THE DIGESTION AND INACTIVATION OF MALTASE BY TRYPSIN AND THE SPECIFICITY OF MALTASES

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In 1925 Leibowitz (1) proposed the theory that there are two kinds of maltases,—one kind present in yeast, having the power of hydrolyzing maltose and α-methylglucoside and another found in moulds (2–4) with only maltose-splitting power. Thus he would differentiate between glucosidomaltase and glucomaltase. But Weidenhagen (5) has taken quite a different view. He thinks that sucrose may be hydrolyzed by α-glucosidase and by a β-*h*-fructosidase, and maltose only by α-glucosidase. This he explains by his steric configurative theory, according to which one and the same enzyme, α-glucosidase, must be able to hydrolyze sucrose, maltose, and α-glucosides since each has an α-glucosido rest bound to a glycone or an aglycone. In other words this means that the classical nomenclature of maltase, sucrase, etc., would have to be dropped, since such separate enzymes would not exist. Recently we (6) have shown the maltase of the mammary gland to be unmistakably a glucomaltase, hydrolyzing maltose but not sucrose or α-methylglucoside, thus corroborating the theory of Leibowitz. Doubt has been cast upon Weidenhagen's work (7) from another angle by Karstroem (8), Myrbaeck (9), and Virtanen (10) who found that the enzyme of a certain strain of *B. coli* can hydrolyze maltose but not cane sugar. Experiments on moulds by Pringsheim, Borchard, and Loew (11) speak also against the theory of Weidenhagen.

Since, in enzyme chemistry, the question whether there are distinct maltases and sucrases is of considerable importance, we have undertaken to procure additional data by studying the maltase of saliva and the maltase produced by *Escherichia coli*. For the determination of enzyme activity we have used an improved technic. We have utilized our new method (12) which permits of the determination of minute

amounts of monoses in the presence of bioses. We have also investigated the chemical nature of the maltase of *E. coli*. We shall show that in contrast to the difficulty with which some other enzymes respond to tryptic digestion, this maltase is digested by trypsin with remarkable ease within a relatively short time.

EXPERIMENTAL

The organism used was a laboratory strain of *E. coli* (*B. coli communis*), obtained in 1930, which ferments glucose, maltose, lactose, with acid and abundant gas formation. The enzyme (maltase) was prepared[1] according to the directions

TABLE I

Experiments Showing That the Maltase of E. coli (B. coli communis) and That of Saliva Are Glucomaltases

Temperature 37°

Nature of preparation	Hydrolysis of maltose	Hydrolysis of sucrose	Hydrolysis of α-methyl-glucoside	Time of incubation
	per cent	*per cent*	*per cent*	*hrs.*
E. coli	14	None	None	2
" "	40	"	"	20
" "	51	"	"	72
Saliva	21	"	"	20
"	30	"	"	40

of Karstroem (8) with the only difference that we have used 600 ml. of culture material instead of 6 liters. A sterility test was made from the enzyme material. The bacteria-free enzyme preparation was dissolved in 10 ml. of distilled water. 1 ml. of this solution had a dry weight of 6 mg. To 2 ml. of this or to 2 ml. of saliva,1 ml. of buffer (pH 6.9 N/10 acetate) and 1 ml. of 1.42 per cent maltose, 1.42 per cent sucrose, or 1 per cent α-methylglucoside, respectively, were added. Toluene was used as an antiseptic in all experiments. At intervals 0.5 ml. samples were taken from the digests and the protein precipitated with 95 per cent ethyl alcohol. In the case of maltose 10 volumes of alcohol and in that of sucrose 5 volumes were used for the precipitation. Boiled samples of the enzymes were run as controls. In 1 ml. of the clear filtrate the degree of hydrolysis was determined by our monose method (12). The results of these experiments are summarized in Table I. It is quite evident that the maltase of both *E. coli* and saliva

[1] For this preparation we are indebted to the Department of Bacteriology of this College.

hydrolyzes maltose fairly easily while having no effect upon sucrose or α-methylglucoside.

Inactivation of Maltase by Tryptic Digestion.—To 2 ml. of the maltase solution of *E. coli*, there were added 2 ml. of 0.3 per cent trypsin (Fairchild Bros. and Foster) dissolved in N/10 acetate buffer of pH 6.9. It was incubated at 37° for 2 days. After this 1 ml. maltose (1.42 per cent) was added and it was again incubated for 2 days. We found that after 2 days of tryptic digestion all the maltase was inactivated. A boiled trypsin buffer solution, and a boiled maltase buffer solution, respectively, served as controls. It should be noted that there is a spontaneous hydrolysis of substrates even in water solutions, which should never be neglected when testing for enzyme activity. Since the 0.3 per cent trypsin solution gave no immediate precipitate with 95 per cent alcohol and the maltase solution gave a heavy precipitate it was easy to follow proteolysis by comparing the precipitates given by the digest and the original maltase solution. After 2 days tryptic digestion the inactive maltase solution gave no precipitate with 95 per cent ethyl alcohol.

DISCUSSION

In Table I it is shown that the maltase of saliva does not act in accordance with the theory of Weidenhagen. It only hydrolyzes maltose—not sucrose and not α-methylglucoside. Nor does the maltase of *E. coli* (*B. coli communis*) hydrolyze anything but maltose, which is in confirmation of the work of Karstroem, of Myrbaeck, and of Virtanen, who have also studied the enzyme-producing power of some of the organisms of this group. Our experiments on the specificity of "saccharases" support the theory of Leibowitz, who claims the existence of two kinds of maltases: glucomaltases, of which the maltase produced by *E. coli*, the maltase of saliva, and the maltase of the mammary gland are examples; and glucosidomaltases, of which yeast-maltase is an example, hydrolyzing both maltose and α-methylglucoside. The use of the special monose reagent which is very sensitive to monosaccharides but is not changed by disaccharides (12) has given us an extremely definite analytical procedure for testing the point mentioned.

Contrary to the findings of Pringsheim and Leibowitz (13), who think that there is no maltase at all in saliva, it was found in a series of experiments (14) that maltase is a constant constituent of the saliva. This was believed to be the case by earlier workers. It varies greatly in different individuals, some having only a trace of maltase

in their saliva. However, even such a trace is not negligible if it is remembered that the organic dry weight of the saliva is not more than 0.5 per cent.

As regards the digestibility of maltase, it is well known that much of the maltase of yeast is inactivated during the autolysis of the yeast cells, which is a part of the procedure used in preparing yeast-maltase. This has been attributed to the increasing acidity of the autolysate (15). However, in view of our experiments which show that maltase is digested and inactivated by trypsin, it is possible that the inactivation of yeast-maltase during autolysis really may have been due to the action of proteases, which are found in abundance in yeast, and which act best at a pH ranging between 4.0 to 7.8. Some enzymes (16) are not digested by trypsin, and crystalline urease is inactivated by trypsin only if a gum is present (17, 18). Sumner and Kirk (19) could not digest crystalline urease with trypsin, with or without gum, but Sumner, Kirk, and Howell (20) digested crystalline urease with pepsin and with papain. It has been shown by Northrop and Kunitz (21) that crystalline trypsin is digested and inactivated quite rapidly by dilute solutions of crystalline pepsin. It was found by us (18) that concentrated solutions of trypsin may prevent the digestion of crystalline urease, by acting as a protective colloid for the urease. For this reason dilute trypsin solutions were used in these experiments on maltase.

SUMMARY

1. The maltase of saliva and that of *E. coli* (*B. coli communis*) hydrolyze maltose but not α-methylglucoside or sucrose and are therefore to be considered glucomaltases.

2. Maltase is rapidly and completely inactivated and digested by trypsin.

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