LXXI. THE MECHANISM OF DEGRADATION OF STARCH BY AMYLASES.

II. KINETICS OF ACTION OF α - AND β -AMYLASES ON STARCH, ITS COMPONENTS AND PARTIAL DEGRADATION PRODUCTS.

BY GEORGE GROSVENOR FREEMAN AND
REGINALD HAYDN HOPKINS.

From the Department of Industrial Fermentation, University of Birmingham.

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VAN KLINKENBERG [1934] suggested that α -amylases promote hydrolysis of " α -starch" (which is a fraction resembling Wijsman's "erythrogranulose" or Baker's " α -amylodextrin" and constitutes about 36% of the starch). β -Amylases were correspondingly specific for β -starch (the remainder). Hydrolysis beyond 36% in presence of α -amylase was explained by the supposed slow transformation of β - into α -starch. Now starch in solution contains certain distinct components which may be fractionated by electrodialysis yielding respectively amyloamylose (blue with iodine) and erythroamylose (red with iodine) [Samec and Mayer, 1921]. Amyloamylose is hydrolysed by pancreatic (α -), barley (β -) and unfractionated malt (mixture) amylases relatively easily and completely, but erythroamylose only with difficulty [Samec and Waldschmidt-Leitz, 1931, this paper Table I]. Thus Van Klinkenberg's so-called α - and β -starches are not identical with amylo- and erythro-amylases, though they might represent fractions of the erythroamylose, which constitutes at least 60% (recovered by us) to 83% (Samec) of the total starch.

α -Malt-amylase does not selectively hydrolyse the erythrogranulose or α -amylodextrin fraction of starch [Hanes, 1935; this paper Table I]. In this communication are reported observations on the kinetics of hydrolysis promoted by α - and β -amylases or by their mixtures. The substrates included starch, amyloamylose, erythroamylose (components of starch), α -amylodextrin and certain fission products of the enzyme actions. α -Amylodextrin may be regarded either as a component of the original starch or as a fission product of the action of β -amylase.

EXPERIMENTAL.

Substrates.

Lintner soluble starch as described in Part I. *α -Amylodextrin* was prepared from the soluble starch by the action of alcohol-precipitated barley amylase at p_H 4.6 and 50° by a method closely resembling that described by Baker [1902]; yield 33%, $[\alpha]_D^{20} + 188^\circ$, reducing power (R) 1% as maltose. *Glycogen* (Pfanzstiehl), $[\alpha]_D^{20} + 185^\circ$, R 0.7%. *Achroodextrin* was prepared from soluble starch by the action of commercial (B.D.H.) pancreatin at p_H 6.8 and 25° until the reducing power corresponded to 51% as maltose, the dextrin being precipitated in and washed by 80% boiling alcohol until the washings were free from reducing power and finally dried over H_2SO_4 at 60°; yield 46%, $[\alpha]_D^{20} + 160.6^\circ$, R 32.8%.

Amyloamylose and erythroamylose were prepared by repeated electro-dialysis at 220 v. of potato starch (autoclaved at 120°) [Samec and Mayer, 1921], using parchment membranes and platinum electrodes.

Table I. *Hydrolysis of soluble starch, α -amylodextrin and glycogen by α -malt-amylose, β -barley-amylose and mixtures of the two.*

Amylase	Equivalent weight of barley or malt per 100 ml. g.	Substrate	Dry weight per 100 ml. of reaction mixture g.	Hydrolysis as percentage of theoretical maltose							
				Minutes					Hours		
				7	20	40	90	210	8	48	
α	1.25	Soluble starch	2.00	3.06	8.74	16.3	27.4	35.5	41.8	54.0	
	2.5			6.20	15.9	25.8	32.8	40.6	47.0	64.7	
	5.0			11.7	25.4	31.8	37.4	46.3	52.7	76.6	
β	1.25	Soluble starch	2.00	2.16	4.91	9.0	18.3	34.3	47.7	55.6	
	2.5			3.54	8.94	17.5	33.0	50.1	56.4	58.3	
	5.0			6.09	16.7	31.9	48.6	56.7	60.0	64.0	
	13.0			10.5	36.8	49.0	—	—	—	—	
$\alpha + \beta$	$\left\{ \begin{array}{l} 1.25 \alpha \\ 1.25 \beta \end{array} \right\}$	Soluble starch	2.00	5.08	13.5	23.7	43.4	62.6	—	76.3	
	$\left\{ \begin{array}{l} 1.25 \alpha \\ 5.00 \beta \end{array} \right\}$			9.90	26.3	47.8	66.3	70.6	—	79.0	
	$\left\{ \begin{array}{l} 1.25 \alpha \\ 13.0 \beta \end{array} \right\}$			18.5	49.4	66.6	—	—	—	—	
	$\left\{ \begin{array}{l} 5.0 \alpha \\ 5.0 \beta \end{array} \right\}$			9.25	23.3	39.3	60.6	69.8	—	80.8	
α	1.25	α -Amylo-dextrin	2.00	3.27	8.45	14.7	20.3	25.6	29.8	42.1	
	2.5			6.27	14.0	19.1	23.9	28.6	33.7	50.1	
	5.0			10.9	18.8	22.9	26.6	31.7	37.5	60.6	
β	1.25	α -Amylo-dextrin	2.00	—	—	1.1	—	—	1.6	2.3	
	2.5			—	1.0	2.0	—	—	2.0	2.5	
	5.0			—	—	—	—	—	—	—	
$\alpha + \beta$	$\left\{ \begin{array}{l} 1.25 \alpha \\ 1.25 \beta \end{array} \right\}$	α -Amylo-dextrin	2.00	4.2	11.2	18.5	29.2	35.4	—	49.4	
	$\left\{ \begin{array}{l} 1.25 \alpha \\ 5.0 \beta \end{array} \right\}$			6.15	16.8	26.2	32.5	36.7	—	50.8	
	$\left\{ \begin{array}{l} 2.5 \alpha \\ 2.5 \beta \end{array} \right\}$			8.03	18.9	27.9	34.1	38.5	—	62.0	
α	1.25	Glycogen	2.00	1.53	3.80	6.12	10.3	14.4	20.0	33.9	
	2.5			2.98	6.11	9.53	13.7	19.1	25.3	45.6	
	5.0			5.00	9.15	12.9	18.1	24.2	31.0	68.3	
β	1.25	Glycogen	2.00	—	—	—	4.79	8.16	12.1	20.5	
	2.5			1.13	—	—	8.16	12.6	18.1	25.7	
	5.0			1.97	—	—	12.9	18.9	23.8	29.1	
$\alpha + \beta$	$\left\{ \begin{array}{l} 1.25 \alpha \\ 1.25 \beta \end{array} \right\}$	Glycogen	2.00	3.12	7.72	11.9	22.7	33.2	—	54.0	
	$\left\{ \begin{array}{l} 1.25 \alpha \\ 5.0 \beta \end{array} \right\}$			5.17	13.2	20.7	30.4	38.8	—	56.7	
Hydrolysis by β -amylase (barley) of amyloamylose and erythroamylose.											
β	11.8	Amylo-amylose	0.256	19.8	40.5	64.0	89.0	—	94.8	96.8	
β	22.2	Erythro-amylose	1.60	38.0	46.2	48.4	49.9	—	53.8	57.7	
β	22.2	Soluble starch	1.60	45.4	54.3	56.6	57.8	—	60.5	63.4	

The enzymes were prepared as described in Part I, and were free from maltase. The quantities used are expressed in terms of the equivalent quantities

Table II. *Hydrolysis by β -amylase (barley) of α -amylodextrin previously degraded to 20% of theoretical by α -malt-amylase.*

Amylase	Equivalent weight of barley per 100 ml.	Substrate	Dry weight per 100 ml. of reaction mixture	Results as percentage of theoretical maltose					
				Minutes				Hours	
				7	30	90	210	8	23
β	0.6	Degraded dextrin	2.20	2.70	8.62	11.4	12.9	13.8	14.0
	5.0		2.20	10.6	12.6	14.3	14.8	15.4	16.0
β	0.6	Original dextrin	2.20	0.2	0.8	1.0	1.5	—	2.3
	5.0		2.20	0.2	1.1	1.7	3.2	—	—

Table III. *Hydrolyses of achroodextrin by α - and β -malt-amylase.*

Amylase	Equivalent weight of malt per 100 ml.	Substrate	Dry weight per 100 ml. of reaction mixture	Results as percentage of theoretical maltose				
				Minutes		Hours		
				20	90	4	8	24
α	2.5	Achroo-dextrin	0.69	6.87	9.74	12.7	16.3	22.4
	5.0		0.69	7.91	11.5	14.8	17.3	23.9
	2.5	Starch	2.0	17.2	32.9	41.2	—	56.3
β	2.5	Achroo-dextrin	0.69	13.1	18.0	21.9	24.9	32.2
	5.0		0.69	15.5	20.5	24.7	28.5	38.6
	2.5	Starch	2.0	13.9	51.2	—	—	64.9

of malt or barley from which they were prepared. Exps. 1-5 (Table I) were performed with the same enzyme preparations, which were stored in a refrigerator and showed little change in activity.

All reactions were performed at p_H 4.6 (0.015 *N* acetate) and 25°, the enzyme solutions having been brought to p_H 4.6 by the addition of acetic acid. The progress of hydrolysis was followed iodimetrically, controls for the reducing power of the enzyme preparation and of all substrates being deducted. There is doubt whether, in the case of "non-reducing" substrates, such as starch, α -amylodextrin, glycogen and the amyloses, this deduction is justified, but it seemed desirable in this instance to adopt a uniform procedure throughout. All results are expressed as percentage of maltose theoretically equivalent to the quantity of substrate used. In some experiments control hydrolyses of starch were performed. In that reported in Table IV the appropriate quantities of maltose were added to the controls. The results in Table IV were calculated on the total concentration of carbohydrate in the reaction mixture less that of maltose equivalent to the reducing power. To indicate percentages in terms of the original starch the final percentage hydrolysis stated should therefore be

Table IV. *Hydrolysis by β -malt-amylase of starch previously degraded to 20% hydrolysis by (a) α -amylase, (b) β -amylase and the enzymes destroyed.*

Amylase	Starch previously degraded by	%	Minutes					Hours	
			7	20	40	90	210	8	24
β (dil.)	α -amylase	2.0	2.44	5.95	10.8	22.9	40.0	44.8	48.6
	β -amylase	2.0	2.05	5.19	9.93	18.6	27.3	31.0	36.9
	Control	2.0	2.21	5.83	10.9	21.3	36.3	43.4	48.3
β (conc.)	α -amylase	2.0	38.4	44.3	46.0	49.9	53.5	58.7	—
	β -amylase	2.0	28.7	34.3	37.1	43.5	49.5	55.5	—
	Control	2.0	34.2	41.8	44.5	50.6	54.5	60.1	—

multiplied by $(100 - x)/100$, where x is percentage of the original conversion. The values of x for the four hydrolyses quoted in Table IV were respectively α 21.1, β 21.1, control 21.1, α 21.9, β 18.7, control 21.6.

DISCUSSION.

The kinetics of hydrolysis of starch by α - and β -amylases separately, confirm the results reported by Van Klinkenberg and by Hanes. When employed together in low concentrations, the α - and β -amylases function additively. However, with higher concentrations, particularly of the β -enzyme, the rate of hydrolysis in the early stages exceeds the sum of the component rates (Table I). The effect on starch may be partly attributed to the presence of α -amylodextrin (but see also discussion of Table IV). The observation that the limit of hydrolysis with mixed amylases is only 10–12 % greater than with single amylases of similar total activity and never approaches 100 %, suggests that at least half of the starch constitutes a substrate common to both enzymes.

Greater activity was shown in the late stages and higher limiting hydrolyses attained than in the experiments of Hanes at 35°, or of Van Klinkenberg at 40°. Inactivation of the enzyme is the probable explanation. The β -amylase by itself exercised a negligible effect on α -amylodextrin, but acting in conjunction with the α -enzyme it evidently hydrolysed some of the early fission products formed by the latter, since the rates of hydrolysis by the combined enzymes greatly exceeded the sums of the component hydrolyses [see also Hanes, 1935]. This conclusion was confirmed by allowing β -amylase to attack α -amylodextrin previously hydrolysed to 20 % by α -amylase, after destruction of the latter enzyme, with controls (Table II). An appreciable further hydrolysis took place.

Results similar to those with α -amylodextrin were obtained with glycogen, which in this respect behaves as if intermediate in character between starch and α -amylodextrin (Table I).

Achroodextrin, the maltose-free product of hydrolysis of starch to 50 % by pancreatin, was more rapidly hydrolysed by β - than by α -amylase (of about equal activities on starch). The limit, about 40 % in the case of β -amylase, leaves 60 % of the dextrin unhydrolysed; this is equivalent to 30 % of the original starch, and probably corresponds to a large part of the α -amylodextrin fraction of the latter. β -Amylase hydrolyses α -amylodextrin, previously slightly degraded by the α -enzyme, to about 15 % (Table II), leaving perhaps 70 % of the dextrin, equivalent to 28 % of the original starch. This agrees in quantity with the undegraded residue of the achroodextrin. The achroodextrin probably contains a high proportion of α -amylodextrin, the hydrolysis of which by the pancreatic (α -) amylase has not progressed far. That it should succumb more rapidly to the action of β - than α -amylase is in agreement with the greatly reduced rate of starch hydrolysis promoted by the latter enzyme at the 50 % stage, as compared with that of the β -enzyme when of equal initial activity (see Table I).

The products of the actions on starch of the α - and β -amylases to the 20 % stage can be understood from the results of certain experiments described in Part I. When β -amylase acted on these products and a control of starch + maltose and enzyme concentration was the limiting factor, the three substrates were hydrolysed at approximately the same rates, except in so far as modified by the approach of the limit. With very high concentrations of the β -amylase, the starch previously degraded by the α -enzyme was more rapidly attacked than the others. This may be due either to the higher molecular concentration of substrate,

which had in this case been broken up into dextrans by the α -enzyme, or to the relative absence of maltose, an inhibiting agent. On the whole there is no clear evidence that, at this stage of the hydrolysis, the dextrinous fission products of the α -amylase are more susceptible to the action of β -amylase than is starch.

The conclusions to be drawn from these experiments and discussion are as follow.

(1) Both α - and β -amylases readily attack and hydrolyse completely the amyloamylose component (about 20 %) of starch, and a portion of the erythroamylose component.

(2) Both enzymes hydrolyse much the same fraction, 60 %, of the starch; but whereas the remainder (α -amylodextrin) is almost if not completely resistant to the β -amylase, it can be, with difficulty, hydrolysed by the α -enzyme.

(3) α -Amylodextrin, the hydrolysis of which has been commenced by the α -amylase, can be further degraded to a certain extent by the β -amylase. The α -enzyme holds the key to further progress.

(4) In degrading starch to the 50 % stage, the α -enzyme does so mainly at the expense of the portion which is other than amylodextrin, since action on the residue by β -amylase still leaves about 30 % of the original starch unattacked.

SUMMARY.

The velocities of hydrolysis of soluble starch, amyloamylose, erythroamylose, α -amylodextrin, achroodextrin and glycogen by α -malt-amylase and β -malt- or barley-amylase have been measured. The results confirm those of previous workers. A mixture of the α - and β -amylases in low concentration functions additively in hydrolysing starch. A mixture with β -amylase in high concentration promotes hydrolysis of starch at a rate greater than the sum of the component rates. Mixtures at either concentration hydrolyse α -amylodextrin and glycogen at rates greater than the sums of the component rates.

Achroodextrin, a fission product of the action of α -amylase, is hydrolysed more rapidly by β -amylase than by α -amylase. α -Amylodextrin previously degraded by α -amylase to about 20 % hydrolysis, is (after destroying the α -enzyme), hydrolysed by β -amylase. The residues left when α -amylodextrin is attacked successively by α - and β -amylases and when achroodextrin is hydrolysed by β -amylase correspond in each case to about 28–30 % of the original starch. Amyloamylose is readily hydrolysed by β -amylase to nearly 100 % maltose, whereas erythroamylose is degraded with greater difficulty than starch, and to a limit within 60 %.

The suggestion of Van Klinkenberg of selective hydrolysis of different components of the starch by the respective amylases is not supported by the evidence. It appears that both amylases hydrolyse the same (non- α -amylodextrin) portion of the starch, amounting to about 60 %. Only the α -amylase can attack the α -amylodextrin portion, which however is not appreciably degraded by the enzyme until the hydrolysis of the starch is fairly advanced.

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