SOME ASPECTS OF THE INDUCED BIOSYNTHESIS OF ALPHA-AMYLASE OF *PSEUDOMONAS SACCHAROPHILA*[1]

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In a paper on the facultative autotroph, *Pseudomonas saccharophila*, Doudoroff (1940) noted that this organism forms an extracellular amylase when grown with starch as a source of carbon. Later studies showed that this enzyme could be formed also in resting cellular suspensions simply by the addition of starch or maltose (Klein and Doudoroff, *unpublished experiments*). In this respect, *P. saccharophila* appears to be similar to *P. fluorescens* and *P. putrefaciens*, both of which can be induced to form enzymes in the absence of added nitrogenous compounds (Stanier, 1951; Klein and Doudoroff, 1950). These bacteria thus resemble yeasts in their capacity to form new enzyme protein from endogenous sources.

The purpose of the experiments reported here is to describe and characterize the amylase system of *P. saccharophila*, to test the effect of various factors on the induction of this enzyme, and to study possible interactions between the induction of amylase and that of another inducible system.

MATERIALS AND METHODS

Organism and media used. P. saccharophila (Doudoroff, 1940) was maintained on agar slants containing the following: 0.033 M KH_2PO_4-Na_2HPO_4 buffer (pH 6.64), NH_4Cl, 0.1 per cent; $MgSO_4$, 0.05 per cent; $FeCl_3$, 0.005 per cent; $CaCl_2$, 0.0005 per cent; sodium lactate, 0.2 per cent; and agar 1.5 per cent. Solutions of carbohydrates or sodium lactate, in distilled water, were sterilized separately by autoclaving for 20 min at 13 lb pressure. Thirty-ml quantities of media contained in 125-ml Erlenmeyer flasks, or 500-ml quantities of media contained in 2-L Erlenmeyer flasks, were aerated by shaking on a rotary shaker at approximately 25 C. When incubation at 30 C was desired, the flasks were shaken in a water bath at that temperature.

Resting cellular suspensions were prepared by centrifuging 24-hr-old cultures in a Serval centrifuge at 10,000 RPM for 5 min, washing the cells twice in 0.033 M phosphate buffer of the desired pH and resuspending the organisms in buffer of the same composition to a volume of approximately one-fourth that of the original culture.

Assay for sucrose phosphorylase. Washed cells were ground in a mortar and pestle with twice their wet weight of alumina (McIlwain, 1948), after which the intact cells, cell debris and alumina were removed by centrifugation, and the supernatant was assayed for sucrose phosphorylase, using the method of Doudoroff (1943). Under the conditions employed in this study, the rate of disappearance of inorganic orthophosphate was linear for 40 min at 30 C.

Assay for amylase. The source of enzyme for these assays was the supernatant liquid from experiments in which cells of *P. saccharophila* were incubated with starch or maltose in phosphate buffer. The activity of the enzyme was measured by one of the following methods: *Starch-iodine method:* The following modification of the method of Smith and Roe (1949) was used. One ml of the enzyme solution is added to 8 ml of a buffered (pH 5.5) solution of starch (final starch concentration, 0.11 per cent). Another tube, containing water instead of the enzyme, is used as a control. After the appropriate incubation period at 37 C, 1 ml of 1 N HCl is added to each tube to stop enzyme action, and a 1-ml aliquot is transferred to a tube containing 0.5 ml of 1 N HCl. One ml of the iodine reagent (0.3 per cent

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KI, 0.03 per cent I_2) is added and the solution is diluted to 25 ml with distilled water, after which the color is read at 660 mμ. Activity is expressed as amylase units. One unit of α-amylase is the amount of enzyme causing the loss of color equivalent to 0.5 mg of starch in 30 min under the assay conditions. *Reducing sugar method:* α-amylase activity was also measured by the increase in reducing groups resulting from the hydrolysis of starch (Noelting and Bernfeld, 1948). With this method activity is expressed as mg maltose liberated per 3 min at 37 C at pH 5.5. Using this procedure, 1 unit of α-amylase was found to release 0.021 mg reducing sugar (as maltose) in 3 min at 37 C at pH 5.5.

Chemical analyses. Inorganic phosphate was determined directly on samples, after removal of proteins by treatment with 1 volume of 10 per cent trichloracetic acid, by the method of Fiske and SubbaRow (1925). The method of Stadtman *et al.* (1951) was used to estimate protein, using a preparation of crystalline bovine serum albumin as the standard.

Chemicals. Merck soluble starch was used as a substrate-inducer for growing the organisms, as well as for the starch-iodine assay, while "Noredux" soluble starch (Ziegfried, A. G., Zofingen, Switzerland) was used for the enzyme assays in which reducing sugar was measured. All other chemicals were of C. P. grade.

RESULTS

Kinetics of induced α-amylase biosynthesis in resting cells. To test for the rate of enzyme formation, resting cell suspensions were prepared, and at zero time maltose was added to a final concentration of 0.2 per cent. Samples were removed at intervals, and at each interval the cells were removed by centrifugation for 5 min in the Serval centrifuge, after which the supernatants were diluted in 0.067 phosphate buffer (pH 5.5) and assayed for α-amylase activity. The results of these analyses are illustrated in figure 1. No lag in enzyme formation was observed in this system although an initial lag period of up to 15 min is possible, since it took that much time to separate the cells and supernatant for each sample examined. Under the conditions employed here, therefore, enzyme synthesis is linear for a period of at least 3 hr.

Using starch as the inducing substance, essentially similar results were obtained. Starch was added to a final concentration of 0.3 per cent at zero time. Samples were removed at various times and transferred to 12-ml Spinco centrifuge tubes which were immediately swirled in an ice bath for a period of 1½ min. This procedure rapidly lowered the temperature of the mixture to 5 C. By 13 min after removal of each sample, the supernatant and cells had been separated. Assays for amylase were performed on the supernatants and the results obtained are given in figure 2. It can be seen that no lag period was observed in the synthesis of the amylase. The maximum possible lag period in enzyme production in this experiment is 13 min. Thus it is reasonable to conclude that, under the conditions employed, synthesis of this protein commences very soon, if not immediately, after addition of the inducer, and continues at a constant rate for several hours. Of course, since the enzyme investigated here is largely extracellular, figures 1 and 2 represent merely the rate of appearance of enzyme in the supernatant. The kinetics of amylase appearance may thus be a summation of synthesis and secretion unless, as is not unlikely, synthesis takes place at the cell surface.

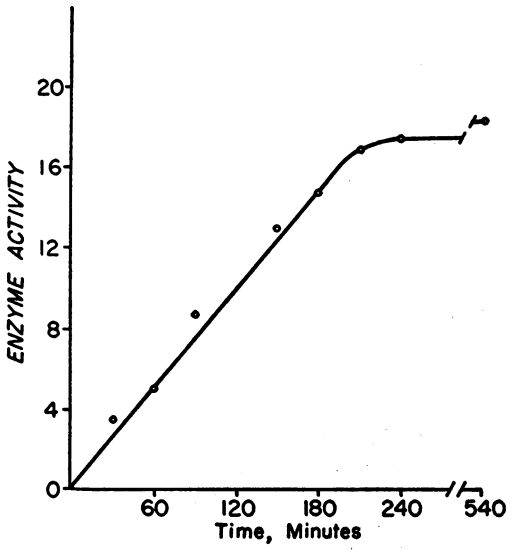

Figure 1. The rate of appearance of the α-amylase of *Pseudomonas saccharophila* with maltose as an inducer. Resting cellular suspensions were prepared in M/30 phosphate buffer (pH 6) as described under Methods, and maltose (0.2 per cent final concentration) was added. Enzyme assays performed by the starch-iodine method.

The effect of varying concentrations of maltose or starch on the synthesis of amylase. Resting cellu-

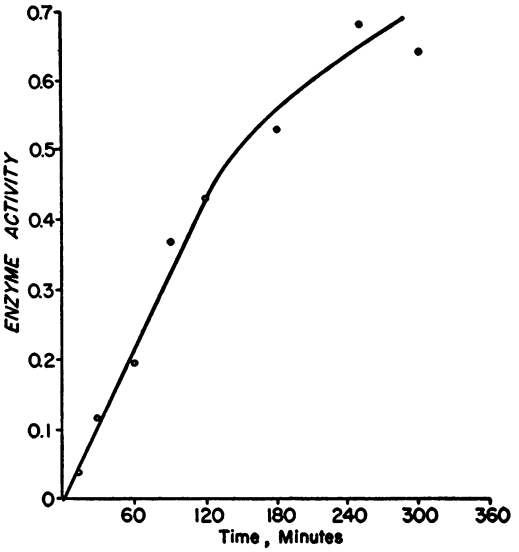

Figure 2. The rate of appearance of the α-amylase of *Pseudomonas saccharophila* with starch as an inducer. Resting cellular suspensions were prepared in M/30 phosphate buffer (pH 6) as described under Methods and starch (0.3 per cent final concentration) was added. Enzyme assays performed by reducing sugar method.

lar suspensions were prepared in 0.033 M phosphate buffer and varying concentrations of maltose or starch were added. The suspensions were then aerated at room temperature on a rotary shaker for 8 hr, after which the cells were removed and the supernatants assayed for amylase activity. The results, illustrated in figure 3, demonstrate that maltose and starch are essentially equivalent inducers on a weight basis. Furthermore, the data indicate that induction with these compounds is a function of inducer concentration. It should be noted, however, that starch and maltose serve the dual functions of inducer and energy source in these experiments. Therefore, these data show the over-all effect of inducer concentration on enzyme production and do not necessarily reflect the influence of maltose or starch as inducers.

In the absence of a "gratuitous inducer" (Monod and Cohn, 1952) of amylase formation, we have attempted to obtain further information on the effect of the inducer concentration in the following manner. In the series of inductions with maltose, chemical analyses for maltose were performed on the supernatants at the time of assay for amylase. These determinations showed that, using initial concentrations of maltose above 0.1 per cent, the amount of reducing sugar utilized during the 8-hr incubation period was constant, regardless of the initial maltose concentration. It is therefore probable that the systems for generating energy were saturated at the higher concentrations. For this reason, the increased levels of amylase produced at higher substrate concentrations may be considered to be a consequence of an increase of inducer concentration, rather than of an energy or carbon source.

The effect of added ammonium chloride or acid-hydrolyzed casein on the biosynthesis of α-amylase. Since suspensions of *P. saccharophila* were found to be capable of forming enzyme in the absence of added nitrogenous compounds, it was of interest to test the effect of such substances on the formation of amylase. Accordingly, ammonium chloride or acid-hydrolyzed casein, over the concentration range of from 0.01 to 0.5 per cent, were added to the cells during adaptation. These substances had no stimulatory effect on resting cellular suspensions. However, when the salts

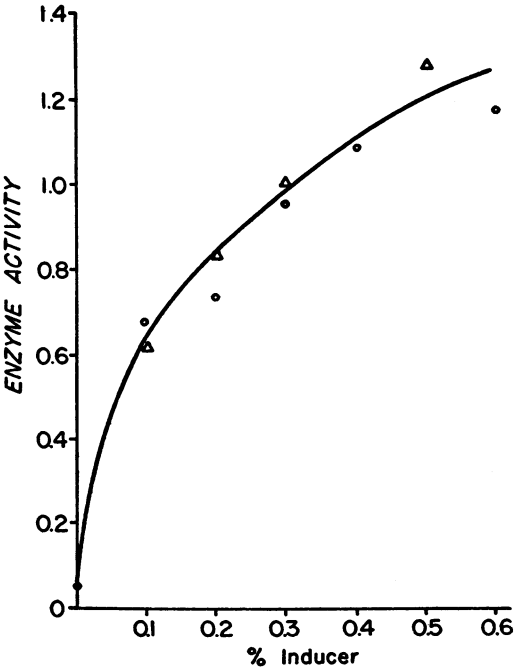

Figure 3. The effect of varying concentrations of maltose or starch on the induced biosynthesis of α-amylase. Open circles = starch; triangles = maltose. Conditions as in figures 1 and 2 except for substrate concentration. Enzyme assays performed using reducing sugar method.

TABLE 1

*The effect of ammonium chloride and acid-hydrolyzed casein on the synthesis of α-amylase by suspensions of Pseudomonas saccharophila**

NH_4Cl Added		Acid-Hydrolyzed Casein Added	
Final per cent	Amylase activity	Final per cent	Amylase activity
0	5.3	0	5.3
0.0001	6.4	0.0001	3.0
0.001	11.6	0.001	3.2
0.005	15.5	0.005	6.4
0.01	20.8	0.01	8.8
0.05	18.6	0.10	11.0
0.1	6.7	0.25	11.7
1.0	0.0	0.50	12.7

* In this experiment, washed cells were suspended in M/30 phosphate buffer (pH 6) and salts (see text). Enzyme assays performed by starch iodine method.

needed for growth were also added (0.05 per cent $MgSO_4$, 0.005 per cent $FeCl_3$, and 0.0005 per cent $CaCl_2$), increased levels of amylase were obtained. Table 1 shows the effect of ammonium chloride or acid-hydrolyzed casein on the final enzyme content of the supernatants after 4½-hr aeration on a rotary shaker at room temperature. Under the conditions of these experiments 1 to 2 cell divisions may occur (Whelton and Doudoroff, 1945). Thus these apparent stimulatory effects may be attributable to the growth of the cells rather than to an actual increase in the amount of enzyme produced per cell. Indeed the lack of stimulation of enzyme synthesis by these compounds in the absence of added salts makes this interpretation seem reasonable.

The effect of pH on the synthesis of amylase. Several aliquots of a 24-hr-old culture of *P. saccharophila*, grown on glucose, were washed twice in M/15 phosphate buffer at various pH values, and each was resuspended finally in buffer of the same composition. Maltose was added to these suspensions to a final concentration of 0.3 per cent, and the cells were aerated for 8 hr at room temperature on a rotary shaker. After removal of the cells, the pH of each supernatant was determined with a Beckman pH meter and amylase assays were carried out on aliquots of each supernatant. These were first diluted with 3 volumes of acetate buffer in order to bring the final pH to between 5.4 and 5.5.[4]

[4] Thayer (1953) stated that the optimal pH for this enzyme is 5.4. Using crystalline preparations of the enzyme, we have found the optimal range to be between 5.25 and 5.75 (Markovitz *et al.*, 1955).

TABLE 2

The effect of pH on the synthesis of α-amylase

Initial pH	Final pH	α-Amylase Activity*	Per Cent Maximum Activity
5.0	4.5	0.12	27
5.5	5.2	0.28	64
6.0	6.0	0.44	100
6.5	6.4	0.36	82
7.0	6.8	0.28	64
7.5	7.3	0.28	64
8.0	7.5	0.20	45

* Enzyme assays performed by reducing sugar method.

Table 2 is a summary of the results obtained in this experiment. It is evident that the optimum pH for the induced biosynthesis of the α-amylase is approximately 6, which is rather close to the optimal value for the activity of the enzyme.

Inhibition of the synthesis of α-amylase by azide, arsenate, and 2,4-dinitrophenol. The effect of varying concentrations of sodium azide, sodium arsenate, and 2,4-dinitrophenol on the induced biosynthesis of the α-amylase was studied. Preliminary experiments, to test the effect of these substances on the enzyme itself, showed them to be non-inhibitory at the concentrations used in the following experiments. Resting cellular suspensions containing 0.3 per cent maltose, 0.033 M phosphate buffer (pH 6.0), and varying concentrations of the inhibitor were used. The suspensions were shaken for a period of 8 hr on the rotary shaker at room temperature, and afterwards the cells were removed by centrifugation and the supernatants assayed for amylase activity. The results obtained are reported in table 3 and demonstrate that all three compounds inhibit the synthesis of amylase and that this inhibition is a function of the concentration of the inhibitor employed.

Interactions in the induced biosyntheses of α-amylase and sucrose phosphorylase in resting cellular suspensions. In order to study possible interactions between the sucrose phosphorylase and the α-amylase synthesizing systems in resting cellular suspensions, it was necessary first to determine optimal conditions for sucrose phosphorylase synthesis. The results of experiments

TABLE 3

Inhibition of the synthesis of α-amylase by sodium arsenate, sodium azide, and 2,4-dinitrophenol

Additions	Per cent inhibition
A. Sodium arsenate	
0.004 M	18
0.04 M	69
0.08 M	80
B. Sodium azide	
0.000025 M	12
0.00025 M	32
0.0025 M	63
C. 2,4-Dinitrophenol	
0.0000025 M	10
0.000025 M	49
0.00025 M	60

bearing on this problem are summarized in the following paragraph.

For this purpose, studies were conducted using conventional manometric techniques[5] to measure the rate of sucrose oxidation under various circumstances. These experiments showed pH 7.5 and 0.05 M sucrose to be optimal for the adaptation to sucrose oxidation. The age of the cells at the time of harvesting proved to have little bearing on their subsequent ability to adapt to sucrose. In addition, it was found that maximal sucrose phosphorylase synthesis had usually been obtained in resting cellular suspensions by the end of 3 hr.

Having obtained information pertinent to sucrose phosphorylase synthesis, experiments were performed to test for interactions between the formation of this enzyme and amylase.

To determine what effect the sequential induction of these two enzymes would have on the final enzyme levels, cells were grown in lactate medium for 24 hr, washed, and divided into five aliquots. The first aliquot was then shaken with sucrose for a period of 4 hr, and after this the cells were washed and resuspended in buffer plus 0.2 per cent starch for an additional 4 hr (suspension A). A similar process was performed with a second aliquot of cells except that during the initial period of 4 hr the cells were in starch, and during the second 4 hr they were in sucrose (suspension B). Two additional aliquots of cells were shaken for 4 hr in buffer only. The cells were then washed twice, after which 1 aliquot was induced with starch (suspension C), and the other with sucrose (suspension D). A final aliquot of cells was induced to form sucrose phosphorylase during the first 4 hr and, after washing, these cells were shaken in buffer for an additional 4 hr (suspension E). Enzyme assays for both enzymes were performed on appropriate suspensions, as indicated in table 4. The results show that cells first induced to form sucrose phosphorylase can subsequently form only 12 per cent as much amylase as a suitable control. In addition, cells first induced to form amylase can subsequently form only 25 per cent as much sucrose phosphorylase as a suitable control. It is interesting to note that

TABLE 4

Interactions in the induced biosyntheses of α-amylase and sucrose phosphorylase in resting cell suspensions of Pseudomonas saccharophila

Suspension	Treatment	Enzyme Activity	
		Sucrose Phosphorylase*	α-Amylase†
A	Induced to form sucrose phosphorylase; then induced to form α-amylase	0.51	0.45
B	Induced to form α-amylase; then induced to form sucrose phosphorylase	0.14	3.65
C	Shaken in buffer; then induced to form α-amylase	—	3.65
D	Shaken in buffer; then induced to form sucrose phosphorylase	0.55	—
E	Induced to form sucrose phosphorylase; then shaken in buffer	0.50	—

[5] Although invertase activity undoubtedly contributes to the dissimilation of sucrose by these organisms (Doudoroff, 1943), direct determinations of sucrose phosphorylase activity, using extracts of cells induced under these conditions, showed a good correlation between the levels of this enzyme and oxygen uptake of intact cells.

* Sucrose phosphorylase activity is expressed as the disappearance of inorganic phosphate in μg/mg of protein per 40 min at 30 C. Sucrose phosphorylase inductions were carried out at pH 7.5.

† α-Amylase assays were done by the starch-iodine method. The α-amylase inductions were performed at pH 6.8.

the assay for sucrose phosphorylase in suspension D, performed 4 hr after the addition of sucrose, was very nearly identical with that of suspensions A and E, both of which were shaken in the absence of sucrose for 4 more hours. This demonstrates that this enzyme is relatively stable and that the cells do not easily readapt. This behavior is even more emphatically shown in the results obtained with suspension A. In this case, cells that had initially formed sucrose phosphorylase, and later small amounts of amylase, showed no significant drop in sucrose phosphorylase. Thus, with respect to sucrose phosphorylase at least, the enzyme once formed is not easily lost, even upon the subsequent induction of another enzyme.

One further point is worth noting. This is the observation that cells shaken for 4 hr in buffer and then washed (suspensions C and D) were still capable of forming as much enzyme as cells freshly harvested from the growth medium. If, in the resting cellular suspension, some cells were undergoing autolysis or were releasing endogenous cellular materials in some other manner, the cells remaining after the washing process should have had a reduced capacity to adapt to sucrose or starch utilization. Since this did not prove to be the case, we consider it unlikely that the induced synthesis of these enzymes occurs in some cells at the expense of materials released from other cells.

Another way to study the interaction between the synthesis of these two proteins is to induce the synthesis of both enzymes simultaneously rather than sequentially. Unless some kind of a "diauxie" effect (Monod, 1949) occurs, it should be possible to induce the simultaneous formation of both amylase and sucrose phosphorylase. To study this possibility, a resting cell suspension was divided into three portions; sucrose was added to one, starch was added to another, and sucrose plus starch were added to the third group of cells. After 4 hr aeration on a rotary shaker at room temperature, the cells were centrifuged and extracted for sucrose phosphorylase assays and the supernatants were used for α-amylase assays. The results obtained are presented in table 5, from which it is seen that the induced biosynthesis of both enzymes is depressed by the simultaneous induction of the other enzyme. Forty-seven per cent as much α-amylase and 62 per cent as much sucrose phosphorylase are produced in the double induction as compared with their respective controls.

TABLE 5

*Simultaneous induction of sucrose phosphorylase and α-amylase in resting cell suspensions**

Inductor(s) Used	Sucrose Phosphorylase Activity	α-Amylase Activity
Sucrose	0.40	—
Starch	—	3.46
Sucrose plus starch	0.25	1.62

* In these experiments cells were suspended in M/30 phosphate buffer (pH 6.8). This pH is intermediate between the optima for each of the inductions. Final concentration of starch used was 0.2 per cent and of sucrose 0.05 M. Enzyme assays as in table 4.

DISCUSSION

The kinetics of the induced biosynthesis of α-amylase have been shown to be linear, using maltose or starch as inducers. Although no lag was actually observed, a lag in enzyme formation of the order of 13 min is possible. As pointed out by Monod and Cohn (1952), the lag time in the induced biosynthesis of the β-galactosidase of *E. coli* can and does vary according to the inductor used and, under certain conditions, is virtually non-existent. Thus, according to these authors, the kinetics of enzyme formation should be considered from the moment enzyme first begins to appear. The kinetics of the induced biosynthesis of the β-galactosidase of *E. coli* (Monod and Cohn, 1952) and of the penicillinase of *Bacillus cereus* (Pollock, 1952) are linear. On the other hand, Halvorson (1953) has reported an autocatalytic type of induced biosynthesis with the maltase of *Saccharomyces cerevisiae* which cannot be attributed to the energy derived from the inducer since the inducer, α-methylglucoside, is not utilizable under the experimental conditions employed.

It was demonstrated that the ability of cells to form α-amylase was reduced by 88 per cent when they had previously been induced to form sucrose phosphorylase. Similarly, when cells were first induced to form amylase, their subsequent ability to form sucrose phosphorylase was reduced by 75 per cent. This decreased ability to form enzyme is not due to the depletion of the en-

dogenous carbon reserves of the cell, since the carbon content of induced cells is higher than that of freshly harvested cells and the endogenous respiration of induced cells is higher than the endogenous respiration of freshly harvested cells (Markovitz and Klein, 1954). It is more likely that the deficiency is related to a reduced supply of nitrogenous compounds available within the cell for enzyme biosynthesis. This interpretation is supported by the fact that resting cells, grown on lactate, can be rendered incapable of induced enzyme biosynthesis after a period of oxidation of additional lactate, and that such cells regain the capacity to form enzymes when acid-hydrolyzed casein is added in appropriate concentrations (Markovitz and Klein, *unpublished experiments*).

Studies on enzyme formation in certain strains of *S. cerevisiae* (Spiegelman and Dunn, 1948; Halvorson and Spiegelman, 1953) suggest that these yeasts are capable of turnover of nitrogenous compounds in order to supply nitrogen for enzyme synthesis. The present results, which show that sucrose phosphorylase does not decrease upon the subsequent formation of α-amylase, are in agreement with the results of experiments on the β-galactosidase of *E. coli* (Rotman and Spiegelman, 1954; Hogness *et al.*, 1955).

SUMMARY

An extracellular α-amylase has been shown to be formed in resting cellular suspensions of *Pseudomonas saccharophila*, using maltose or starch as inducers. The kinetics of α-amylase appearance have been shown to be linear. Both substrates are essentially equivalent on a weight basis, and the level of enzyme produced is proportional to substrate concentration.

The synthesis of α-amylase is stimulated by the addition of acid-hydrolyzed casein or ammonium chloride only in the presence of inorganic salts essential for growth.

Interactions between the systems synthesizing sucrose phosphorylase and α-amylase have been observed.

REFERENCES

Doudoroff, M. 1940 The oxidative assimilation of sugars and related substances by *Pseudomonas saccharophila*. Enzymologia, **9**, 59–72.

Doudoroff, M. 1943 Studies on the phosphorolysis of sucrose. J. Biol. Chem., **151**, 351–361.

Fiske, C. H. and SubbaRow, Y. 1925 The colorimetric determination of phosphorus. J. Biol. Chem., **66**, 375–400.

Halvorson, H. O. 1953 The biosynthesis of enzymes. U. Mich. Med. Bull., **19**, 229–244.

Halvorson, H. O. and Spiegelman, S. 1953 Net utilization of free amino acids during the induced synthesis of maltozymase in yeast. J. Bacteriol., **65**, 601–608.

Hogness, D. S., Cohn, M., and Monod, J. 1955 Studies on the induced synthesis of beta-galactosidase in *Escherichia coli*: The kinetics and mechanism of sulfur incorporation. Biochim. et Biophys. Acta, **16**, 99–116.

Klein, H. P. and Doudoroff, M. 1950 The mutation of *Pseudomonas putrefaciens* to glucose utilization and its enzymatic basis. J. Bacteriol., **59**, 739–750.

Markovitz, A. and Klein, H. P. 1954 Studies on the biosynthesis of the alpha-amylase of *Pseudomonas saccharophila*. Abstracts Am. Chem. Soc. 126th Meeting, page 67C.

Markovitz, A., Klein, H. P., and Fischer, E. H. 1955 Crystallization and properties of the alpha-amylase of *Pseudomonas saccharophila*. Biochim. et Biophys. Acta. *Accepted for publication.*

McIlwain, H. 1948 Preparation of cell-free extracts with powdered alumina. J. Gen. Microbiol., **2**, 288–291.

Monod, J. 1949 The growth of bacterial cultures. Ann. Rev. Microbiol., **3**, 371–394.

Monod, J. and Cohn, M. 1952 La biosynthèse induite des enzymes. Advances in Enzymol., **13**, 67–119.

Noelting, G. and Bernfeld, P. 1948 Sur les enzymes amylolytiques. III. La beta-amylase: dosage d'activité et contrôle de l'absence d'alpha-amylase. Helv. Chim. Acta, **31**, 286–290.

Pollock, M. R. 1952 Penicillinase adaptation in *Bacillus cereus:* An analysis of three phases in the response of logarithmically growing cultures to induction of penicillinase formation by penicillin. Brit. J. Exptl. Pathol., **33**, 587–600.

Rotman, B. and Spiegelman, S. 1954 On the origin of the carbon in the induced synthesis of beta-galactosidase in *Escherichia coli*. J. Bacteriol., **68**, 419–429.

Smith, B. W. and Roe, J. H. 1949 A photometric method for the determination of alpha-amylase in blood and urine with use of the starch-iodine color. J. Biol. Chem., **179**, 53–59.

SPIEGELMAN, S. AND DUNN, R. 1948 Interactions between enzyme-forming systems during adaptation. J. Gen. Physiol., **31**, 153–173.

STADTMAN, E. R., NOVELLI, G. D., AND LIPMANN, F. 1951 Coenzyme A function in and acetyl transfer by the phosphotransacetylase system. J. Biol. Chem., **191**, 365–376.

STANIER, R. Y. 1951 Enzymatic adaptation in bacteria. Ann. Rev. Microbiol., **5**, 35–56.

THAYER, P. S. 1953 The amylases of *Pseudomonas saccharophila*. J. Bacteriol., **66**, 656–663.

WHELTON, R. AND DOUDOROFF, M. 1945 Assimilation of glucose and related compounds by growing cultures of *Pseudomonas saccharophila*. J. Bacteriol., **49**, 177–186.