[CONTRIBUTION FROM THE CHEMICAL LABORATORIES OF COLUMBIA UNIVERSITY. No. 225.]

STUDIES ON AMYLASES, VI.[1] A COMPARISON OF AMYLOCLASTIC AND SACCHAROGENIC POWERS.

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The action of amylases has been studied in two general ways; by observations, first, upon the amyloclastic power (splitting of starch into products which do not react blue with iodine) and, second, upon the saccharogenic (sugar forming) power. The terms "liquefying" and "saccharifying" have often been used to designate these two phases of the action of amylases but the words amyloclastic and saccharogenic seem more accurately descriptive.

From time to time, for several years past, various observers have noted that in the action of amylase upon starch the amount of reducing sugar formed is not always proportional to the amount of starch apparently digested. This fact has sometimes been expressed in statements to the effect, that amylase does not always form maltose and dextrin in the same quantitative proportions. At other times the splitting of starch into dextrin, and of dextrin into maltose, have been attributed to two distinct enzymes; and the varying proportions of maltose and dextrin formed in starch-digestion experiments have been explained on the hypothesis that ordinary diastatic preparations contain both these enzymes, but not always in the same proportions, or that the optimum conditions are different for the amyloclastic and saccharogenic enzymes. The phenomenon has also been attributed to lack of homogeneity of starch, and to "retrogradation" or "coagulation" of a portion of the starch during the action of the enzyme.

Among the investigators who have given attention to the distinction between amyloclastic and saccharogenic (liquefying and saccharifying) activities may be mentioned: Dubrunfaut,[2] Schwarzer,[3] O'Sullivan,[4] Brown and Heron,[5] Brown and Morris,[6] Chittenden and Smith,[7] Harris and Gow,[8] Lintner,[9] Effront,[10] Seyffert,[11] Stone and Wright,[12] Sykes and Hussey,[13] Takamine,[14] Vernon,[15] Pollack,[16] Fernback and Wolff,[17] Frankel and Hamburg.[18] Johnson,[19] Chrzaszcz,[20] Chrzaszcz and Terlikowski.[21] Evans,[22] Pribram.[23]

[1] For earlier papers see THIS JOURNAL, **32**, 1073, 1087; **33**, 1195; **34**, 1104; **35**, 1617. [2] *Dingl. polyt. J.*, **187**, 491. [3] *J. prakt. Chem.*, (n. f.) **1**, 212. [4] *J. Chem. Soc.*, **30**, 125. [5] *J. Chem. Soc.*, **35**, 596; *Proc. Roy. Soc.*, **30**, 393; *Ann.*, **204**, 228. [6] *J. Chem. Soc.*, **47**, 527. [7] *Trans. Conn. Acad.*, 6. [8] *J. Physiol.*, **13**, 469. [9] *Woch. Brau.*, **1892**, 699. [10] *Compt. rend.*, **120**, 1281; **141**, 626. [11] *Z. ges. Brauw.*, **21**, 195, 207, 211, 611. [12] THIS JOURNAL, **20**, 639. [13] *J. Fed. Inst. Brew*, **1898**, 527. [14] *J. Soc. Chem. Ind.*, **17**, 437. [15] *J. Physiol.*, **28**, 156. [16] *Woch. Brau.*, **21**, 317. [17] *Compt. rend.*, **140**, 1067. [18] *Hofmeister's Beitrage*, **8**, 389. [19] THIS JOURNAL, **30**, 798. [20] *Woch. Brau.*, **27**, 69, 89, 98, 120, 126, 134. [21] *Woch. Brau.*, **29**, 590, 607, 623, 636. [22] *J. Physiol.*, **44**, 220. [23] *Biochem. Z.*, **44**, 293.

Without attempting any exhaustive critical discussion or final interpretation at this time, it seems desirable to place on record certain data bearing on this subject, which have been determined in this laboratory during the past two years, and which, we hope, may be of service to other students of enzyme action.

In all the experiments here described "soluble" starch, prepared according to the Lintner method, has been used under the conditions and with the precautions described in our earlier papers.[1]

All iodine color reactions here recorded are designated strictly according to the Milton Bradley Color Standards from Mulliken's "Identification of Pure Organic Compounds."

Pancreatic Amylase.

Pancreatin 4, a commercial sample, showed a diastatic power of 150 (new scale) and a Wohlgemuth figure of 76,000. Expressed in more easily comparable terms, it digested 760 times its weight of starch into products giving no blue or violet color with iodine in 30 minutes at 40° (amyloclastic power, 760); in the same time, at the same temperature, it formed 380 times its weight of maltose (saccharogenic power, 380). Thus the maltose formed amounts to one-half of the weight of the starch apparently digested (disappearance of blue or violet reaction with iodine).

Table I shows the amyloclastic and saccharogenic powers of pancreatins 4 and 5 (commercial samples) and of pancreatic amylase preparations 24, 21, 23, 34, 36, and 57, which varied in power from about that of the best commercial pancreatin to about eight times this activity. Preparations 34 and 36 originally had powers about 10% higher than those here shown, the decrease in activity having occurred during storage of several months in air-dry condition.

TABLE I.—AMYLOLYTIC PREPARATIONS OF PANCREAS.

Material.	Diastatic power. "New scale."	Wohlgemuth figure.	Amyloclastic power.	Saccharogenic power.
Pancreatin 4	150	76,000	760	380
Pancreatin 5	395	178,000	1,780	1,030
Preparation 24	510	232,000	2,320	1,310
Preparation 21	862	555,000	5,550	2,250
Preparation 23	912	418,000	4,180	2,310
Preparation 36	2,250	1,000,000	10,000	5,830
Preparation 34	2,560	1,176,000	11,760	6,420
Preparation 57	3,570	1,609,000	16,090	8,940

It will be seen that in all these cases the amount of starch apparently digested (amyloclastic power) is about twice the amount of maltose produced (saccharogenic power).

When a portion of preparation 57 was allowed to stand in solution until it had lost about one-sixth of its activity, its amyloclastic power

[1] THIS JOURNAL, 32, 1073, 1087.

was found to be 12,500 and the saccharogenic power 7,370, the ratio being very little changed.

When an active preparation of purified pancreatic amylase acted upon 500,000 times its weight of starch until the end point of the Wohlgemuth method (red reaction with iodine) was reached (23–24 hours), it formed 211,600 times its weight of maltose; in digesting 1,000,000 times its weight of starch to the same end point (70–72 hours) it formed 408,000 times its weight of maltose.

When the digestion was carried to the "colorless end point" (*i. e.*, through the erythrodextrin stage) preparation 34 digested 500,000 times its weight of starch in 46–48 hours with the production of 290,000 times its weight of maltose; preparation 50 in the same time digested 500,000 times its weight of starch and produced 276,000 times its weight of maltose. The same preparation within 96 hours digested 1,000,000 times its weight of starch to the colorless end point, with production of 516,000 times its weight of maltose. In another experiment which differed from the last in that the digestion mixture contained only one-half the quantities of sodium chloride and disodium phosphate ordinarily added in our experiments with pancreatic amylase,[1] the enzyme preparation in 93–96 hours digested 1,000,000 times its weight of starch and produced 512,000 times its weight of maltose.

From the standpoint of the present discussion, the significant feature of all these results is that the amount of starch digested is about twice the amount of maltose formed, whether the pancreatic enzyme be in the form of an ordinary or an exceptionally good commercial pancreatin, a partially purified or highly purified laboratory preparation of pancreatic amylase, and whether the amylase be tested in its condition of maximum activity or after partial deterioration either in the dry state or in solution. Similar ratios also hold for digestion mixtures containing a different concentration of the activating salts, and for experiments so arranged that the time of digestion varied from 20 or 30 minutes to 24, 48 or 96 hours. This fairly stable relation, between the amount of starch apparently digested by pancreatic amylase (as indicated by the iodine reaction) and the amount of maltose actually formed as shown by quantitative determination, probably explains why the method of testing with iodine for disappearance of starch reaction has given satisfactory results in the hands of investigators who have worked wholly or mainly with pancreas preparations or with other amylases from the animal body whose behavior is probably similar.

On the other hand, an attempt to apply this same method to amylase preparations from malt may yield entirely misleading results.

[1] THIS JOURNAL, 32, 1082.

Malt Amylase.

Comparative determinations of amyloclastic and saccharogenic powers of malt extracts and amylase preparations made from them have given results showing no such tendency toward a fixed ratio as was observed with the pancreas preparations above.

Table II shows data for malts and malt preparations arranged in the same manner as are the pancreas preparations in Table I.

The malt preparations acted upon the "soluble" starch in the presence of M/250 monosodium phosphate.

TABLE II.—MALTS AND MALT AMYLASE PREPARATIONS.

Material.	Diastatic power. "New scale."	Wohlgemuth figure.	Amyloclastic power (apparent).	Saccharogenic power.
Malt 1, extracted with pure water.	5.7	1,560	15.6	14.7
Malt 6, extracted with mol/250 NaH_2PO_4	4.4	1,790	17.9	11.5
Malt 7, do	7.5	2,380	23.8	19.2
Malt 3, do	7.6	2,080	20.8	19.3
Malt 4, do	6.0	1,800	18.0	15.6
Do., do. after dialysis	5.25	1,470	14.7	13.5
Alcohol precipitate[1] from undialyzed malt extract (6A)	25	2,040	20.4	53
Alcohol or acetone precipitates from dialyzed malt extracts[2]				
32 C	238	20,800	208.0	620
32 B	250	20,800	208.0	650
14 IA	270	45,400	454	700
12 A	475	112,000	1,120	1,240
33 D	555	100,000	1,000	1,430
13 A	570	125,000	1,250	1,470
35 AII	590	50,000	500	1,530
34 BIII	640	41,700	417	1,630
35 BII	665	100,000	1,000	1,710
46 B	690	156,250	1,563	1,780
44 B	755	143,000	1,430	1,940
58	830	167,000	1,670	2,140
64 I	940	167,000	1,670	2,400
64 IV	935	125,000	1,250	2,370

From the examination of the data of Table II, it is plain that under the conditions obtaining in the methods here used (the gravimetric method of Sherman, Kendall and Clark for the saccharogenic, and the Wohlgemuth method for amyloclastic power) the ratio of maltose formed to starch apparently digested is much higher for the malts and malt preparations than for the pancreatic products. With the simple or dialyzed extracts of malt, the maltose formed ranges from two-thirds to nine-tenths, or more, of the weight of starch apparently digested, while with the pre-

[1] This was the fraction which precipitated between 68% and 75% alcohol.

[2] For further description see THIS JOURNAL, 35, 1619–22.

cipitated malt amylase preparations the amount of starch apparently digested is in every case less than the amount of maltose known to have been formed. Hence it is evident that, in the case of malt amylase, the disappearance of the starch-iodine color reaction in the digestion mixture is not adapted to serve as a criterion for the starch-digesting power of the enzyme; with malt extracts the quantitative relation between the amyloclastic and saccharogenic figures is possible though not probable; with precipitated malt amylase[1] the indicated relation is plainly impossible.

Discussion.

The reason for this discrepancy is doubtless to be found in the fact that the "saccharogenic power" is an expression of the amount of maltose produced when the enzyme acts on a liberal excess of starch, whereas the "amyloclastic power" expresses the amount of starch *all of which* is digested to a certain point within a certain time. If, either because of lack of homogeneity in the starch or through reactions which exclude a portion of the starch from full exposure to the digestive hydrolysis, the whole of the starch is not digested with equal speed there results a delay in the disappearance of the iodine reaction.

This delayed end point makes the Wohlgemuth figure and apparent amyloclastic power misleadingly low.

It is hoped that experiments, now in progress in this laboratory, may show which of the suggested hypotheses affords the best explanation of this delayed digestion of a portion of the starch by certain amylases.

The object of the present paper is rather to point out the marked difference, in this respect, between pancreatic and malt amylases and to call attention to the necessity of a different interpretation of observations upon the iodine reaction in dealing with these two types of amylases. With amylases of the pancreatic type the observations based on the iodine reaction have been of great service, notably in the investigations of Roberts, Vernon, Wohlgemuth, and Long and Johnson. With malt diastase the indications of the iodine reaction are much more difficult and less certain of interpretation, and in the case of the precipitated enzyme preparations they are often quite misleading.

If the enzyme were allowed to act upon an excess of starch, as in the determination of saccharogenic power, and the amount of starch remaining unchanged at the end of the half-hour allowed for the reaction were de-

[1] In the preparation of these precipitated amylases the dialyzed malt extract is first mixed with an equal volume of alcohol (or acetone) and the "50% precipitate" thus obtained is rejected. The filtrate is then mixed with more alcohol (or acetone) up to a final concentration of 65 or 70% and the precipitate obtained at this point is collected and dried and constitutes the "amylase preparation." The "50% precipitate" appears to contain little amylase. Its apparent amyloclastic power is sometimes higher, sometimes lower, than the saccharogenic power, *i. e.*, the indicated relation is sometimes a possible and sometimes an impossible one.

termined, a much better indication of the true amyloclastic power would probably be obtained.

The delayed disappearance of the iodine reaction, when the hydrolysis is catalyzed by precipitated malt amylase, is quite as pronounced in experiments of relatively long duration as in the 30-minute determinations of amyloclastic and saccharogenic powers.

It has been shown above that, when pancreatic amylase acts for from one to four days upon large quantities of starch, the iodine test shows a red reaction whenever the maltose amounts to from two-fifths to one-half the original weight of starch, and no color reaction with iodine is obtained when the maltose amounts to from one-half to three-fifths the weight of starch originally present. On the other hand, we find that, when precipitated malt amylase (preparation 52) in similar experiments upon relatively large amounts of starch has carried the digestion to the point where the maltose produced equals half the weight of starch originally present, the solution still shows a deep blue color reaction with iodine, and when the maltose reaches two-thirds the original weight of starch the digestion mixture still shows with iodine a violet-blue to violet-red reaction.

In all the experiments above quoted, the amylases acted in the presence of such added electrolytes as had been found to furnish a favorable environment for its activity,—as measured by the method chiefly used in this laboratory, which depends upon the saccharogenic power.

The discrepancy between the saccharogenic power and the apparent amyloclastic power of the malt preparations may be due, in some measure, to a difference in the optimum conditions for these two phases of the diastatic action of the malt amylase. In this connection, certain results obtained in this laboratory by Mr. A. Gross, in a preliminary study of the behavior of pancreatin and takadiastase in the presence of different added electrolytes, are of interest. With pancreatin the optimum concentration of sodium chloride appeared to be the same for the amyloclastic and saccharogenic powers, while with takadiastase the amyloclastic action was augmented by concentrations considerably beyond the optimum for the saccharogenic action; in testing the takadiastase by the Wohlgemuth method for amyloclastic power the persistence of a slightly bluish color reaction with iodine (reading red-violet or violet-red on the color chart) was especially noticeable in the absence of added salt. The optimum concentration of neutral phosphate (Sörensen mixture) also appeared to be higher for the amyloclastic than for the saccharogenic action of takadiastase. In experiments in which both sodium chloride and neutral phosphate were added, the optimum seemed to be the same for amyloclastic and saccharogenic action in the case of pancreatin, but in case of takadiastase it appeared much higher for the amyloclastic than for the saccharogenic action. The mixture of sodium chloride and disodium

phosphate which is regularly used in this laboratory in determining the activity of pancreatic amylase, and which gives favorable conditions for both its amyloclastic and saccharogenic action, seemed, in the case of takadiastase, to activate amyloclastic and at the same time to retard saccharogenic action.

It is evident that observations upon the iodine color reaction of digestion mixtures as an indication of the amyloclastic power require very different interpretations when dealing with different amylases.

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[CONTRIBUTIONS FROM THE CHEMICAL LABORATORIES OF COLUMBIA UNIVERSITY. No. 226.]

STUDIES ON AMYLASES. VII. THE FORMS OF NITROGEN IN AMYLASE PREPARATIONS FROM THE PANCREAS AND FROM MALT, AS SHOWN BY THE VAN SLYKE METHOD.

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Previous papers from this laboratory[1] have described the purification and some of the properties of amylase preparations from the pancreas and from barley malt, which preparations, while not chemically pure, are believed to represent high concentrations of the respective enzymes.

In the case of pancreatic amylase, the product obtained in many independent preparations is so uniform in diastatic power, as well as in other properties, as to justify the belief that we are here dealing with material which, while not absolutely free from impurities, is essentially a definit substance; and since the substance is able to digest at least one million times its weight of starch it seems not unreasonable to consider these preparations as essentially composed of pancreatic amylase.

The attempts to purify malt amylase have given products which are less uniform in composition and much less uniform in activity than the preparations from the pancreas, but which show considerably higher diastatic power than has previously been described for any amylase preparations, except the pancreatic amylase just mentioned.

Our amylase preparations show the nitrogen content and color reactions of protein substances, but the yields obtained are so small that it would be quite impracticable to prepare sufficient quantities for complete analysis of the hydrolytic products. We have therefore sought to gain a further insight into the chemical nature of these substances by determining the principal forms of nitrogen present according to the method of Van Slyke.

[1] THIS JOURNAL, 33, 1195; 34, 1104; 35, 1617.