with a given solution measured at the lower and higher temperatures are not markedly different and that the measurements made at room temperature are also a measure of the hydrogen-ion activities of the substrates at the temperatures of hydrolysis. This would seem to indicate that malt amylase exerts its optimal activity under different conditions of hydrogen-ion activity at different temperatures.

TABLE I

INFLUENCE OF TEMPERATURE UPON E.M.F. OF THOSE SOLUTIONS WHICH ENABLE MALT AMYLASE TO EXERT ITS OPTIMAL ACTIVITY AT 30, 50, 60 AND 70°

Temps. of enzymic hydrolyses °C.	Electrometric Measurements and Sørensen Values: At room temperature E.m.f.[a]	Temperature of e.m.f. reading, °C.	Corresponding P_H value[b]	At temperatures of enzymic hydrolyses E.m.f.[a]	Temperature of e.m.f. reading, °C.	Corresponding P_H value[b]
30	0.5137	24	4.53	0.5158	30	4.56
50	.5292	26	4.76	.5290	50	4.79
60	.5598	25	5.28	.5564	60	5.22
70	.5800	24.5	5.62	.5745	70	5.50

[a] The barometric reading for the above e.m.f. values was 761.

[b] Calculated as explained in the text.

Malt Amylase in the Presence of Acetate-Acetic Acid Mixtures.—In view of the work of Chrzaszcz, Bidzinski and Krause[8] and of the more

[5] Fales, "Inorganic Quantitative Analysis," The Century Co., New York, 1925, p. 257.

[6] Fales and Mudge, THIS JOURNAL, **42**, 2434 (1920).

[7] Wilson and Kern, *Ind. Eng. Chem.*, **17**, 74 (1925).

[8] Chrzaszcz, Bidzinski and Krause, *Biochem. Z.*, **160**, 155 (1925).

recent work of Luers and Nichimura,[9] we have carried out a second series of experiments with malt amylase under the same conditions described for the first series, except that we used 0.01 *M* sodium acetate-acetic acid buffer mixtures instead of phosphate to regulate the hydrogen-ion activities of the substrates. It was found that in the presence of acetate and in experiments of the same duration, malt amylase exerts its optimal activity at 40° and at 60° in solutions of about P_H 4.6 for half an hour and at about P_H 4.8 for two-hour periods of hydrolysis. This confirms the findings of Luers and Nichimura and indicates that the effects of acetate and phosphate are somewhat different in this respect. The enzyme is more sensitive to changes in the hydrogen-ion activity of its environment at the higher temperature, whether it acts in the presence of phosphate or of acetate, but in the presence of acetate there is a tendency for the optimal enzymic activity to occur in slightly less acid solutions as the experimental period is lengthened.

Within the range covered by our experiments, heat does not appreciably influence the hydrogen-ion activities of our systems. This is shown for the phosphate solutions by the experiments summarized in Table I and has also been experimentally demonstrated for the acetate solutions. Thus the e.m.f. value of the starch solution, containing 0.01 *M* acetate, which permitted malt amylase to exert its optimal activity at 60° was 0.5215 (or P_H 4.65) when measured at 23.5° and 0.5240 (or P_H 4.72) when measured at 60°, the temperature of the hydrolysis.

Parallel hydrolyses of the phosphate and acetate substrates carried out at 60°, with determinations of the hydrogen-ion activities of the solutions before and after the hydrolyses, showed that neither of the solutions had changed in hydrogen-ion activity during the experiment.

Pancreatic Amylase.—Experiments similar to those described with malt amylase were also carried out with pancreatic amylase, measuring both its saccharogenic and amyloclastic activities. Starch solutions, two per cent. for the former and one per cent. for the latter measurements, were adjusted to Sørensen values systematically varied at close intervals from P_H 6.3 to P_H 7.7. The enzymic activity was measured in periods of one-half, one and two hours at 30 to 70°.

In the first series of experiments, a commercial pancreatin was used in the presence of 0.05 *M* sodium chloride and 0.0005 *M* disodium phosphate. In the second series of experiments a purified enzyme preparation obtained according to the method of Sherman and Schlesinger was used in the presence of 0.03 *M* sodium chloride and 0.01 *M* phosphate mixtures which had very recently been found[10] to afford slightly more satisfactory conditions.

[9] Luers and Nichimura, *Wochenschrift für Brauerei*, **43**, No. 38, p. 415–416, Sept., 1926.

[10] Adams, *Dissertation*, Columbia University, 1927.

The results of the two series of experiments are in agreement and show that under these conditions the hydrogen-ion activities for the optimal enzymic activity expressed as Sørensen values were P_H 7.0 to P_H 7.2 for one-half to two hours at 30 to 50°, P_H 6.9 for half an hour and P_H 6.7 for two-hour periods at 60°. The rapid destruction of the enzymic activity at 70° made it difficult to obtain any satisfactory results at this temperature.

This difference between the hydrogen-ion activities for the optimal activity of pancreatic amylase at the lower temperatures and at 60°, while very slight, appears to be a true difference and not due to the influence of heat on the hydrogen-ion activities of the solutions themselves, for the hydrogen-ion activity of the solution affording optimal enzymic activity at 60° was found to be the same when measured at room temperature and at 60°.

Summary

As the temperature of hydrolysis is increased from 30 to 70°, malt amylase exerts its optimal activity at different hydrogen-ion activities when acting in the presence of 0.06M phosphate mixtures; whereas, in the presence of 0.01M acetate-acetic acid mixtures, this amylase exerts its optimal activity in solutions of the same hydrogen-ion activity at the different temperatures.

In the presence of 0.06 M phosphate, the hydrogen-ion activities of the solutions affording optimal activity of the enzyme did not show appreciable change as the experimental period was lengthened from one-half to two hours at any given temperature (within the range 30–60°) while in the presence of 0.01 M acetate there is a tendency for the enzyme to exert its optimal activity in slightly less acid solutions as the period of hydrolysis is increased.

The difference in the behavior of the enzyme in the presence of phosphate and of acetate has been shown by e.m.f. measurements of the solutions at room temperature and at the temperatures of hydrolysis, and by measurements before and after hydrolysis, not to be due to the influence of heat or of the products of hydrolysis upon the hydrogen-ion activities of the solutions themselves.

Whether in the presence of phosphate or of acetate, a higher temperature seems to render malt amylase more sensitive to changes in the hydrogen-ion activity of its environment.

Pancreatic amylase, in experiments of one-half to two hours at 30–50°, exerted its optimal activity at P_H 7.0–7.2; but in experiments at 60° the optimal activity for half-hour periods was found at P_H 6.9 and for two-hour periods at P_H 6.7.

From the standpoint of accurate investigation of enzyme action, the results here reported upon two typical amylases indicate that the factors

which induce optimal enzymic activity are even more dependent upon each other than has previously been realized and that none of them should be regarded as fixed if any of the others is changed.

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CATALYTIC OXIDATIONS IN AQUEOUS SOLUTIONS I. THE OXIDATION OF FURFURAL

BY NICHOLAS A. MILAS[1]

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Introduction

The present investigations are part of the applications of catalysts such as osmium tetroxide, vanadium pentoxide, etc., to the oxidation of organic compounds by means of chlorates.

In previous communications[2] it has been shown that small quantities of osmium tetroxide can induce the oxidation of relatively large quantities of organic substances by means of chlorates. The use of vanadium pentoxide with chlorates has been heretofore limited only to a very few special cases[3] in which vanadium salts were used rather than the oxide in the presence of strong acids. The present paper describes several experiments in which vanadium pentoxide has been successfully used in neutral as well as in dilute acid solutions to induce the oxidation of furfural and pyromucic acid by means of chlorates.

Furfural has been oxidized with dil. potassium permanganate solution to pyromucic acid by Volhard,[4] with bromine water at the temperature of the water-bath to mucobromic acid by Simonis,[5] with hydrogen peroxide in the presence of ferrous salts to δ-hydroxyfurfural by Cross, Bevan and Heiberg,[6] and with Caro's acid to succinic acid by Cross, Bevan and Briggs.[7]

In the Experimental Part, it will be shown that with a mixture of sodium chlorate and small quantities of osmium tetroxide in dilute acid solution, furfural yields principally mesotartaric acid, while fumaric acid is the chief product formed when the reaction is carried out in neutral solution with osmium tetroxide replaced by vanadium pentoxide. Similar results

[1] National Research Fellow in Chemistry.

[2] (a) Milas and Terry, THIS JOURNAL, **47**, 1414 (1925). (b) Terry and Milas, *ibid.*, **48**, 2647 (1926).

[3] Guyard, *Bull. soc. chim.*, [2] **25**, 58 (1876); *Chem. News*, **33**, 70 (1876). Willstätter and Dorogi, *Ber.*, **42**, 4128 (1909).

[4] Volhard, *Ann.*, **261**, 379 (1891).

[5] Simonis, *Ber.*, **32**, 2084 (1899).

[6] Cross, Bevan and Heiberg, *J. Chem. Soc.*, **75**, 747 (1899).

[7] Cross, Bevan and Briggs, *Ber.*, **33**, 3132 (1900).