LXX. THE MECHANISM OF DEGRADATION OF STARCH BY AMYLASES.

I. NATURE OF THE EARLY FISSION PRODUCTS.

BY GEORGE GROSVENOR FREEMAN
AND REGINALD HAYDN HOPKINS.

From the Department of Industrial Fermentation, University of Birmingham.

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OHLSSON [1926; 1930] concluded from experiments on the action of the "dextrinogenamylase" and "saccharogenamylase" of malt on starch that the former enzyme breaks starch into progressively smaller molecules until maltose is produced, whereas the latter enzyme detaches maltose molecules successively from the starch. This conclusion was based mainly on the relative permanence of the starch-iodine reaction during digestion by the respective enzymes at equal saccharifying concentrations. The argument is not wholly reliable, since the nature of the starch-iodine reaction is not understood, and it is not certain that colour is a function of molecular dimensions. Therefore an investigation has been made of the actual reducing products present in starch partly degraded by these and similar enzymes. As the dextrinogenamylase of malt is an α -amylase [Ohlsson, 1930] in the sense that the products of its action on starch exhibit mutarotation in the α -sense [Kuhn, 1925], this enzyme will be referred to herein as α -malt-amylase. Similarly the saccharogenamylase, a β -amylase, will be termed β -malt-amylase.

The investigation is concerned with hydrolytic products at a comparatively early stage in the degradation of starch. A preliminary experiment showed that the velocity of hydrolysis of soluble starch by α -malt-amylase in low concentration became inconstant at 15–20 % hydrolysis whilst at 25 % hydrolysis the iodine reaction had changed to red. Therefore stages corresponding to 15, 20 and 25 % hydrolysis were selected as likely to be informative. β -Amylase from malt and from ungerminated barley showed no colour transition until the hydrolysis was too far advanced to be of use.

EXPERIMENTAL.

Lintner soluble starch was used. Its reducing power expressed as percentage maltose was 3.9 (iodimetric titration) and 1.53 (Bertrand); $[\alpha]_D^{20} + 198.4^\circ$.

The malt-amylases were prepared from a highly diastatic Canadian malt by the method of Ohlsson [1930]. For the α -enzyme, an aqueous extract was heated to 70° for 15 min. with stirring, filtered cold, precipitated in 60 % alcohol, freed from alcohol, dissolved in water and filtered clear after adjustment of the reaction to the desired value of p_H . For the β -enzyme, the aqueous extract was brought to p_H 3.3 at 0° for 15 min., restored to p_H 4.6, warmed to normal temperature and further treated as above. Both enzymes could be stored at -5° and p_H 6.4 without appreciable loss of activity. The amylase of ungerminated barley was prepared by precipitation of 20 % alcoholic extract in alcohol *etc.* Commercial pancreatin was used as a source of pancreatic amylase.

Control tests under the conditions of reaction employed in the experiments, indicated the absence of any maltase activity from all of the amylase preparations.

To 480 ml. lots of 5.6 % (dry weight) starch were added respectively:

(1) α -malt-amylase equivalent to 10 g. malt	} at p_H 4.6.
(2) β -malt-amylase equivalent to 8 g. malt	
(3) β -(barley)amylase equivalent to 10 g. barley	
(4) α -(pancreatic)amylase equivalent to 0.1 g. pancreatin at p_H 6.8.	
- Total volume 500 ml.

Each reaction was allowed to proceed at 25° until iodimetric titration of an aliquot indicated the percentage hydrolysis desired. The remaining reaction mixture (usually about 450 ml.) was then poured slowly into 300 ml. of boiling water, boiling and stirring being maintained. The mixture was concentrated to 500 ml. and 20 ml. withdrawn to determine afresh the total reducing power. 250 ml. of each were now evaporated to 50 ml. and dried on a water-bath with washed, ignited kieselguhr until syrupy. The mass was suspended in 500 ml. of boiling absolute alcohol, decanted and the residue extracted under reflux 5 times with 150 ml. of 95 % alcohol. The final extracts contained no reducing matter. The combined extracts were evaporated to dryness, treated with water and re-evaporated thrice to remove alcohol, aldehyde *etc.* and finally dissolved in water and brought to 25 ml. The residue, after freeing from alcohol *etc.*, was heated with water in an autoclave at 120° for 30 min. The filtrate was finally contained in 500 ml. of aqueous solution.

Analyses of these fractions gave the results shown in Table I.

Table I. *Analysis of amylolytic hydrolysates.*

	α -Amylase				β -Amylase		
Apparent maltose in hydrolysate:	Malt			Pancreas	Barley		Malt
Percentage theory	25.6	20.6	19.3	14.8	24.3	14.8	20.6
In portion fractionated, g.	3.41	2.31	2.33	2.03	3.27	1.98	2.44
Alcohol-soluble (sugar) fraction:							
Maltose (iodimetric), g.	1.36	0.59	0.60	0.87	3.11	1.40	2.01
Maltose (polarimetric), g.	2.71	—	—	1.18	3.12	1.41	—
Maltose osazone	Small amounts				Large amounts		
Alcohol-insoluble (dextrin) fraction:							
Dry weight, g.	9.39	9.58	8.44	11.60	9.28	10.03	—
Reducing power (Bertrand):							
As maltose, %	23.0	20.3	21.1	11.4	4.5	7.1	—
$[\alpha]_D$	187°			191°	189°	195°	
Iodine reaction	Violet	Red-violet		Blue-violet	Blue	Blue	
Retrogradation after 7 days	None	None	None	None	Retrogradation		
Osazone	No crystalline osazone formed						

Velocity of fermentation of alcohol-soluble fractions.

Aliquots of the alcohol-soluble fraction possessing reducing powers equivalent to 0.5 g. of maltose were fermented at 30° with 8 g. of pressed brewer's yeast in 50 ml. total volume [Hopkins and Roberts, 1935; 1936]. The rates of fermentation at intervals and the total CO₂ evolved up to the time of practical cessation are recorded in Table II.

Table II. *Rates of fermentation of alcohol-soluble fractions (mg. CO₂/min.).*

Fraction	Apparent maltose g.	Min.								Total CO ₂ mg.
		5	10	15	20	25	30	40	50	
Pure maltose	0.500	7.2	6.2	6.1	5.9	5.7	5.6	3.3	0.8	232
α (25.6% hydrolysis)	0.546	7.2	4.3	4.0	3.2	2.5	2.5	1.2	0.5	157
β (24.3% hydrolysis)	0.477	7.0	6.4	6.0	5.9	5.5	4.9	2.0	0.2	205
Pure maltose	0.500	7.4	6.8	5.9	5.2	4.6	4.3	3.0	1.2	228
α (14.8% hydrolysis)	0.545	7.0	5.3	4.8	4.1	3.5	3.0	2.1	1.2	181
β (14.8% hydrolysis)	0.516	7.0	6.7	5.9	5.2	5.0	4.4	3.0	1.6	224

DISCUSSION.

The alcoholic extracts from the β -amylase hydrolysates contained maltose in quantity nearly equivalent to the total reducing value of the original hydrolysates. It was identified by its specific rotation, reducing power, osazone and in certain instances by the characteristic fermentation by brewer's yeast under conditions described by Hopkins and Roberts [1935; 1936]. The β -amylase products fermented throughout at approximately the same rate as pure maltose of approximately the same concentration, the total CO₂ evolution confirming the identity. After alcohol extraction the "dextrin" residue, dispersed in water at 120°, exhibited properties (Table I) characteristic of slightly degraded starch.

Hydrolysis by α -malt-amylase yielded an alcoholic extract which contained maltose (confirmed by osazone). However, the reducing power was only half of that which would correspond with the specific rotation, assuming the usual relationship between R and $[\alpha]_D$ for maltose. No further fractionation or analysis of this obvious mixture was attempted. It fermented at rates much lower than maltose, although employed in slightly higher concentration. From the known (unpublished) relationship between velocity of fermentation and initial maltose concentration (compare Hopkins and Roberts [1935] for glucose), it is possible to deduce that the fermentation products of α -amylase (apparent maltose 0.55 g.) actually contained not more than 0.25 g. maltose. The total CO₂ from the α -products is fictitiously high owing to slow fermentation of dextrins. This persisted long after pure maltose or the corresponding β -products had been completely fermented; nevertheless the total CO₂ clearly indicates that only a fraction of the extractable reducing power was due to maltose. In one experiment pancreatic α -amylase was used with similar results.

If α -amylases break down starch or the major part of it into reducing dextrins, then, assuming the reducing groups of the latter to exercise reducing power equal to corresponding groups in maltose, the 20–25 % stage of hydrolysis (as percentage maltose) means that the products have an average chain-length of about 8–10 glucose units. Those, including maltose, of shorter length are present presumably in the alcohol-soluble fraction and apparently all slowly fermentable. The more complex ones, of longer chain-length, which appear in the other fractions give a violet colour with iodine and possess appreciable reducing powers whereas the corresponding fraction from the products of β -amylase gives a blue iodine reaction, exhibits relatively little reducing power and in general character rather resembles starch than dextrin.

SUMMARY.

1. α -Malt-amylase and pancreatic amylase produce some maltose in the early stages of hydrolysis of soluble starch. The major part of the reducing material formed has a higher $[\alpha]_D$ than maltose and the alcohol-soluble fraction

is rather slowly fermented by brewer's yeast. The conclusion of Ohlsson that these enzymes split starch initially into dextrins is confirmed.

2. β -Malt-amylase and the amylase of ungerminated barley produce maltose in the early stages of starch hydrolysis in an amount virtually equivalent to the reducing power. The remaining material resembles amylose or starch. The view that β -amylases remove successive molecules of maltose from the starch molecule is confirmed.

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