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Some Auxoamylases

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SOME AUXOAMYLASES.

(ABSTRACT.)

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The amylases, or starch splitting enzymes, are aided, or have their activity increased, by certain nitrogenous compounds particularly those which contain the NH_2 group. These amylolytic stimulating substances I have called auxoamylases. All amino compounds are not auxo substances, however. The experiments described here have been made to ascertain what compounds do activate the amylases and the conditions which affect their action.

Method.—Boiled starch solution was digested at 38° with dilute enzyme solutions under toluene, portions being removed at intervals for testing. The degree of hydrolysis was determined by heating the digested solution on a steam bath with an excess of Fehling's solution, filtering off the cuprous oxide formed, dissolving this washed precipitate in nitric acid, boiling off the nitrous acid, neutralizing the excess of acid, making acid with acetic acid and precipitating the copper as CuCl with KI. The iodine thus liberated was determined by titration with tenth-normal $Na_2S_2O_3$ solution. The quantity of reducing sugar formed by digestion of the starch is, consequently, proportional to the $Na_2S_2O_3$ used. Since the hydrogen ion has a marked effect on amylases, when the amino compounds had an acid reaction they were carefully neutralized before the digestion.

The following classification of the nitrogen compounds was found:

AUXOAMYLASES.

Glycine, $NH_2CH_2CO_2H$.
Tyrosine, $HOC_6H_4CH_2CH(NH_2)CO_2H$.
Hippuric acid, $C_6H_5CONHCH_2CO_2H$.
Anthranilic acid, $NH_2C_6H_4CO_2H$.
Asparagin, $NH_2COCH_2CH(NH_2)CO_2H$.

INACTIVE NH_2 COMPOUNDS.

Sulphanilic acid, $NH_2C_6H_4SO_2H$.
Acid amids like,
Urea, $(NH_2)_2CO$.
Acetamid, CH_3CONH_2.
Propionamid, $CH_3CH_2CONH_2$.

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Table 1 gives some of the details of such a digestion showing that asparagin acts as an auxoamylase but acetamid does not.

TABLE NO. 1.

ACETAMID AND ASPARAGIN.

Each contained 5 cc. toluene and 180 cc. 1 per cent starch.

A. 10 cc. water and 10 cc. of 3 per cent saliva.

B. 0.5 grm. acetamid in 10 cc. water and 10 cc. saliva.

C. 0.5 grm. asparagin, (neutralized) in 10 cc. of water and 10 cc. of 3 per cent saliva.

Degree of digestion shown by cc. of $Na_2S_2O_3$ used:

Digested one hour.	Digested two hours.
A= 4.45 cc. $Na_2S_2O_3$	A= 8.6 cc. $Na_2S_2O_3$
B= 4.18 cc. "	B= 8.9 cc. "
C= 5.90 cc. "	C=10.1 cc. "
Digested four hours.	**Digested six hours**
A=15.1 cc. $Na_2S_2O_3$	A=17.9 cc. $Na_2S_2O_3$
B=14.85 cc. "	B=17.0 cc. "
C=18.35 cc. "	C=19.1 cc. "

Digested 24 hours.
A=25.0 cc. $Na_2S_2O_3$
B=24.5 cc. "
C=27.8 cc. "

In the succeeding tables the details are omitted but the figures, as before, represent the amounts of sugar formed from the starch.

TABLE NO. 2.

ACTION OF GLYCINE.

200 cc. of starch-saliva solution used.

Glycine used	0.5 hour	1.5 hours	4.0 hours	5.5 hours
None	3.95	9.70	16.1	18.75
0.1 grm.....	6.00	11.25	17.2	20.40
0.3 grm.....	9.00	12.80	18.7	21.20
0.5 grm.....	8.20	16.50	22.2	23.25

TABLE No. 3.

TYROSINE.

Tyrosine used	40 min.	1.75 hrs.	4 hrs.	6.25 hrs.	24 hrs.
None	1.25	5.35	9.65	11.15	18.55
0.05 grm.....	2.85	6.20	11.30	13.72	22.25
0.1 grm.....	3.35	6.55	12.10	13.65	24.00

The volume of the digestion solution was 200 cc.

The amylase was ptyalin.

TABLE NO. 4.

HIPPURIC ACID (neutralized).

Digested hours	No hippuric acid	1.2 grm. hippuric acid in 200 cc. solution
1	4.5	9.3
2	9.2	14.8
4	14.05	18.35
24	18.8	20.2

TABLE NO. 5.

ANTHRANILIC ACID (neutralized).

200 cc. of starch-saliva solution used.

Anthranilic acid used	1 hour	3 hours	5 hours	25 hours
None	5.5	12.2	15.1	23.9
0.1 grm.....	7.1	14.3	17.1	25.8
0.3 grm.....	8.3	15.6	18.5	25.9
0.5 grm.....	8.8	16.4	18.7	25.1

TABLE NO. 6.

ACTION OF GLYCINE ON PANCREATIC AMYLASE.

1 cc. of pancreas solution.

Glycine	30 min.	1 hr. 40 min.	2 hr. 20 min.
None	14.6	23.9	24.8
0.5 grm.....	21.1	27.4	30.9

5 cc. of pancreas solution.

None	....	28.7	29.1
0.5 grm.....	....	32.2	35.9

TABLE NO. 7.

ACTION OF UREA ON PTYALIN.

200 cc. starch-saliva solution.

Digested hours	No urea	0.5 grm. urea
2	1.8	2.1
4	3.5	3.6
6	4.0	4.0
24	9.1	9.1

TABLE NO. 8.

SULPHANILIC ACID (neutralized).

Digested hours	No sulphanilic acid	1.3 grm.Na Sulphanilate in 200 cc. of solution
0.5	7.4	7.5
1.5	14.3	14.6
4.0	18.9	19.8
6.0	20.8	21.0

The work is being continued. Its importance is seen from the fact that amino acids are produced by digestive proteolysis and that they must act in the intestine as hormones to the amylolytic enzymes.