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A QUANTITATIVE COMPARISON OF THE INFLUENCE OF NEUTRAL SALTS ON THE ACTIVITY OF PANCREATIC AMYLASE[1]

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In previous papers[2] the optimal hydrogen-ion activities and concentrations of salts for pancreatic amylase in the presence of different salts have been reported. With these conditions established it was possible to continue the investigation and obtain direct comparisons of the influence of these salts (each under its own optimal conditions) on the activity of the enzyme. The salts studied were the chlorides of sodium, potassium and lithium and the bromide, nitrate, chlorate, sulfocyanate, fluoride, sulfate and phosphate of sodium. Mixtures of sodium chloride with either sodium nitrate or sulfate were also studied.

Experimental

The results for the individual salts are summarized in Table I, and are given in terms of the effect of sodium chloride, taken as 100, because this salt was found to have the most favorable influence upon the enzyme.

TABLE I

COMPARISON OF THE ACTIVITY OF PANCREATIC AMYLASE IN THE PRESENCE OF VARIOUS NEUTRAL SALTS EACH AT ITS OPTIMAL HYDROGEN-ION ACTIVITY AND CONCENTRATION[a]

	Activity, compared to that obtained in the presence of 0.03 *M* NaCl		
Salt	Pancreatin, power 280	Purified preparation, power 940	Purified preparation, power 1130
Sodium chloride	100	100	100
Potassium chloride	100	100	100
Lithium chloride[b]	80–90	..	80–90
Sodium bromide	77	77	77
Sodium nitrate	41	40	40
Sodium chlorate	29	27	27
Sodium sulfocyanate	29	..	28
Sodium fluoride	24	..	21
Sodium sulfate	0	0	0
Sodium phosphate	0	0	0

[a] Mixtures of acid and alkaline sodium phosphates corresponding to a total concentration of 0.01 *M* phosphate were present in all cases.

[b] As described in the text the procedure was slightly different with this salt.

Because of the insolubility of lithium phosphate it was necessary to follow a different procedure in determining the influence of lithium chloride on the activity of pancreatic amylase than that followed with the other

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

[2] Sherman, Caldwell and Adams, THIS JOURNAL, **50**, 2529, 2535 (1928).

salts.[2] Sodium hydroxide, 0.01 *M*, was used instead of mixtures of sodium phosphate to adjust the solutions to different hydrogen-ion activities. It was not possible to measure experimentally the hydrogen-ion activities of these unbuffered, nearly neutral, solutions or to plan to reproduce any one of them exactly for use in comparisons. However, by planning parallel and overlapping determinations of enzymic activity, it was possible to eliminate the influence of hydrogen-ion activity and to obtain relative values for the optimal activity of the enzyme in the presence of different concentrations of lithium chloride, which were comparable with each other and with the optimal activity of the enzyme in the presence of sodium chloride. Thus 0.005, 0.02, 0.03 and 0.05 *M* lithium chloride solutions were studied and it was found that the optimal activity of the enzyme in the presence of 0.02 or 0.05 *M* lithium chloride was 80 to 90% of the optimal activity of the enzyme in the presence of sodium chloride. This was found to be true whether the hydrogen-ion activities of the sodium chloride solutions were adjusted with mixtures of sodium phosphates or with sodium hydroxide. This would indicate that the lower activity of the enzyme in the presence of lithium chloride was not due to the absence of phosphate in the case of lithium chloride.

From the results summarized in Table I, it would seem that, of the ions considered here, the anions are by far the most important in their influence upon this enzyme. Pancreatic amylase may be activated to the same extent by potassium or sodium chlorides but the concentration of potassium chloride necessary fully to activate pancreatic amylase is slightly greater than the concentration of sodium chloride needed. Lithium chloride activates pancreatic amylase but even when optimal concentrations are used the activity obtained is not equal to that obtained in the presence of either sodium or potassium chlorides. The cations, therefore, seem to have some slight influence.

Of the anions studied, chloride is the most efficient ion and the salts considered here may be placed in the following order in their influence on the hydrolysis of starch by pancreatic amylase: sodium and potassium chlorides, lithium chloride, sodium bromide, sodium nitrate, sodium chlorate and sodium sulfocyanate, sodium fluoride. The results obtained with the purified and the commercial enzyme preparations have been found to be similar throughout these experiments.

A Study of the Possible Interrelation of the Influence of Different Neutral Salts.—The next series of experiments was planned to study the influence on the activity of pancreatic amylase of the presence of more than one of these salts. The activity of the enzyme was measured in the presence of 0.02 *M* sodium chloride with additions of 0.01 to 0.20 *M* sodium nitrate or 0.01 to 0.10 *M* sodium sulfate. Direct comparisons of the activity of the enzyme in the presence of each combination of salts

were made with the activity of the enzyme in the presence of 0.02 *M* sodium chloride alone. In these series of experiments 0.02 *M* sodium chloride solutions were used instead of 0.03 *M* as in the previous experiments in order to reduce the sodium chloride concentration to the minimum capable of producing complete activation of the enzyme. Each solution was adjusted to the hydrogen-ion activity previously found[2] to be most favorable under the conditions maintained in this investigation. The results are summarized in Table II and are given in terms of the effect of sodium chloride taken as 100.

TABLE II

INFLUENCE OF THE CONCENTRATION OF SODIUM SULFATE OR SODIUM NITRATE ON THE ACTIVITY OF PANCREATIC AMYLASE IN THE PRESENCE OF SODIUM CHLORIDE[a]

	Activity compared to that obtained in the presence of NaCl	
Concentration of sodium nitrate, *M*	Purified preparation, power 1130	Pancreatin, power 280
0.01	91	92
.10	66	68
.20	55	54
Concentration of sodium sulfate, *M*		
0.01	100	100
.05	94	94
.10	90	92

[a] Mixtures of acid and alkaline sodium phosphates corresponding to a total concentration of 0.01 *M* phosphate were present in all cases.

From the data given in Table II it is seen that as the concentration of sodium nitrate or sulfate is increased the activity of pancreatic amylase decreases. The addition of sodium nitrate has a much more noticeable effect in decreasing the activity of the enzyme than additions of sodium sulfate. The activity of pancreatic amylase in the presence of 0.02 *M* sodium chloride is reduced by the addition of sodium nitrate even when the concentration of sodium nitrate present is much lower than the concentration of sodium chloride.

Possible Explanations of the Influence of Neutral Salts upon Enzymic Activity.—The results of these investigations show that there is some decided specific influence of these salts on the activity of pancreatic amylase but how this influence is exerted is not yet known.

Several previous investigators[3] have reported indications of a relation between the concentration of enzyme and the concentration of sodium chloride necessary to activate it. For this reason a series of experiments was carried out to see whether decreasing the concentration of enzyme to one-half that previously used would change the results obtained. A series of starch solutions containing graded concentrations of sodium

[3] Starkenstein, *Biochem. Z.*, **24**, 210 (1910); **47**, 300 (1912); Norris, *ibid.*, **7**, 622 (1913).

chloride from 0.005 to 0.05 *M*, each adjusted to the optimal hydrogen-ion activity previously determined, were hydrolyzed in parallel using for every 100 cc. of 2% starch 0.03 instead of 0.06 mg. of the purified preparation which had a power of 1130. The optimal activity of the enzyme was found to occur in the presence of 0.02 to 0.05 *M* sodium chloride, just as had been observed in the presence of the more concentrated enzyme solutions.

Other investigators[4,5] state that the concentration of starch influences the amount of salt that is essential. Experiments with 4% instead of 2% starch were therefore also carried out.

The optimal hydrogen-ion activity for the activity of pancreatic amylase in the presence of 4% starch solutions containing 0.01 to 0.05 *M* sodium chloride was first determined and was found to be P_H 6.8 to 7.2, which is similar to the optimal P_H 7.1 to 7.2 observed[2] for the enzyme in the presence of 2% starch. The concentration of sodium chloride permitting the optimal activity of pancreatic amylase was found to be 0.02 to 0.05 *M*, which is the same as that found for the enzyme in the presence of 2% starch.

These results do not necessarily indicate that there is no relation between concentration of starch, enzyme and salt, because there is already present in 2% starch solutions a large excess of starch and further increase in the concentration of starch would not necessarily call for further increase in the concentration of sodium chloride. It is evident, however, that for the conditions regularly used in determining amylase activity in this Laboratory, solutions of 0.02 to 0.05 *M* sodium chloride and hydrogen-ion activities corresponding to P_H 7.0 to 7.2 will permit optimal enzymic activity when the substrate is 2 to 4% starch and when the concentration of enzyme is such that the activity is within the limits usually maintained here, the formation of 100 to 240 mg. of maltose in thirty minutes at 40°.

There are several possible explanations of the influence of neutral salts on the activity of pancreatic amylase but the experimental evidence substantiating most of these explanations is not conclusive.

It is possible that the influence of neutral salts on the activity of pancreatic amylase may be due to some influence on the colloidal state of the enzyme. Sherman, Thomas and Caldwell[6] found that the isoelectric point of malt amylase coincides with the optimal hydrogen-ion activity for the enzymic activity of malt amylase. This would indicate that malt amylase is most active in a state in which it is least soluble and most readily salted out.

If the optimal hydrogen-ion activity and the isoelectric point also coin-

[4] Kübel, *Pflüger's Arch.*, **76**, 276 (1899).

[5] Ambard, Pellois and Bricka, *Bull. soc. chim. biol.*, **2**, 42 (1920).

[6] Sherman, Thomas and Caldwell, THIS JOURNAL, **46**, 1711 (1924).

cide for pancreatic amylase, as has been indicated,[7] it would be expected that those neutral salts which are most effective in precipitating it would have the most favorable influence on its activity. When the anions which have been considered in this investigation are arranged in the order of their favorable effect upon the hydrolysis of starch by pancreatic amylase, the following order results: chloride, bromide, nitrate, chlorate and sulfocyanate, fluoride. Except for sulfate, which was found to exert no influence on the activity of pancreatic amylase, this is the same order that has been observed in the influence of neutral salts on the precipitation of proteins and other neutral substances and the reverse of that obtained by Pfeiffer and Würgler[8] on the influence of these anions on the solubility of amino acids. This salting out factor which depends upon the specific properties of the ions is generally observed in more concentrated solutions of salt than we are considering in this investigation. In the lower concentrations of salt with which we are concerned here, the influence exerted by different ions as determined by freezing point and solubility measurements depends upon the valence type of the salt. This does not seem to hold here as the activity of pancreatic amylase is very different in the presence of salts of the same valence type and appears to be uninfluenced by the divalent sulfate ion. Moreover, the cations do not seem to fall into the order expected if their influence is one of salting out the enzyme. The order of their favorable influence upon enzymic activity is sodium and potassium, lithium, instead of lithium, sodium, potassium.

This makes it seem probable that we are dealing with some specific reaction between the enzyme, and the salt or substrate, or both, rather than merely with an influence of salt on the total ionic strength and activity of the solution.

Summary

The influence of sodium, potassium and lithium chlorides and of the bromide, fluoride, nitrate, chlorate, sulfocyanate and sulfate of sodium upon the activity of pancreatic amylase has been quantitatively investigated and compared.

The influence of neutral salts on the activity of pancreatic amylase was found to be the same for the purified preparations of the enzyme and for commercial pancreatin and seems therefore, to be a property of the enzyme itself rather than of any impurities that may be present.

The salts which are less favorable to the activity of pancreatic amylase are required in higher concentration and in turn exert their most favorable influence in solutions of higher hydrogen-ion activities than do those salts which are more efficient activators.

The activity of pancreatic amylase in the presence of two anions which

[7] Sherman, Caldwell and Adams, THIS JOURNAL, **48**, 2947 (1926).

[8] Pfeiffer and Würgler, *Z. Physiol.*, **97**, 128 (1916).

exert a specific influence on its activity, such as the nitrate and chloride ions, is intermediate between the activity of the enzyme in the presence of each taken separately. The activity of the enzyme in the presence of such a mixture of salts is dependent on the relative concentrations of the ions. Anions which alone do not influence the activity of the enzyme seem to have comparatively small influence on the activity of the enzyme in the presence of anions which exert a decidedly specific influence.

Reducing the concentration of enzyme by one-half or using 4% instead of 2% starch does not appreciably influence the concentration of sodium chloride necessary for complete activation of the enzyme or the optimal hydrogen-ion activity.

The presence of neutral salt is essential to the activity of pancreatic amylase. The influence exerted by different salts appears to be very specific.

The anion is far more influential than the cation, although the latter seems to have some slight influence on the activity of the enzyme.

Of the ions studied, chloride is the most efficient ion and the salts may be placed in the following order in their influence on the hydrolysis of starch by pancreatic amylase: sodium and potassium chlorides, lithium chloride, sodium bromide, sodium nitrate, sodium chlorate and sodium sulfocyanate, sodium fluoride. Sodium sulfate and phosphate were found to be without influence on the activity of pancreatic amylase.

A consideration of the influence of the different anions on the activity of pancreatic amylase indicates that these have specific effects on the enzyme or substrate.

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[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE OHIO STATE UNIVERSITY]

THE MECHANISM OF CARBOHYDRATE OXIDATION. X. (a) THE ACTION OF POTASSIUM HYDROXIDE ON MANNOSE. (b) A COMPARISON WITH THAT OF GLUCOSE AND FRUCTOSE

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It has been shown[2] that glucose, fructose, mannose and galactose may be oxidized with alkaline potassium permanganate solutions into carbon dioxide, oxalic acid and small amounts of acetic acid. At lower alkali normalities and at a temperature of 25°, it was observed that the oxalic acid–carbon dioxide ratios obtained from both glucose and galactose were not of the same value. However, as the normality of the alkali was in-

[1] Read at the Detroit Meeting of the American Chemical Society, September 6, 1927.

[2] (a) Evans and co-workers, THIS JOURNAL, **47**, 3085 (1925); (b) Evans and Buehler, *ibid*, **47**, 3098 (1925).