BIOCHEMICAL STUDIES ON RICE STARCH. III. THE ACTION OF ENZYMES ON RICE STARCH. I.

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The experimental results of researches into the complex metabolic processes which take place in the reserved starch during the germination of the seeds have been described briefly in the previous article, and the transformation of the starch molecule into simpler sugars was assumed to take place successively by the action of the hydrolytic enzymes such as amylase or diastase which, according to the opinion of Brown and Morris, seems to occur in the seedlings by a direct glandular secretion of the columnar epithelium of the embryo.

The idea that the conversion of starch into sugars by the action of the diastase formed and diffused slowly out of the epithelium during germination, was confirmed by the experiment upon rye by Haberlandt.(1) The process of germination thus shows that as it commences, active metabolism, synchronous with the dissolution of the cell-walls and the transformation of starch, goes on in the epithelium.

There are two kinds of vegetable diastase, one of which named translocation diastase acts on starch grains to convert them slowly into sugar, the optimum temperature being said to be 45–50°C., and the other one is "diastase of secretion," which corrodes starch grains and disintegrates them before dissolution, and the working temperature is around 50–55°C., and the latter enzyme plays an important part for the conversion of starch into sugars during the germination.

As a matter of fact, the diastase from germinated barley was found to be capable of corroding and dissolving the starch of barley, wheat, rice and maize but to have no evident action on that of the potato, and the kidney-bean.

The optimal temperature for the diastase in germinated grains is said to be 54–56°,(2) and the activity of the enzymes is usualy increased or retarded by the presence of various salts.(3)

The optimum temperature for the germination of the rice grain is said to be 30° to 35°, and actually the transformation velocity of the starch in

(1) *Ber. Deut. botan. Ges.*, **8** (1890), 40.

(2) J. Effront, *Compt. rend.*, **174** (1922), 18.

(3) C. Oppenheimer, "Die Fermente u. Ihre Wirkungen," (1924-6); H. C. Sherman and A. W. Thomas, *J. Am. Chem. Soc.*, **37** (1915), 623.

the plant tissues is noticed to be greatest at 35°, where the diastase occurs in the embryo, and this fact seems apparently to contradict the experimental results in vitro that the optimal temperature of diastase extracted from germinated grains is situated at 54–56°, and the discrepancy between the two cases will be attributed to the presence of some accelerating agents for the action of the enzymes in vivo.

The action of the diastases prepared from malted barley and rice on the starch solution in vitro was investigated.

In the experiments, diastatic enzymes were prepared by extraction with 100 c.c. of distilled water at room temperature for 3 hours from 5 grams of the malted barley and germinated rice which were previously powdered, the insoluble residue being filtered off, and the brown coloured filtrate was used.

The substrate which was used in studying the action of the enzymes, was prepared with many precautions, as the impurities associated with starch, such as protein, exert some influence on the diastatic action of enzymes.[1]

The various starch solutions were prepared as follows:

(1) The rice starch was prepared from ground rice-powder after treating with petroleum ether, and a 0.3% NaOH solution to remove the fat and the protein. The 1% solution of this starch was used, the reducing power of the solution is zero.

(2) Soluble starch was prepared by Lintner's method. Digest the starch with 7.5% HCl for 7 days at room temperature, wash with water till the washing shows a neutral reaction to litmus, filter and dry. The reducing power of this solution is 1.5.

(3) Amylose and amylopectin were prepared by the method proposed by Gatin and Gruzewska, from the rice starch by means of a 1% NaOH solution. The reducing power of amylose and amylopectin is 3.1 and zero respectively.

To 20 c.c. of the 1% starch solutions, 4 c.c. of the extracts were added, and the mixture was left to stand for 15 minutes at 20°C., 30°C., 40°C. and 50°C. respectively. After a certain time of reaction, the reducing power of each solution was determined on one hand, and on the other the colour test for the iodine solution was studied.

In the following tables, the reducing power of the starch solution examined was shown by subtracting the value original to the extracts from that of the whole of the reaction system. The conversion of the starch solutions into simpler sugars by the enzymes was carried out with progressive velocity at a higher temperature.

(1) S. A. Waksman and W. C. Davison, "Enzymes," (1926), p. 154.

Rice Diastase.

	Rice starch		Soluble starch		Amylose		Amylopectin	
	I.	R.P.	I.	R.P.	I.	R.P.	I.	R.P.
Without enzymes	Blue	0	Blue	1.5	Blue	3.1	Blue	0
With enzymes 20°C.	blue	3.1	violet blue	5.5	purple	4.7	blue	3.9
With enzymes 30°	violet blue	7.8	violet blue	9.5	purple	7.8	blue black	6.9
With enzymes 40°	violet blue	9.5	red violet	16.4	red violet	16.4	blue	12.4
With enzymes 50°	violet blue	17.3	red violet	25.0	red violet	21.0	blue	18.9

Barley Diastase.

	Rice starch		Soluble starch		Amylose		Amylopectin	
	I.	R.P.	I.	R.P.	I.	R.P.	I.	R.P.
Without enzymes	Blue	0.	Blue	1.5	Blue	3.1	Blue	0
With enzymes 20°C.	violet blue	42.9	violet blue	51.5	violet	39.9	blue	35.8
With enzymes 30°	,,	46.1	,,	55.5	red violet	44.6	violet blue	42.7
With enzymes 40°	,,	49.2	,,	59.3	light violet	45.2	,,	46.9
With enzymes 50°	violet	52.6	violet	84.2	yellow	50.1	light violet	56.8

The malted rice acts on soluble starch more easily than on the other starch solutions, and this fact would be ascribed to the occurrence of a subtance promoting the enzyme activity in the soluble starch. The case is also noticed in the action of the malted barley extract on the starch solutions. However, there was noticed a marked difference between the two malt extracts in their hydrolytic action on the starch solution, especially in the temperature effect; the activity of the malted rice extract was increased markedly by increase of temperature, while the malted barley extract remains almost constant in its activity throughout the different temperatures. The optimal temperature for the action of these extracts in vitro seems to lie at about 50°, as is described in the literatures.

In the next experiment, both the rice amylase and the barley amylase were prepared in a purified state by the method of Sherman from both extracts, which show no reducing power, and acted on the starch solutions.

2.3 mg. of the barley diastase and 2.9 mg. of the rice diastase acted on the 1% solutions of 50 mg. of the starches for 15 minutes at 24°C., the reducing power of the solution, and the colour reaction for the iodine solution, were studied.

Rice Diastase.

Substrate	Rice starch solution		Soluble rice starch solution	
	Iodine-colour	R.P.	Iodine-colour	R.P.
	light violet	50.0	light violet	51.6

Barley Diastase.

Substrate	Rice starch solution		Soluble rice starch solution	
	Iodine-colour	R.P.	Iodine-colour	R.P.
	violet	57.8	violet	63.1

As will be seen from the above results, the hydrolytic action of the barley diastase on both the rice starch and the soluble starch shows no marked difference between them, and also the diastases prepared fairly in a pure state from both seeds show a similarity in the hydrolytic action on rice starch at 24°C., though they behave in their malt extracts with a different activity toward the same substrates.

In conclusion, the writer states that the diastases from different kinds of germinated grains behave in a different manner in their activity toward the same starch, and that the activity of the enzyme depends upon its purity.

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