tion is probably a simple addition to the —C=C— bond and does not involve the ketone group.

2. This compound was reduced to 1-amino-2-chloro-1,3-diphenylpropanone-3 by concentrated hydrochloric acid and on further reduction with sodium amalgam 1-amino-1,3-diphenylpropanol-3 was obtained.

IOWA CITY, IOWA

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, COLUMBIA UNIVERSITY, No. 556]

A QUANTITATIVE STUDY OF THE INFLUENCE OF SODIUM ACETATE, SODIUM BORATE, SODIUM CITRATE AND SODIUM PHOSPHATE UPON THE ACTIVITY OF PANCREATIC AMYLASE

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In continuation of our study of the nature and properties of pancreatic amylase it became important to obtain further quantitative information on the influence of electrolytes upon its action. The first series of experiments described briefly here deal with the sodium salts of acetic, boric, citric and phosphoric acids.

Data dealing with the influence of these salts upon enzymic activity have been reported from time to time by various investigators but unfortunately they are conflicting and hard to interpret because of failure to recognize the simultaneous importance of other factors such as the hydrogen-ion activities of the solutions, the concentrations of substrates and electrolytes and the value of strictly comparable data obtained under parallel conditions.

Experiments were, therefore, planned in which each of these factors was carefully considered.

Experimental

The general plan was to study the influence upon the amylase activity, of each salt alone in solutions in which the hydrogen-ion activities differed at close intervals (by about 0.1 P_H) and then to make a quantitative parallel comparison of the influence of the different salts, the solutions being adjusted in each case to the hydrogen-ion activity which had been found to favor the optimal activity of the enzyme in the presence of that salt.

The same preparation of pancreatin was used throughout and its activity measured under constant conditions, half-hour hydrolyses of 2% starch at 40°. The hydrogen-ion activity of each starch solution used was measured electrometrically at room temperature.

As has been shown in earlier papers from this Laboratory,[1] amylase

[1] Sherman and co-workers, THIS JOURNAL, **32**, 1073, 1087 (1910); **33**, 1195 (1911); **34**, 1104 (1912); **35**, 1617, 1784 (1913); **37**, 623 (1915); **41**, 231 (1919); **43**, 2461 (1921).

activity may be measured quantitatively by determining, under carefully controlled conditions, the increase in the reducing action of the hydrolysis mixture caused by the formation of reducing sugar from the starch by the enzyme. The details of the method will not be repeated here.

All salts were recrystallized and air-dried. Sodium acetate, tertiary sodium citrate and mono- and disodium phosphates were used as such. Sodium borate was obtained in the starch solutions by adding recrystallized boric acid and adjusting the hydrogen-ion activities with 0.01 *M* sodium hydroxide. The influence of the salts was measured in the presence of 0.05 *M* sodium chloride.

Discussion of Results

Experiments with Sodium Phosphate.—The total concentration of sodium phosphate in the solutions was varied from 0.0005 to 0.05 *M* and the hydrogen-ion activities were adjusted by the use of suitable proportions of equimolar solutions of mono- and disodium phosphate. The activity of pancreatic amylase was found to be independent of these concentrations of phosphate provided the hydrogen-ion activities of the solutions were suitably adjusted to P_H 7.0 to 7.2 for the lower concentrations and to P_H 7.0 for solutions containing 0.05 *M* sodium phosphate. A concentration of 0.004 *M* was chosen as suitable for the comparison of sodium phosphate with the other salts studied.

Experiments with Sodium Citrate.—In the presence of 0.004 *M* sodium citrate the enzyme was found to exert its optimal activity in solutions of P_H 7.0 to 7.2. Direct comparisons showed that the amylase was practically as active in the presence of citrate as in the presence of phosphate.

Experiments with Sodium Acetate.—As this salt is not effective as a buffer in solutions of about P_H 7.0 it was difficult to control the hydrogen-ion activities of the solutions as accurately as is desirable for work with enzymes. The data obtained, however, indicate no marked difference in the influence of acetate and phosphate upon pancreatic amylase. In comparable experiments, the activity of the enzyme although more irregular was nearly if not quite as high in the presence of acetate as in the presence of phosphate and the differences and irregularities in the results with the acetate solutions can readily be explained by slight departures from the optimum conditions in the less adequately buffered solutions.

Experiments with Sodium Borate.—Experimental difficulties in controlling the hydrogen-ion activities of the solutions containing sodium borate were also encountered, and probably here too because the solutions were inadequately buffered. It was found, however, that when the solutions were adjusted to about P_H 7.0, ten-fold variations in the borate concentration from 0.001 *M* to 0.01 *M* did not influence the activity

of the enzyme and that the activity of the enzyme, in the presence of borate, while less regular and slightly lower, was within the experimental limits of its activity in the presence of phosphate.

Experiments in the Absence of any "Buffer" Salt.—A further comparison of the activity of pancreatic amylase in solutions with and without phosphate was made. The hydrogen-ion activities were adjusted in one series of solutions with phosphate and in the other with sodium hydroxide, no phosphate being added. Sodium chloride, 0.05 *M*, was present in both series. The results in the unbuffered solutions were more irregular and the activity of the enzyme was often, but not always, slightly lower than in the presence of phosphate. The results as a whole indicate that the addition of phosphate to the substrate aids the enzymic activity by its favorable influence in helping to control the hydrogen-ion activities of the solutions rather than because of any "specific" influence of the phosphate itself.

Conclusions

The activity of pancreatic amylase in the presence of 0.05 *M* sodium chloride and under optimal conditions of hydrogen-ion activity is practically the same in the presence of equimolar concentrations of citrate and phosphate. It is slightly lower in the presence of borate and acetate but this decrease in activity seems to be more probably due to less adequate control of hydrogen-ion activity in the less adequately buffered solutions rather than to any specific effect of the acetate or borate ions.

When acting in the presence of 0.05 *M* sodium chloride and 0.004 *M* concentrations of the sodium salts of boric, citric and phosphoric acids for half-hour periods at 40°, pancreatic amylase exerts its optimum activity in solutions of P_H 7.0 to 7.2.

In the presence of 0.05 *M* sodium chloride the activity of the enzyme appears to be independent of the concentrations of phosphate from 0.0005 to 0.05 *M* and of borate from 0.001 to 0.01 *M* provided the optimal conditions of hydrogen-ion activity are maintained.

Given optimal hydrogen-ion activity, the activity of the enzyme appears to be practically the same in starch solutions containing 0.05 *M* sodium chloride and no phosphate, as it is in solutions containing phosphate as well.

In view of the fact that the activity of the enzyme in the absence of any salt except sodium chloride may be as great as in the presence of phosphate and that the activity is practically the same in the presence of acetate, borate, citrate or phosphate, it seems improbable that these salts have any marked specific effect upon the enzyme.

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