STUDIES ON THE ENZYMES OF SILKWORM. I.

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For the properties of enzymes of silkworm we have yet only a few reports, so I intended to study the properties of enzymes of various organs of silkworm. At first the enzymes of the intestine has been studied. In the present paper some properties of amylase in the middle intestine are described.

Preparation of Enzyme. The intestinal tube from 1000 matured silkworms has been collected by dissolution, and washed thoroughly by distilled water. The organ is then ground up in an ordinary mortar with sands. The mass is then treated with acetone, acetone-ether and lastly with ether, then spread out on filter paper in the air for 30 minutes to evaporate the ether. The preparation is dried in vacuo over sulphuric acid, powdered and preserved.

The active enzyme is obtained by extraction of the powder with water, glycerin solution or buffer solution (for example glycocoll-NaCl-NaOH mixture $P_H=9.62$). From extracted solution the enzyme have been precipitated with acetone or alcohol, the precipitate is washed with absolute alcohol, then with ether and lastly is dried in vacuo over sulphuric acid. For the further purification the resulting precipitate is redissolved in water and absorbing the amylase with kaolin (at $P_H=9.62$). Absorbed amylase can be washed out with ammonium-phosphate mixture solution. The elution is then diluted with water and the phosphate is precipitated with magnesium mixture and again precipitated.

The amylase which is obtained in this way has the activity (saccharifying action) $\overset{25^\circ C}{\underset{24\text{ hour}}{D}}=1500$, per gram in wohlgemuth unit.

Optimal Temperature. The velocity of the amylase reaction is accelerated as the temperature is raised until 60°C. On further elevation of the temperature the reaction velocity begins to diminish until it cease completely (80°C).

It is generally believed that each enzyme has an optimal temperature characteristic of that enzyme. This optimum is, however, greatly influenced by its concentration, the nature and concentration of substrate upon which the enzyme acts, the reaction of medium, and the period of action, etc.

In the case of this amylase, the optimal temperature observed are as follows, the value of P_H being 9.6.

Table 1.

Optimal Temp.	Period of action
55–60°C.	6 hours or less
55°C.	10 hours
50–55°C.	20 hours
50°C.	24 hours

Reaction velocity, temperature coefficient and temperature constant are shown in Table 2 and Fig. 1.

Table 2.

The rate of saccharifying of soluble starch by amylase at various temperature (P_H=9.6).

Time (hour) / Temp.	Sugar produced (mg.)							Velocity constant $k=\frac{1}{t}\log\frac{a}{a-x}$	Temperature coefficient $k_{t+10}:k_t$	Temperature constant $A=\frac{\log k_2-\log k_1}{0.4343}$ $R\frac{T_2T_1}{T_2-T_1}$
	1/2	1	2	3	4	5	24			
80°C.	0	0	0	—	—	—	—	—	—	—
70	1.2	1.6	2.6	3.4	4.0	4.4	7.4	—	—	—
65	1.25	2.9	5.7	7.5	9.0	11.4	15.0	0.00150	—	—
60	1.5	3.3	6.7	9.0	11.4	13.0	20.0	0.00217	—	—
55	1.3	3.0	5.2	7.5	9.5	12.2	21.0	0.00151	1.85	13271
50	1.0	2.1	4.1	6.3	7.8	9.4	21.7	0.00117		
40	0.6	1.1	2.0	3.7	5.2	6.3	17.4	0.00060	1.94	13486
30	—	0.55	1.3	2.4	3.0	3.6	15.8	0.00030	2.00	13088

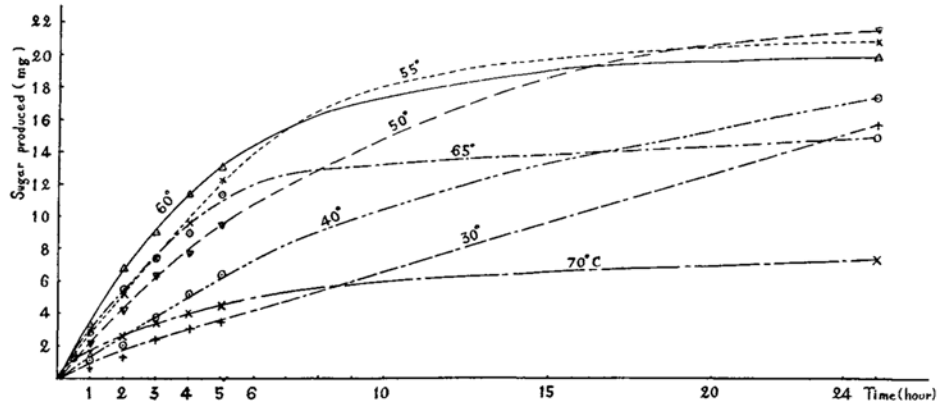

Fig. 1.

Optimal Hydrogen Ion Concentration for the Amylase Action. Most enzymes are greatly influenced by the concentration of hydrogen ion of the medium in which they act. That is, there is an optimal hydrogen ion concentration for the action of each enzyme.

The intestinal amylase of silkworm has an optimal hydrogen ion concentration at P_H=9.4 to 11. This result has been obtained by comparing the activity at various values of P_H, other conditions being held constant, and at lower temperature 30°C., so as to avoid the destruction of the enzyme. The activity has been measured by determining the reducing action of the decomposed starch, e.g. with respect to Fehling's solution according to Bertrand. The results of experiments for the optimal P_H are as follows. (Table 3, Fig. 2).

Table 3.

Milli-grams of sugar produced by the action of amylase at various values of P_H. (Period of action=10 hours)

Temp. \ P_H	5.3	5.9	6.8	7.4	8.04	8.6	9.4	9.7	11.3	12.9
30°C.	2.4	2.6	2.9	3.5	4.5	5.1	9.1	9.0	9.1	1.4
40	2.6	2.9	3.2	3.8	5.0	5.6	10.3	10.1	10.2	1.5

Thus, the P_H optimum of the amylase lies between 9.4–11.3. The optimal P_H for an enzyme action often depend upon the type of buffer solution used, so the several types of buffers, have been examined, for example, phosphate mixture, glycocoll-NaOH mixture and ammonium mixture, etc. and it was observed that the P_H optimum for this amylase is independent of the nature of buffer solution used.

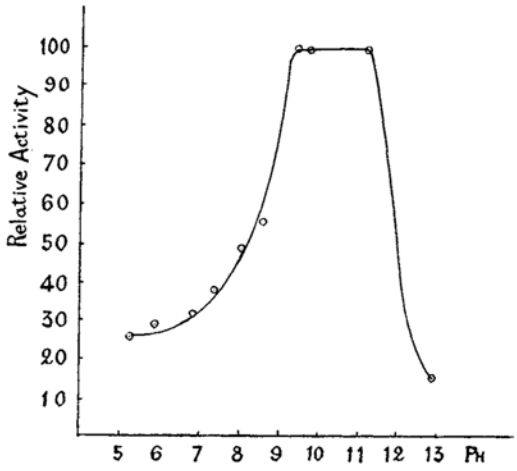

Fig. 2.

Isoelectric Point of the Amylase. Amphoteric substances, such as proteins, to which most enzymes are probably related, are charged positively or negatively depending upon the ion with which they are combined or upon the reaction of the solution in which they are suspended. These colloid particles travel toward the pole of opposite sign to that of the charge.

In the case of this amylase, I observed that the enzyme travel toward the negative pole in alkaline solution and toward the positive pole in acid reaction, and neither negative nor positive pole at about P_H=5.7.

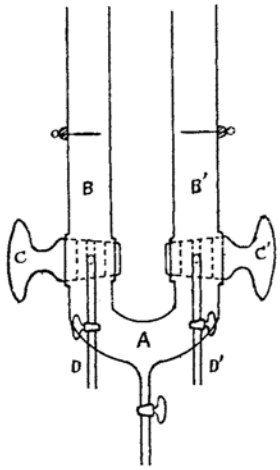

Fig. 3

The apparatus for this experiment is shown in Fig. 3. The middle part (50 c.c.) of U shaped tube is filled by the enzyme solution (A). After closing the cocks C and C′ each limbs of U-tube is filled with 50 c.c. of distilled water. When the water has settled, the cocks C and C′ are slowly opened and connect the electrodes with the circuit of 100 volt direct current. The whole apparatus should be kept as free from shaking as possible. After one hour, the cocks C and C′ are closed and the solutions at B and B′ are drawn out by the cocks D and D′, and determined the enzymatic activity of each solution.

Table 4 shows the number of milli-grams of sugar produced by the action of 5 c.c. of the solution at B and B′ with 10 c.c. of 1% soluble starch solution at 40°C. (P_H=9.7 for 4 hours). The enzymatic power of the original enzyme solution (A) is 51 under the same condition.

From the result of the observation one can conclude that the amylase is positively charged in alkaline reaction, and negatively charged in acid reaction, and its isoelectric point is at about P_H=5.7.

Table 4.

Cataphoresis of amylase.

P_H	Positive pole B	A	Negative pole B′
9.7	0.0	7.1	39.5
7.2	0.0	15.2	.30.4
6.6	0.0	39.7	6.2
5.7	trace	50.0	0.0
3.9	4.0	42.0	0.0

On the other hand it was observed that the amylase is absorbed quantitatively by kaolin, and not by infusorial earth in alkaline solution. On the contrary, the amylase is absorbed by infusorial earth, and not by kaolin in acid solution (P_H is less than 5.7), and is absorbed both by kaolin and by infusorial earth at P_H=5.7. This result coincide with the observation of cataphoresis.

Summary.

1. The optimal temperatures for the action of amylase obtained from the middle intestine of silkworm have been determined.

2. The optimal hydrogen ion concentration for the amylase action has been determined to be P_H=9.4–11.3 at 30–40°C.

3. The amylase charges positively in alkaline reaction, and negatively in acid reaction, the isoelectric point being at about P_H=5.7.

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