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STUDIES ON AMYLASES. X. COMPARISON OF CERTAIN PROPERTIES OF PANCREATIC AND MALT AMYLASE PREPARATIONS.

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Notwithstanding the numerous investigations of recent years, no consensus of opinion has yet been reached as to how far the enzymes of similar activity, but different origin, are to be regarded as distinct substances.

The best amylase preparations obtained in this laboratory from the pancreas and from malt are found to be very similar in physical properties and in chemical nature, so far as shown by their qualitative reactions, the percentage of nitrogen which they contain, and the distribution of this nitrogen among the different types of amino-acid radicals. That they are nevertheless not identical appears to be established by certain differences in their behavior.

Activity.

Optimum Reaction.—Perhaps the most noticeable difference between these two enzymes is that malt amylase is most active in an acid, and pancreatic amylase in an alkaline solution.

Electrometric measurements recently made in this laboratory[1] have shown that the optimum acidity for malt amylase (saccharogenic action in 30 minutes at 40°) is P_H^+ 4.2 to 4.6, the optimum action being found at this point, whether the activating agent be a strong acid, a weak acid, or an acid phosphate. Solutions of this hydrogen ion concentration give a distinct (but not strong) red reaction with azolitmin, while methyl orange gives an orange color.

Exact measurements of the optimum alkalinity for pancreatic amylase have not yet been made, but the reaction which has seemed best in the regular testing of these preparations has been that obtained by the use of 7 cc. of 0.02 *M* disodium phosphate and 5 cc. of *M* sodium chloride in each 100 cc. of previously neutral 2% starch. Such a solution gives a distinct (but not strong) blue reaction with azolitmin and is colorless with phenolphthalein. According to Sörensen this indicates a P_H^+ of 8.0 to 8.5. We hope soon to confirm this by the electrometric method.

Diastatic Power.—When each is tested in the presence of its proper activating electrolyte but with time, temperature, and substrate the same, the diastatic powers of the best preparations thus far obtained are fully twice as high for the pancreatic as for the malt amylase. The most active preparations of pancreatic amylase show diastatic powers around

[1] Sherman and Thomas, THIS JOURNAL, 37, 623 (1915).

4000 (new scale) corresponding to Lintner figures of about 6000, which means that the enzyme preparation forms 10,000 times its weight of maltose in 30 minutes at 40°. The most active preparations of malt amylase yet obtained showed powers of 1570 (new scale) corresponding to a power of about 2350 on Lintner's scale, and forming about 4000 times its weight of maltose in 30 minutes at 40°.

The foregoing statements refer in all cases to the relative amounts of reducing sugar formed under the action of the enzyme (saccharogenic activity or saccharogenic power). That the amyloclastic activity, as usually measured by determining the relative amount of starch *all* of which is digested under specified conditions into products giving no blue or violet color with iodine, runs approximately parallel with the saccharogenic power among amylase preparations from the pancreas and not among the corresponding preparations from malt, constitutes another interesting point of difference between the two amylases. This point was discussed recently[1] and therefore need not receive further attention here.

Proteolytic Activity.—Another pronounced difference between our amylase preparations from the two sources is that in those from pancreatin, proteolytic activity, as shown in the digestion both of casein[2] and of gelatin,[3] was concentrated with the concentration of the diastatic power, whereas in our purified malt amylase preparations we have not yet succeeded in demonstrating any proteolytic activity.

Deterioration.—Our pancreatic and malt amylase preparations also differ strikingly in the rate at which their solutions lose diastatic power on standing.[4] Under the conditions obtaining in our experiments, malt amylase is much the more stable in pure water solution, while pancreatic amylase is the more stable in 50% alcohol and remains active longer than the malt amylase when allowed to act upon starch.

The following are illustrative of numerous experimental data on which the foregoing statement is based:

Pancreatic and Malt Preparations in Solution at Ice-box Temperature.—The results of deterioration experiments with commercial pancreatin and malt extract, with purified amylases of high activity, and with intermediate products, are shown in Table I. (When a value is given in parenthesis with the designation of the preparation at the head of a column, it represents the original diastatic power of the preparation when first obtained.)

[1] THIS JOURNAL, 35, 1784–90 (1913).

[2] *Ibid.*, 34, 1109–10 (1912).

[3] Wahl, *Orig. Com. 8th Intern. Congr. Appl. Chem.*, 14, 215 (1912).

[4] The rate of deterioration when kept in the dry state varies too much with conditions to permit definite conclusions to be drawn from the data at hand. On the whole, the pancreatic preparations appear to have retained their diastatic power rather better than those from malt, when kept dry at the same temperature.

TABLE I.—DETERIORATION OF PANCREATIC AND MALT AMYLASES IN WATER AT 5–10°.

Diastatic powers found when solutions were tested as below.	From pancreas.			From malt.									
	Pancreatin 5 (400).	Preparation 21.	Preparation 60 (3560).	Malt extract.	Preparation 101 (780).	Preparation 53 (1220).	Preparation 53 with toluene.	Preparation 53 with tricresol.	Preparation 13B (1060).	Preparation 112 (900).	Preparation 111B (1470).	Preparation 52 (1470).
At once	400	2150	3560	5.5	240	490	480	490	570	900	1150	1470
After 1 hour	...	..	2200	...	...	...	...	...	...	...	..	..
After 3 hours	...	1520	1290	...	...	...	...	...	...	...	..	..
After 24 hours	380	1440	0	6.1	...	500	...	...	...	...	1060	1230
After 48 hours	370	1080	..	5.3	...	...	...	...	...	900	1050	..
After 72 hours	265	..	..	5.0	...	...	...	...	...	...	..	..
After 4 days	...	..	..	4.9	260	...	...	...	410	...	..	..
After 6 days	...	..	..	4.7	260	440	...	...	440	...	..	..
After 8 days	...	..	..	...	...	440	440	380	...	...	..	..
After 13 days	...	..	..	...	...	430	430	330	...	...	1150	..
After 14 days	...	..	..	...	250	380	420	310	190	...	..	..
After 17 days	...	..	..	...	260	...	...	...	0	...	..	..
After 24 days	...	..	..	...	260	...	340	260	...	...	..	..
After 27 days	...	..	..	...	260*	0	320	230	...	...	..	..

It will be seen from the table that, whether crude or purified material be compared, the malt amylase is much the more stable in cold water solution (5° to 10°). The solution of commercial pancreatin lost one-third of its activity in 3 days, while the malt extract showed a smaller loss after 6 days, its activity when 3 days old being nearly equal to that when freshly prepared. The highly purified pancreatic amylase (No. 60) lost all of its activity within 24 hours, while the highly purified malt amylase preparation (No. 52) lost less than one-fifth of its activity under the same conditions. Pancreatic preparation No. 21 (having about three-fifths the activity of No. 60) lost half its diastatic power in 48 hours, while malt preparation No. 112 (having three-fifths the activity of No. 52) showed no appreciable loss in the same length of time.

In the case of the pancreatic amylase it is plain that the rapidity of deterioration is proportional to the purity of the preparation and, in general, a similar relationship appears among the malt preparations. The latter, however, show irregular results, possibly because they are less homogeneous products, and probably also because the malt amylase solutions retain their activity so long that (when no antiseptics are used) there is sometimes bacterial action even at the low temperature of these observations. More experiments upon the influence of antiseptics are contemplated.

Both the great stability of the malt amylase solutions as compared

* In this case only, the activity was studied beyond the 28th day. The last observation was taken on the 53d day and showed a diastatic power of 230, indicating that in this case there was little loss of activity in over seven weeks.

with those of pancreatic amylase, and the fact that the former may show increased activity after standing for some time may be due, in part at least, to the presence in the malt preparations of some malt albumin which retards hydrolysis of the enzyme when in solution, or possibly protects it from deterioration in some other way. This point was discussed in our last paper.

Effect of Heat upon Dry Enzyme.—It is well known that enzymes, when dry, are much more stable at high temperatures than are their solutions. In our experience, however, it has not been found possible to heat the dry enzyme preparations at 100° without considerable loss of diastatic power. Pancreatic amylase preparation 35, which originally had a power of 3450, showed after heating for one hour at 100° a power of 1020. Malt amylase preparation 111B, which originally had a power of 1150, showed after the same treatment a power of 510. Thus the heating destroyed about two-thirds of the activity of the pancreatic and about one-half the activity of the malt amylase.

Statements in the literature to the effect that dry enzymes can be heated at 100° "without loss of activity," are probably based (for the most part at least) upon qualitative experiments and should perhaps be construed to mean "without becoming inactive," *i. e.*, the loss of activity is only partial and in qualitative experiments would very likely not be apparent.

Effect of Temperature and of Activating Salts upon Deterioration of Pancreatic and Malt Amylase in Water Solution.—The effect of temperature upon the rate of deterioration of diastatic power in commercial and purified amylase preparations, and the relative stability of these preparations in pure water solution and in the presence of "activating" salts, are shown in Table II. It will be seen that in pure water solutions the deterioration is always more rapid the warmer the solution. For both pancreatic and malt amylase the deterioration is more rapid the purer the enzyme. Pancreatic amylase in water solution is to an important extent preserved from deterioration by the presence of the mixture of salts which is favorable to its action (sodium chloride and secondary sodium phosphate); on the other hand, purified malt amylase may deteriorate more rapidly in the presence of its "activating" salt (primary sodium phosphate) than in pure water, probably because the deterioration is due to an hydrolysis of the enzyme, which is accelerated by increasing the hydrogen ion concentration of the solution. As will be more fully explained beyond, the enzyme is, in these deterioration experiments, dissolved in the water or salt solution in larger quantity (concentration) than would be used in determinations of diastatic power or in long-digestion experiments, since portions of the deteriorating solution must be

withdrawn from time to time in order to determine the proportion of enzyme still remaining active.

TABLE II.—DETERIORATION AT DIFFERENT TEMPERATURES IN WATER WITH AND WITHOUT "ACTIVATING" SALTS.

Diastatic power found when solution had stood at temperature and for time given below.	From pancreas.								From malt.						
	Pancreatin 4 in pure water at 20° to 25°.	Pancreatin 4 with NaCl and Na_2HPO_4 at 20° to 25°.	Preparation 21 in pure water at 5° to 10°.	Preparation 21 in pure water at 20° to 25°.	Preparation 21 with NaCl and Na_2HPO_4 at 20° to 25°.	Preparation 57 in pure water at 25°.	Preparation 57 in pure water at 40°.	Prep. 60 with NaCl and Na_2HPO_4 at 40°.	Malt extract at 7°.	Malt extract at 23°.	Malt extract at 40°.	Prep. 67 in pure water at 7°.	Prep. 67 in pure water at 20° to 25°.	Prep. 139 in pure water at 40°.	Prep. 139 with NaH_2PO_4 at 40°.
15 mins.	...	...	..	..	..	3120	1230	..	5.5	5.7	5.7	...	...	...	...
20 mins.	133	189	..	1240	2115	..	..	..	...	...	...	...	...	...	...
30 mins.	...	...	..	..	..	2430	..	3270	...	...	...	...	...	...	...
45 mins.	...	...	..	..	..	1740	270	..	...	...	...	...	...	...	...
60 mins.	111	...	..	1020	..	1610	..	2850	...	...	5.1	...	...	...	...
2 hrs.	81	...	..	675	..	..	0	1970	...	...	...	...	...	800	770
3 hrs.	57	165	1520	535	1720	390	..	..	...	...	5.0	870	930	...	...
18 hrs.	...	...	..	..	..	..	..	0	...	...	...	...	...	...	...
24–27 hrs.	0	124	1440	0	1200	0	..	..	6.1	5.3	3.9	870	870	780	480
2 days	...	..	1080	..	..	..	..	..	5.3	5.0	2.1	...	...	750	390
3 days	...	...	..	..	..	..	..	..	5.0	...	0.6	...	...	750	360
4 days	...	...	..	..	..	..	..	..	4.9	4.7	0.2	870	600	750	300
5 days	...	...	..	..	..	..	..	..	4.9	4.3	0	830	490	730	290
6 days	...	...	..	..	..	..	..	..	4.7	4.1	...	830	230	560	290

In water solutions the deterioration is more strikingly accelerated by rise of temperature in the case of the pancreatic than of the malt amylase.

A further comparison of the rate of deterioration of pancreatic preparations of different degrees of purity, when kept in pure water at room temperature, may be summarized briefly as follows:

Initial diastatic power	3430	2000	1185	425	150
Percentage of initial power lost in 20 minutes	45.5	32.5	25.8	19.0	11.3
Percentage of initial power lost in 3 hours	?	73.3	72.3	62.5	62.0

All of these solutions became entirely inactive within 24 hours. These data seem sufficiently suggestive to warrant fuller investigation at accurately controlled temperatures.

Behavior in the Presence of Substrate.—In marked contrast to their deterioration in water solutions (with and without activating salts), is the behavior of the two amylases in the presence of a sufficient amount of starch to test the duration of their activity, when allowed to come in contact with their substrate, and the maximum amount of reducing sugar calculated as maltose which the enzyme can form in relation to its own weight. In Table III are shown the data obtained when different amylase preparations were allowed to act upon many times their weight of soluble starch, the latter being usually in the form of a 1% solution. Toluene

TABLE III.—LONG-DIGESTION EXPERIMENTS WITH SOLUBLE STARCH AT 40°.

Enzyme.	Initial relation of enzyme to starch, 1.	Color reaction with iodine, and maltose formed (enzyme to maltose as 1 to figure given below). 24 hours.	48 hours.	72 hours.	96 hours.	120 hours.	144 hours.	168 hours.	192 hours.
Malt amylase preparation No. 52.....		Red-violet	Violet-red	Violet-red	Violet-red				
	25,000	17,000	17,000	17,000	.....				
			Red-violet	Red-violet					
	50,000	....	34,000	34,000					
		Blue	Violet-blue	Violet	Violet				
	75,000	40,000	49,000	50,000					
		Blue	Blue	Blue	....	Blue			
	150,000	64,000	75,000	....	....	..			
		Blue	Blue	Blue	....	Blue			
	300,000	90,000	93,000	93,000	....	..			
		Blue	Blue						
	1,000,000	76,000	76,000	....	....				
Pancreatic amylase, preparation No. 34.....		Red	Colorless						
	400,000	182,000	211,000	....	....				
		Violet-red	Red	Colorless					
	800,000	273,000	379,000	455,000	....				
		Blue	Red-violet	Red	Orange				
	1,000,000	203,000	305,000	408,000	470,000				

Pancreatic amylase preparation No. 50.....		Orange-red	Colorless		Colorless					
	500,000	211,000	276,000	....	292,000					
		Violet-red	Yellow-orange		Colorless					
	1,000,000	356,000	440,000	....	516,000					
		Blue	Red-violet	Red	Colorless		Colorless			
	1,000,000[1]	.....	.....	388,000	....	..	512,000			
Pancreatic amylase preparation No. 57.....			Orange-yellow	Colorless						Colorless
	1,000,000	.....	581,000	581,000	....	..	....	.....		590,000
			Violet-red	Light orange	Colorless				Colorless	Colorless
	2,000,000[2]	.....	823,000	950,000	1,040,000	..	.....		1,200,000	1,200,000
			Violet-red	Light orange	Colorless	..			Colorless	Colorless
	2,000,000[3]	.....	822,0000	950,000	1,040,000	..	.....		1,165,000	1,165,000

[1] In this case the digestion mixture had one-half the usual concentration of salt and phosphate.

[2] In this experiment a 2% starch solution was used.

[3] In this experiment a 4% starch solution was used.

N. B. In all except these three cases the digestion mixture contained 1% of starch and the usual concentration of electrolyte; primary phosphate for malt amylase, secondary phosphate and sodium chloride for pancreatic amylase.

was used as antiseptic in all these cases. At intervals of about 24 hours, portions of each solution were withdrawn for testing with iodine and for the determination of maltose. The colors obtained in the iodine test, and the number of times its weight of maltose which the enzyme had formed up to the time of the test, are given together in the table.

On the scale of diastatic power in use in this laboratory,[1] pancreatic amylase preparation 57 showed a power of 3700 (in the air-dry condition, corresponding to about 4000 for the dry substance) which is a higher activity than we had previously[2] observed. Pancreatic preparation 50 had a power of 3200, and preparation 34 a power of 3430 when freshly prepared, and of 2560 at the time of the experiment recorded in Table III. Malt amylase preparation 52 had a power of 1470 when freshly prepared, but at the time of the "long-digestion" experiments recorded in the table it had been kept in powder form for some months and its diastatic power had fallen to 980. This malt preparation, as originally tested, bears about the same relation in diastatic power to the best malt amylase yet obtained, which pancreas preparation 50 bears to 57; in relative activity at the time these tests were made it is similarly comparable to pancreas preparation 34. A comparison of malt preparation 52 with pancreas preparations 50 and 34, as regards duration of activity and relation between initial and total activity, seems therefore logical and brings out the following interesting difference:

Malt amylase preparation 52 produced 2450 times its weight of maltose in 30 minutes, in 48 hours it had produced 93,000 times its weight of maltose, and thereafter it showed no activity.

Pancreatic amylase preparation 50 formed 8000 times its weight of maltose in 30 minutes; 440,000 in 48 hours; 516,000 in 96 hours.

Pancreatic preparation 34 under similar conditions formed 6450 parts of maltose in 30 minutes; 305,000 in 48 hours; 408,000 in 72 hours; 470,000 in 96 hours.

Thus, while the malt amylase had expended its entire activity within 48 hours and produced in all about 38 times as much maltose as it formed in the first 30 minutes, the pancreatic amylases in the same circumstances (presence of large excess of substrate) continued active for 72 to 96 hours and formed, respectively, in the first 48 hours, 55 and 48 times, and in all, 65 and 73 times as much maltose as in the first 30 minutes.

Thus the influence of the substrate in protecting the enzyme from deterioration in aqueous solution was much greater in the case of the pancreatic than of the malt amylase.

Direct comparison of the data for the malt amylases, as given in Table II,

[1] Sherman, Kendall, and Clark, THIS JOURNAL, **32**, 1082–85 (1910); Sherman "Methods of Organic Analysis," 2nd Edition, 117–118.

[2] THIS JOURNAL, **33**, 1202 (1911).

with those in Table III might lead to the impression that this enzyme deteriorates faster in the presence of its substrate, but no such interpretation is justified, because the ratio of water to enzyme is about 1000 times as great in the latter experiments as in the former. It therefore seemed desirable, in the case of the malt amylase, to compare the rate of deterioration at the different dilutions, notwithstanding the fact that only approximate results can be obtained from deterioration experiments in which the ratio of water to enzyme is of such magnitude as obtains in the long-digestion experiments. The data of such a comparison are shown in Table IV.

TABLE IV.—INFLUENCE OF DILUTION UPON DETERIORATION OF MALT AMYLASE AT 40°.

	Malt amylase preparation No. 139 (960).		
Diastatic power found after solution had stood as indicated below.	10 mgs. in 100 cc. pure water (1:10,000) at 40°.	0.01 mg. in 100 cc. pure water (1:10,000,000) at 40°.	0.01 mg. in 100 cc. water containing the "optimum" concentration of NaH_2PO_4 at 40°.
After 15 minutes.....	760	500	500
After 2 hours.....	760	200	200
After 24 hours.....	700	100	100
After 48 hours.....	630	0	0
After 4 days.....	600		
After 7 days.....	270		
After 10 days.....	100		

From these data it will be seen that the malt amylase deteriorated as much in one day at a dilution of 1:10,000,000 as in 10 days at a dilution of 1:10,000. Parallel experiments with pancreatic amylase are impracticable because of its much more rapid deterioration in water.

The significance of the different behavior of the two amylases in water solution, and of the influence of the substrate upon the rate at which the enzyme loses its diastatic power will be discussed further on in this paper after certain chemical and physical characteristics have been described.

Deterioration in Alcohol at Different Temperatures.—Whereas in pure water solutions the pancreatic amylase deteriorates much more rapidly than malt amylase, this is not the case when the preparations are dissolved in 50% alcohol, as will be seen from Table V.

From the data in Table V it is evident that the alcohol solutions of both the amylases are much more stable at low temperature (5° to 10°) than at the ordinary room temperature of about 23°.

While not enough observations have been made to permit detailed comparison, it appears that, as in the case of water solutions, the purer the enzyme the more rapid its deterioration.

Comparing the data of Tables I, II, and V it will be seen that, at similar temperatures, purified pancreatic amylase is more stable in 50% alcohol than in water, whereas purified malt amylase is more stable in water than in 50% alcohol.

TABLE V.—DETERIORATION OF AMYLASES IN 50% ALCOHOL AT DIFFERENT TEMPERATURES.

Diastatic power found when solution had stood as indicated below.	From pancreas.					From malt.			
	Pancreatin No. 5 at 5°.	Pancreatin No. 5 at 23°.	Preparation F? at 5°.	Preparation F? at 23°.	Preparation No. 21 at 23°.	Malt extract at 7°.	Malt extract at 23°.	Preparation No. 67 at 7°.	Preparation No. 67 at 23°.
After 5 minutes	400	330	550	550	2000	5.4	5.4	(800)	570
After 3 hours	...	...	...	...	..	...	...	705	490
After 20 hours	...	...	540	335	1350	...	...	...	...
After 24 hours	385	290	540	...	1200	5.4	5.1	510	66
After 48 hours	370	290	...	260	..	4.9	4.4	...	...
After 3 days	265	150	...	...	..	...	3.3	...	...
After 4 days	...	...	...	...	..	4.7	2.7	350	0

Chemical Composition and Color Reactions.—Our preparations of both pancreatic and malt amylase are essentially proteins in that they show the typical color reactions,[1] contain 15 to 16% of nitrogen,[2] and on hydrolysis are resolved into amino acids. Determinations, by the Van Slyke method, of the distribution of the nitrogen among the different types of amino acid radicals show[3] that these preparations contain the same forms of nitrogen in about the same proportions as found in such typical proteins as casein and edestin.

Amylase preparations from both sources react as ordinary proteins in the Millon, xanthoproteic, tryptophan, and biuret tests.

Physical Properties.—The amylases from malt and from pancreas are both soluble in water and in 50% alcohol; insoluble in strong alcohol or acetone. In general, our preparations from malt are less readily soluble in water or in diluted alcohol than are those from pancreas.

Solutions of preparations from both sources give similar colloidal appearance under the ultramicroscope, but some isolated observations upon one of our malt preparations suggest that a part at least of the colloidal appearance may be due to material other than the active enzyme, possibly inactive protein formed by deterioration of the enzyme during purification as explained later in this paper. We hope to make a more systematic series of ultramicroscopic observations in the near future.

In diffusion experiments both amylases are retained either by parchment paper or by collodion membrane. Occasionally the dialyzate shows some diastatic power, but this is so slight in comparison with that of the dialyzing solution, and so irregular in its occurrence, that it may readily be due to imperceptible defects in the membrane. (See also description of dialysis experiments beyond.)

[1] THIS JOURNAL, **33**, 1203 (1911); **35**, 1622 (1913).

[2] *Ibid.*, **34**, 1106 (1914); **37**, 643 (1915).

[3] *Ibid.*, **35**, 1792–4 (1913).

Coagulation.—One per cent. solution of either amylase when heated above 50° yields a precipitate of coagulated albumin. This coagulum gives in both cases a blue-violet biuret reaction. The filtrate from this coagulum gives a biuret reaction which is rose-red in the case of the pancreatic, and violet-red in the case of the malt preparation. Thus both of these amylase preparations resemble that of Osborne in yielding coagulable albumin and a proteose or peptone, the latter apparently representing a product of somewhat more advanced hydrolysis in the case of pancreatic than of malt amylase.

In order to study further the change which takes place on coagulation, 200 mg. of a malt amylase preparation[1] (equivalent to 0.1836 g. of dry ash-free substance) were dissolved in 20 cc. of water and heated gradually in a beaker with constant stirring. Between 50° and 55°, coagulation took place and the coagulated protein flocculated well, leaving a clear solution. The coagulum after washing and drying weighed 0.1136 g. (61.8% of the dry weight of the sample) and contained 16.1% of nitrogen. The filtrate, containing 0.0700 g. of the original (dry ash-free) substance yielded 0.00959 g. nitrogen, showing 13.7% of nitrogen in the noncoagulable fraction of the enzyme preparation.

In a parallel experiment with pancreatic amylase, 300 mg. of preparation 60, equivalent to 0.2640 g. dry, ash-free substance, was dissolved in 30 cc. of water and heated as above. Coagulation occurred between 50° and 55°. The coagulum after washing and drying weighed 0.0306 g. (11.6% of the original dry weight) and showed 16.9% of nitrogen. The uncoagulable ash-free material of the filtrate weighed 0.2334 g. and showed 14.9% of nitrogen.

Dialysis.—Under the heading of activity, we have described above the rapid deterioration of pancreatic amylase in water solution at room temperature, and the relative stability of malt amylase under the same conditions. In the preceding paper[2] we have shown that the most serious loss of diastatic power encountered in the purification of malt amylase is that due to its deterioration during dialysis, and have suggested that this deterioration may be due to the removal through the dialyzing membrane of a portion of the proteose (or peptone) fraction of the enzyme.

The following experiments were designed to test this view:

1. A solution of 20 mg. of a purified preparation of malt amylase (Preparation 117; power 1070, new scale) in 50 cc. water was dialyzed against 500 cc. water at a temperature of 5° to 10° for 24 hours, at the end of which time the activity of the solution corresponded to a power of 190 (new scale), *i. e.*, four-fifths of the power was lost. The dialyzate alone showed almost no activity, 50 cc. forming only enough maltose to

[1] Preparation 111B described in the preceding paper.

[2] THIS JOURNAL, 37, 643 (1915).

reduce 3 mg. of cuprous oxide; but 50 cc. of the same dialyzate, when added to 0.5 cc. of the dialyzed solution, increased the activity of the latter to the extent of producing enough additional maltose to reduce 15 mg. of cuprous oxide. This corresponds to a restoration of about one-thirtieth of the power which the enzyme had lost during the dialysis. As a difference of one-thirtieth of the total power is hardly larger than the experimental errors involved in such work we should not attach much importance to this apparent restoration of a part of the lost activity, except for the fact that a similar result was again observed in the subsequent trial in which the experimental conditions were quite different. At any rate, it is plain that only a small part of the lost power was restored to the dialyzed solution by the addition of the dialyzate, indicating that, when the two constituents of the enzyme are separated by dialysis, at least one of them undergoes some further change which prevents the regeneration of more than a small fraction of the enzyme when the two solutions are subsequently mixed.

2. A solution of 200 mg. of preparation 111B (power when freshly prepared 1470, new scale, at time of this experiment 1050, new scale) in 20 cc. water was dialyzed against 200 cc. water at a temperature of 5° to 10° for 48 hours, at the end of which time the activity of the solution corresponded to a power of 750 (new scale), *i. e.*, about 30% of the power was lost. That the loss here is less than in the preceding experiment is doubtless due to the much lower ratio of water to enzyme. In this case the dialyzate showed measurable activity, about 6% of the total initial power, and again the mixing of the dialyzate with the dialyzed solution resulted in a small but appreciable restoration of power, somewhat greater than in the preceding experiment, as would be expected in view of the lower ratio of water to enzyme.

The dialyzate was concentrated by evaporation and found to give a distinct biuret reaction which was notably pinker in color than that given by the original enzyme solution. The same amount of (boiled down) dialyzate which gave this biuret reaction failed to give any coloration with triketohydrindene hydrate (ninhydrin).

All of our observations upon the properties of the pancreatic and malt amylase preparations, including their behavior in solution in the presence and absence of substrate and in dialysis and coagulation, appear to be consistent with the following hypothesis as to their chemical nature, based on that suggested by Osborne in 1896:

Pancreatic and malt amylases, although not identical substances, may each be regarded as a complex protein consisting of an albumin fraction and a proteose (or peptone) fraction. In either case, when the enzyme is heated in water solution these parts are separated by hydrolysis and the

albumin fraction coagulated. Probably the same sort of hydrolysis takes place to some extent at room temperatures, or even in cooler solutions.

In the case of malt amylase this cleavage into albumin and proteose appears to be an easily reversible reaction, so that when simply standing in water solution the enzyme molecule is in equilibrium with its cleavage products and there is no rapid destruction of the enzyme by the water at ordinary temperature. If, however, such a water solution is placed in a dialyzer it deteriorates[1] much more rapidly, probably because of the removal of the dialyzable cleavage product. Since the diastatic power thus lost is only to a slight extent restored by subsequently mixing the dialyzate with the dialyzed solution, it appears probable that the dialyzable proteose fraction, when separated from the albumin fraction by the dialyzer membrane and exposed to the action of a relatively large amount of water, undergoes some further cleavage or other change which is evidently not readily reversible.

In the case of pancreatic amylase it would seem that a similar cleavage of the enzyme into an albumin and a proteose or peptone takes place, but that this action is much less readily reversible than in the case of malt amylase, so that on standing in water solution the pancreatic amylase deteriorates very much more rapidly. The fact that the boiled-down solution of the noncoagulable cleavage product gives a pinker color with the biuret reagent in the case of pancreatic than of malt amylase, suggests that the proteose fraction of the enzyme may more readily undergo further cleavage in the case of pancreatic amylase and that this may account for the fact that the enzyme is not (as in the case of malt amylase) protected from rapid deterioration by establishing equilibrium with its primary cleavage products.

Probably in both cases the amylolytic action involves first a combination of the amylase with the starch, and while in such combination the labile constituent of the enzyme is protected from destructive change. With pancreatic amylase this has the effect of greatly prolonging the "life" of the enzyme which would otherwise undergo rapid hydrolytic destruction as outlined in the preceeding paragraph. On the other hand, with malt amylase the labile constituent is protected by the readily reversible character of the primary hydrolysis which results in the establishment of a condition of equilibrium in which most of the enzyme exists intact, so that there is only a very slow deterioration, even in the absence of the substrate, and the influence of the latter upon the duration of activity of the enzyme is therefore not readily demonstrable.

[1] Since in all of our work the activating salts are added to the optimum concentrate at the time of determining diastatic power, the deterioration cannot be attributed simply to the removal of electrolytes.

Summary.

The purified amylase preparations obtained from the pancreas and from malt are similar in many respects but are not identical substances.

Both are amorphous nitrogenous substances soluble in water or in 50% alcohol, but insoluble in concentrated alcohol or acetone.

Both show typical protein reactions in the Millon, xanthoproteic, tryptophane, and biuret tests.

Both contain 15 to 16% nitrogen and when examined by the Van Slyke method are shown to yield the eight forms of nitrogen distinguishable by this method (ammonia, melanine, arginine, lysine, cystine, histidine, and amino and nonamino nitrogen not precipitable by phosphotungstic acid) in proportions within the range of variation shown by such typical protein substances as casein, edestin, hair, and hemoglobin.

Both the pancreatic and the malt amylase preparations, when heated in solution, yield coagulated albumin and a proteose or peptone, the presence of the latter in the filtrate from the coagulum being shown by protein reactions and nitrogen content. The biuret reactions of these filtrates are pinker than those of the original enzyme solutions.

The malt amylase exerted its optimum diastatic (saccharogenic) power in a somewhat acid solution (P_H^+ 4.4 ± 0.2), whereas the optimum for the pancreatic amylase was slightly alkaline (P_H^+ in the neighborhood of 8 to 8.5).

When each amylase is allowed to act upon soluble starch for thirty minutes at 40° under its optimum conditions of reaction and salt concentration, the pancreatic amylase shows a much higher activity than any yet obtained from malt. In such a test the pancreatic amylase forms about 10,000 times its weight of maltose, corresponding to a "new scale" diastatic power of 4,000 or a Lintner figure of about 6,000; the malt amylase forms about 4,000 times its weight of maltose giving a "new scale" diastatic power of 1570 or a Lintner figure of about 2350.

In long-digestion experiments at 40° the malt amylase has thus far been observed to form a total of 93,000 times it weight of maltose, whereas the pancreatic amylase has formed 1,200,000 times its weight of maltose.

The most highly purified preparations of pancreatic amylase show also a pronounced proteolytic action both upon casein and gelatin, while in the purified malt amylase preparations we have been unable to demonstrate any proteolytic activity.

In purified pancreatic amylase, the amyloclastic and saccharogenic powers are concentrated in practically the same ratio; purified malt amylase of high saccharogenic activity does not show a correspondingly high amyloclastic power, at least as measured by present methods.

In a solution of either amylase the activity deteriorates more rapidly the higher the temperature or the greater the dilution, but malt amylase

is always more stable than pancreatic amylase when simply standing in pure water solution.

On the other hand, pancreatic amylase is the more stable in 50% alcohol solution, and shows a more sustained activity in long-digestion experiments, in which there is present a relatively large amount of substrate together with such a concentration of "activating" electrolytes as had been found best adapted to each enzyme in the ordinary determination of diastatic power.

Both amylases deteriorate during dialysis, and much more rapidly at room temperature than at 5° to 10°. The increased deterioration in dialysis over simple standing in water at the same concentration is more readily demonstrable in the case of malt amylase.

The problem of the chemical nature of the enzymes is discussed in the light of the new osbervations on composition, coagulation, dialysis, activity, and deterioration.

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STUDIES ON THE ACTION OF EREPSIN.

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Since the discovery of erepsin by Cohnheim,[1] in 1901, it has been investigated principally from the point of view of the physiologist. The properties of erepsin and the nature of its action as an enzyme, independent of any function it may have in the animal organism, have been less studied.

Erepsin is known to readily attack peptones and polypeptids, resolving them into their constituent amino acids. Only a few proteins have been found to be digested to any great extent, casein being the most notable example.

Maximum ereptic activity is manifested in weakly alkaline media. Vernon,[2] in studying the action of erepsin on Witte peptone, found that, while small quantities of alkali accelerated the digestion by erepsin, in higher concentrations it injured the enzyme to some extent. He concluded, that "over a certain range of alkalinity these two opposite influences nearly neutralize each other."

For ereptic experimentation, pure intestinal juice, or, a solution of

[1] *Z. physiol. Chem.*, **33**, 451–65 (1901); **35**, 134-140 (1902); **49**, 64–71 (1906); **51**, 415–24 (1907).

[2] *J. physiol.*, **30**, 330–70 (1903).