[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, COLUMBIA UNIVERSITY]

Further Studies of the Action of Pancreatic Amylase: A Differentiation of the Products of the Hydrolysis of Potato Starch and of a Linear Fraction from Corn Starch[1]

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Considerable information concerning the mechanism of the enzymic hydrolysis of starch may be obtained from investigations of the action of purified amylases upon different substrates. The work reported briefly here deals with studies of the action of highly purified maltase-free pancreatic amylase[1,2] upon unfractionated potato starch, Lintner soluble potato starch, and upon a linear fraction from corn starch. The linear substrate was kindly supplied by Dr. T. J. Schoch. It was hydrolyzed completely to fermentable sugar by beta amylase and corresponded to 94% "amylose"[3] by potentiometric titration.[4] The studies included comparisons of the rates of the hydrolysis of the different substrates as well as of the products formed from them.

Experimental

The hydrolyses were carried out at 40° as previously described.[2] Portions of the reaction mixtures were removed at intervals during the hydrolyses and examined for total reducing value by iodometric titration.[5] The reducing values were converted into their equivalents of maltose or of glucose and are reported in terms of the percentage yield of maltose or of glucose that could be obtained theoretically from the substrate. Because they give stoichiometric values, iodometric titrations give a measure of the number of glucosidic linkages of the substrate that have been ruptured. Portions of the reaction mixtures were also examined at intervals during the hydrolyses for maltose and for glucose by a modification of the method of Somogyi[6,7] for the selective fermentation of these sugars by washed bakers yeast. Examination of mixtures of known concentrations of glucose and of maltose in the presence of starch showed that these sugars could be accounted for under the conditions of these experiments to within 2%. The reducing value of the reaction mixtures in excess of that due to glucose and maltose was attributed to unfermented reducing dextrins. According to Myrbäck,[8,9] conditions which permit the fermentation of maltose also permit some fermentation of any straight-chain trisaccharide which may be present. No attempt was made in this work to distinguish between maltose and such a trisaccharide.

Average degrees of polymerization have been calculated for the reducing dextrins from their weights and reducing values. In the early stages of the hydrolyses, the values for the average degrees of polymerization of the dextrins cannot be taken as absolute values. Iodometric methods are stoichiometric for reducing sugars of low molecular weights. The application of the method to dextrins formed in the earlier stages of the hydrolyses may introduce certain errors. However, this method seems to be the most satisfactory of those at present available by which to obtain an indication of the breakdown of the substrates.

No evidence of maltase activity was found in composite mixtures of the preparations of purified pancreatic amylase even when the highest concentrations used here reacted for twenty-four hours at 40° with 1% maltose under the conditions used for the hydrolysis of starch.[10] Similarly, attempts to cause the selective inactivation of amylase and of glucosidase (dextrinase) activities failed to give evidence of the presence of traces of carbohydrases other than amylase in the preparations of purified pancreatic amylase.[2] Therefore, the results reported here do not appear to be influenced to any significant extent by the action of contaminating carbohydrases.

Results

Rates of Hydrolysis.—The typical data summarized in Table I show that Lintner soluble

TABLE I

A COMPARISON OF THE ACTION OF PANCREATIC AMYLASE ON POTATO STARCH AND ON LINTNER SOLUBLE POTATO STARCH

	Relative concentrations of amylase[b]					
	1		8		32	
	Reducing values as per cent. theoretical maltose, %					
Reaction[a] time, min.	Potato starch	Lintner soluble potato starch	Potato starch	Lintner soluble potato starch	Potato starch	Lintner soluble potato starch
15	67	69	74	77	83	84
30	71	71	75	79	86	86
60	73	71	79	80	89	91
180	76	73	85	84	99	100
300	77	74	89	86	105	107

[a] Starch, 1%; 0.01 *M* phosphate; 0.02 *M* chloride; *p*H 7.2; 40°.[10] [b] Amylase preparation 1, 8 or 32 mg. per 1000 mg. starch.

TABLE II

A COMPARISON OF THE ACTION OF PANCREATIC AMYLASE ON LINTNER SOLUBLE POTATO STARCH AND ON A LINEAR FRACTION FROM CORN STARCH

	Reducing value as per cent. theoretical maltose	
Reaction[a] time, min.	Lintner soluble potato starch	Linear fraction from corn starch
15	74	95
30	77	96
60	79	99
120	85	100
180	90	108
360	94	111

[a] Starch or linear substrate, 0.5%; 0.01 *M* phosphate; 0.02 *M* chloride, *p*H 7.2; 40°.[10] Amylase preparation 8 mg. per 1000 mg. of carbohydrate.

(1) (a) The authors wish to thank the Corn Industries Research Foundation for generous grants in aid of this investigation. (b) The data reported here are taken from a dissertation submitted by Roslyn B. Alfin in partial fulfillment of the requirements for the degree Doctor of Philosophy in Chemistry under the Faculty of Pure Science of Columbia University.

(2a) H. C. Sherman, M. L. Caldwell and M. Adams, *J. Biol. Chem.*, **88**, 295 (1930); (2b) R. B. Alfin and M. L. Caldwell, THIS JOURNAL, **70**, 2534 (1948).

(3) R. W. Kerr and G. M. Severson, *ibid.*, **65**, 193 (1943).

(4) F. L. Bates, D. French and R. E. Rundle, *ibid.*, **65**, 142 (1943).

(5) M. L. Caldwell, S. E. Doebbeling and S. H. Manian, *Ind. Eng. Chem., Anal. Ed.*, **8**, 181 (1936).

(6) M. Somogyi, *J. Biol. Chem.*, **119**, 741 (1937).

(7) M. Somogyi and I. E. Stark, *ibid.*, **142**, 579 (1942).

(8) B. Örtenblad and K. Myrbäck, *Biochem. Z.*, **303**, 335 (1940).

(9) K. Myrbäck, *ibid.*, **304**, 147 (1940).

(10) H. C. Sherman, M. L. Caldwell and M. Adams, THIS JOURNAL, **50**, 2529, 2535, 2538 (1928).

TABLE III

A COMPARISON OF THE HYDROLYSIS OF POTATO STARCH AND OF A LINEAR FRACTION FROM CORN STARCH BY PURIFIED MALTASE-FREE PANCREATIC AMYLASE

Reaction[a] time, min.	Reducing values as per cent. theoretical maltose: Total, Potato starch	Total, Linear substrate	Maltose[b], Potato starch	Maltose[b], Linear substrate	Glucose[b] as per cent. of theoretical glucose: Potato starch	Glucose[b], Linear substrate	Dextrins: Per cent. of total products by weight: Potato starch	Per cent., Linear substrate	Average degree of polymerization, DP[c]: Potato starch	Average degree, Linear substrate
5	2.94	1.55	1.57	1.32			98.4	98.7	144	860
10	4.08	3.34	1.92	2.29			98.1	97.7	89	464
15	5.36	5.27	2.12	3.56			97.9	96.4	60	113
20	6.40	6.64	2.53	3.83			97.5	96.2	51	68
25	7.49	8.19	2.94	4.09			97.1	95.9	43	47
30	8.58	9.97	3.46	5.27			96.5	94.7	38	40
45	12.8	14.8	4.50	6.42	0.24		95.3	93.6	24	22
60	16.6	19.3	6.00	8.00	.55	0.15	93.5	91.9	20	17
90	23.2	27.9	7.92	10.5	.89	.65	91.2	88.9	13	11

[a] Potato starch, 1% or linear substrate, 0.25%; 0.01 M phosphate; 0.02 M chloride; pH 7.2; 40°.[10] Amylase preparation, 0.01 mg. per 1000 mg. carbohydrate. [b] Glucose or maltose by selective fermentation with washed bakers yeast; dextrins by difference.[6,7] [c] Average degrees of polymerization of dextrins, calculated from their reducing values (maltose equivalents) as follows:

$$DP = \frac{\text{weight of dextrins (mg.)}}{\text{reducing value as maltose (mg.)}} \times 2$$

potato starch and unfractionated potato starch were hydrolyzed at practically the same rates when treated with the same concentrations of pancreatic amylase under comparable conditions. On the other hand, the data given in Tables II and III show significant differences in the rates of the hydrolysis of the linear substrate and of unfractionated potato starch.

In the very early stages of the hydrolyses, in the presence of relatively low concentrations of amylase (Table III), unfractionated potato starch was hydrolyzed more rapidly than the linear substrate. However, after approximately 3% of the glucosidic linkages of the substrates had been broken, the linear substrate was hydrolyzed more rapidly than the unfractionated starch (Tables II and III). These results suggest that there are more points for attack by pancreatic amylase in the branched chain than in the straight-chain components of starch, but that the reducing dextrins formed from the branched chain components are hydrolyzed less rapidly by the amylase than are the reducing dextrins formed from the straight chain components.

Further study of the data given in Table III shows not only that pancreatic amylase hydrolyzes

TABLE IV

PRODUCTS FORMED FROM POTATO STARCH AND FROM THE LINEAR SUBSTRATE BY PURIFIED MALTASE-FREE PANCREATIC AMYLASE[a]

Reducing values as per cent. theoretical maltose: Total	Reducing dextrins[b], Potato starch	Reducing dextrins[b], Linear substrate	Maltose[b], Potato starch	Maltose[b], Linear substrate	Glucose[b] as per cent. of theoretical glucose: Potato starch	Glucose[b], Linear substrate	Dextrins[b]: Per cent. of total products by weight: Potato starch	Per cent., Linear substrate	Average degrees of polymerization[c]: Potato starch DP	Average degrees, Linear substrate DP
2.9	1.33	0.5	1.57	2.0			98.4	98.0	148	392
5	2.8	2.3	2.2	2.7			97.8	97.3	70	85
10	6.5	5.5	3.5	4.5			96.5	95.5	30	35
15	9.5	8.5	4.7	6.5	0.40		94.9	93.5	20	22
20	12.3	11.3	6.5	8.3	0.60	0.2	92.9	91.5	15	16
25	15.0	13.5	8.0	10.5	1.0	0.5	91.0	89.0	12	13
30	18.4	15.9	9.0	12.5	1.3	0.8	89.7	86.7	9.8	11
35	21.3	18.1	10.5	14.5	1.6	1.2	87.9	84.3	8.3	9.3
40	23.9	19.3	12.5	17.5	1.8	1.6	85.7	80.9	7.2	8.4
50	28.7	22.4	16.5	23.0	2.4	2.3	81.1	74.7	5.7	6.7
60	31.0	24.1	21.0	29.5	4.0	3.2	75.0	67.3	4.8	5.6
70	31.3	23.5	25.5	38.5	6.6	4.0	67.9	57.5	4.3	4.9
80	30.2		31.0		9.4		59.6		3.9	
90	22.5		41.5		13.0		45.5		4.0	
100	15.5		50.5		17.0		32.5		4.2	
110	15.2		52.0		21.4		26.6		3.5	

[a] Potato starch, 1% or linear substrate 0.25%; 0.01 M phosphate; 0.02 M chloride; pH 7.2; 40°.[10] [b] Glucose or maltose, by selective fermentation with washed bakers yeast; dextrins by difference.[6,7] [c] Please see Table III, footnote *c*.

the unfractionated starch and the linear substrate at different rates, but also that, for the same time intervals with the same concentration of amylase, the relative concentrations of the products formed from the two substrates differ.

A comparison of the products formed from the two substrates when equivalent numbers of glucosidic linkages had been ruptured is given in Table IV.

Examination of the data summarized in Tables III and IV shows that maltose was present in measurable concentrations from the very early stages of the hydrolysis. It was present in considerably larger concentrations in the hydrolyzates from the linear substrates than in those from potato starch whether the comparisons are made at the same time intervals with equal concentrations of amylase (after the first five minutes, Table III) or at equivalent stages in the hydrolyses of the two substrates (Table IV).

Glucose, also, was liberated by maltase-free pancreatic amylase from potato starch and from the linear components of corn starch although this sugar did not appear in the very early stages of the hydrolyses of these substrates. The data given in Table IV show that even after 15% of the glucosidic linkages of the substrates had been broken, only about 1% of glucose was present. Comparisons at the same time intervals with equal concentrations of amylase (Table III) and also at equivalent stages in the hydrolyses of the two substrates (Table IV) show that glucose appeared somewhat earlier and at equivalent hydrolysis was present in slightly larger concentrations in the hydrolyzates of potato starch than in those of the linear substrate. These differences in the concentrations of maltose and of glucose obtained from the two substrates suggest that the presence of 1,6-α-D-glucosidic linkages in the branched chain components of starch interferes with the formation of maltose and favors the production of glucose by pancreatic amylase. This suggestion has received confirmation in subsequent studies with waxy maize starch, a branched chain substrate.[11]

The data given in Table IV also show that pancreatic amylase causes the rapid breakdown of both substrates to reducing dextrins of low average molecular weights and of relatively high reducing values. When the total reducing values of the hydrolyzates were equivalent to 50% theoretical maltose, reducing dextrins accounted for 57 and 45% of the total reducing value and for 81 and 75% by weight of the total products present in the hydrolyzates from the unfractionated starch and from the linear substrate, respectively. These findings are in accord with the observations of many investigators that pancreatic amylase causes a rapid decrease in the viscosities of its substrates and the rapid disappearance of products which give color with iodine. In the present studies, a clear red color with iodine was obtained when the reducing values of the reaction mixtures were equivalent to approximately 25% theoretical maltose. The achromic stage was reached at approximately 55% theoretical maltose.

Further study of the reducing dextrins (Tables III and IV) is of interest. During the early stages of the hydrolyses, the average molecular weights of the reducing dextrins liberated from the linear substrate were relatively high as compared to the average molecular weights of the dextrins from unfractionated potato starch. These findings again suggest that the branched chain components of starch offer more points of attack for pancreatic amylase than do the linear components. However, reducing dextrins were present in consistently lower concentrations in the hydrolyzates from the linear substrate than in those from the unfractionated starch. Therefore, the dextrins from the linear substrate appear to be hydrolyzed more readily than those from the branched chain components of starch.

Taken all together, the data lead to the conclusion that pancreatic amylase causes the random hydrolysis of both the branched and the linear components of starch.

Comparisons of the data obtained with unfractionated potato starch and with Lintner soluble potato starch, showed that a preliminary acid treatment of potato starch influences appreciably the course of the hydrolysis of starch by pancreatic amylase. As would be expected, the average molecular weights of the reducing dextrins were lower in the earlier stages of the hydrolysis of the acid treated, partially degraded starch. In the later stages of the hydrolyses, more maltose and smaller concentrations of residual dextrins of the same or of slightly higher average molecular weights were present in the hydrolyzates of Lintner soluble potato starch than in those of untreated potato starch.

Summary and Conclusions

A study has been made of the action of purified maltase-free pancreatic amylase upon unfractionated potato starch, Lintner soluble potato starch and upon a linear fraction from corn starch.

No marked differences were observed in the rates of the hydrolysis of unfractionated potato starch, or of Lintner soluble potato starch when comparisons were based upon the total reducing values of the hydrolyzates obtained with the same concentrations of amylase reacting under comparable conditions.

On the other hand, marked differences were observed in the rates of hydrolysis of unfractionated potato starch and of the linear substrate.

The evidence indicates that there are more points for attack by pancreatic amylase in the branched chain components than in the linear components of starch but that the reducing dextrins formed from the branched chain components are hydrolyzed less readily by pancreatic amylase

(11) F. M. Mindell, A. L. Agnew and M. L. Caldwell, THIS JOURNAL, in press.

than are the reducing dextrins formed from the linear components. Marked differences were also found in the relative concentrations of the products formed by pancreatic amylase from unfractionated potato starch and from the linear substrate. These differences were observed whether the comparisons were made for the same time intervals with the same concentrations of amylase or at equivalent stages in the hydrolysis of the two substrates.

Maltose was present in measurable concentrations from the very early stages of the hydrolysis of unfractionated potato starch and of the linear substrate. However, this sugar was present in considerably higher concentrations in the hydrolyzates from the linear substrate than in those from potato starch whether comparisons are made at the same time intervals with equal concentrations of amylase or at equivalent stages in the hydrolysis of the two substrates.

Glucose was liberated by maltase-free pancreatic amylase from unfractionated potato starch and from the linear substrate although this sugar did not appear in the very early stages of the hydrolyses of these substrates. Comparisons made at the same time intervals with equal concentrations of amylase and also at equivalent stages in the hydrolyses of the two substrates show that glucose was liberated somewhat earlier and at equivalent hydrolysis was present in slightly larger concentrations in the hydrolyzates of potato starch than in those of the linear substrate.

A study of the results as a whole indicates that purified pancreatic amylase causes the random hydrolysis of both the straight and the branched chain components of starch.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, OREGON STATE COLLEGE]

Pantothenic Acid Studies. VI. A Biologically Active Conjugate of Pantothenic Acid[1]

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In a previous paper from this Laboratory[2] glutamic acid was reported to enhance the growth promoting property of pantothenic acid in certain strains of yeast. A related observation was made by Woolley,[3] who found that glutamic acid was very active in reversing the inhibitory effect of ketone analogs of pantothenic acid in organisms which require the preformed vitamin in the medium. Although the mechanism of these actions is at present obscure, it appeared possible that glutamic acid might conjugate with pantothenic acid or β-alanine (and possibly with pantoic acid in the proper linkage) to produce a substance which is more active for the growth of microörganisms. Since numerous possibilities exist for combinations of pantothenic acid, glutamic acid and β-alanine, their direct preparation was deferred in favor of biosynthesis of active materials by resting cells.

Glutamic acid was incubated in buffered saline with β-alanine or with pantothenic acid in the presence of resting yeast cells (*S. cerevisiae* L.M. strain, A.T.C.C. No. 9371). The resulting mixture showed extremely great activity, occasionally over a thousand times that of the β-alanine or pantothenic acid present, as measured in a pantothenic acid-free medium using L.M. yeast. The incubated product from β-alanine was always more active than that from pantothenic acid. However, the results were not consistent; in six of eighteen experiments no increase in activity was obtained, and the trend among the later experiments was toward increases of twofold or less. It was not possible to tell whether this was due to inconsistencies in synthesis of active material or to variable requirements by the assay organisms. It was therefore decided to seek another assay organism for the active principle, as well as new sources from which it might be isolated.

Stimulation of Growth in *S. cerevisiae* 2190

Strain 2190 (National Collection of Cultures, London) was observed previously[4] to grow very feebly in a pantothenic acid containing medium which was satisfactory for the growth of sixteen other yeasts. The addition of glutamic acid improved the growth considerably, although further enhancement was obtained with yeast extract. The effects are summarized in Table I. The glutamic acid effect recalls the similar observation in strains L.M. and 2504.[2] However, strain 2190 was not stimulated by the mixture resulting from incubation with resting L.M. yeast cells. The extra stimulatory effect of yeast extract upon 2190 was also manifested by acid-hydrolyzed casein, as well as by amino acid mixtures containing no additional glutamic acid.

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(2) T. E. King and V. H. Cheldelin, *Arch. Biochem.*, 16, 231 (1948).

(3) D. W. Woolley, *J. Biol. Chem.*, 168, 481 (1946).

(4) H. P. Sarett and V. H. Cheldelin, *J. Bact.*, 49, 81 (1945).