DE NOVO SYNTHESIS OF α-AMYLASE BY *BACILLUS STEAROTHERMOPHILUS*[1]

N. E. WELKER[2] AND L. LEON CAMPBELL[2]

Department of Microbiology, School of Medicine, Western Reserve University, Cleveland, Ohio, and Department of Microbiology, University of Illinois, Urbana, Illinois

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ABSTRACT

WELKER, N. E. (Western Reserve University, Cleveland, Ohio and University of Illinois, Urbana), AND L. LEON CAMPBELL. De novo synthesis of α-amylase by *Bacillus stearothermophilus*. J. Bacteriol. **86**:1202–1210. 1963.—The pH optimum for the synthesis of α-amylase by washed-cell suspensions was 6.7. α-Amylase synthesis began soon after the addition of the inducer (maltose, methyl-β-D-maltoside, or phenyl-α-D-glucoside, at 10^{-3} M), proceeded at a linear rate for 60 min, and then leveled off. Cell suspensions without inducer produced small amounts of α-amylase. The addition of glucose (2×10^{-3} M), sucrose (10^{-3} M), or glycerol (4×10^{-3} M) to washed-cell suspensions failed to stimulate the production of α-amylase. Nitrogen starvation of washed cells for 60 min with fructose as a carbon source or by induction with pure maltose showed that the ability to produce α-amylase was lost. Examination of the amino acid pool at this time showed a general depletion of amino acids and the complete disappearance of tyrosine, phenylalanine, proline, and valine. Replenishment of the amino acid pool with casein hydrolysate (0.5%) restored the ability of the cells to produce α-amylase. Chloramphenicol and 8-azaguanine were shown to inhibit α-amylase synthesis. Inhibition was observed immediately upon the addition of chloramphenicol to cell suspensions preinduced for varying periods of time. Actinomycin D and mitomycin C also inhibited α-amylase synthesis when added to induced washed-cell suspensions. The amino acid analogues, norvaline, norleucine, and ethionine, inhibited α-amylase formation by 72, 53, and 38%, respectively. *p*-Fluorophenylalanine inhibited the synthesis of active α-amylase by 92% and the incorporation of proline-C^{14} into α-amylase and cellular proteins by 95 and 74%, respectively.

The kinetics of induction of α-amylase by gratuitous and nongratuitous inducers in growing cultures of *Bacillus stearothermophilus* and the isolation and characterization of the natural inducers (maltodextrins) were described in previous papers (Welker and Campbell, 1963*a*, *b*, *c*). The present paper presents evidence for the de novo synthesis of α-amylase by washed-cell suspensions of this organism.

MATERIALS AND METHODS

Preparation of washed-cell suspensions. The growth media and preparation of cells were the same as those described for growth experiments (Welker and Campbell, 1963*a*) except that the cells were washed two times with and suspended in 10 ml of a sterile salt buffer solution of the following composition: K_2HPO_4, 2.5 g; KH_2PO_4, 1.0 g; NaCl, 1.0 g; and $CaCl_2 \cdot H_2O$, 5 mg per liter of distilled water. The pH was adjusted to 6.7. This buffer is referred to as R buffer.

Cells used for nitrogen starvation experiments, examination of amino acid pools, and proline-C^{14} incorporation experiments were also prepared in this manner.

Induction of α-amylase by washed-cell suspensions. Induction of α-amylase by washed-cell suspensions was carried out in 250-ml nephelometer flasks containing 30 ml of induction medium (R buffer plus inducer, 10^{-3} M). The washed-cell suspensions were adjusted to an absorbancy of 0.5 to 0.66 at 525 mμ and incubated at 55 C in a rotary water-bath shaker at a speed of 133 rev/min. α-Amylase activity was measured as described by Welker and Campbell (1963*a*).

Nitrogen starvation and replenishment of amino acid pools. Nitrogen starvation was accomplished in two ways: by the addition of fructose as a carbon source or by addition of an inducer of α-amylase. The washed cells were placed in a

[1] Part of the dissertation of Neil E. Welker, presented to the Graduate Faculty of Western Reserve University in partial fulfillment of requirements for the Ph.D. degree.

[2] Present address: Department of Microbiology, University of Illinois, Urbana.

500-ml Erlenmeyer flask containing 150 ml of R buffer supplemented with either fructose (2.0%) or inducer (10^{-3} M). The flasks were incubated at 55 C in a rotary water-bath shaker. At 30-min intervals, 5.0-ml samples were removed, centrifuged, and washed two times with R buffer. The washed cells were suspended in 250-ml flasks containing 30 ml of induction medium. The induction flasks were incubated at 55 C in the water-bath shaker, and the formation of α-amylase was followed.

Replenishment of nitrogen-starved cells was accomplished by removing 5.0-ml samples from starvation flasks, washing the cells two times with R buffer, and suspending the cells in 250-ml flasks containing 20 ml of the appropriate replenishment medium (R buffer supplemented with either 0.5% casein hydrolysate or 1.0% NH_4Cl). After 20 min, the cells were centrifuged, washed two times with R buffer, and placed in induction flasks.

Isolation and quantitation of amino acid pools. Amino acid pools were obtained by the procedure of Gale (1947). Cells to be treated for the isolation of amino acid pools were washed two times with deionized water and then brought to a final volume of 20 ml with water. A 0.1-ml sample was removed, taken to dryness in a vacuum desiccator, and weighed.

The cells were heated in a boiling-water bath for 20 min. The cell debris was removed by centrifugation, and the supernatant liquid was decanted. The pellet was washed with water (10 to 20 ml), centrifuged, and the supernatant liquid decanted. The supernatant fluids were combined.

The combined supernatant liquid containing the amino acids was passed through a column, 2.0 cm in diameter, packed to a height of 5.5 cm with Dowex 50W (8X, 200 to 400 mesh) in the H^+ form. The column was washed thoroughly with water, and the amino acids were eluted with 20 ml of 1.5 N NH_4OH. The amino acid mixture was taken to dryness in a vacuum desiccator over NaOH pellets. The amino acids were dissolved in 10 ml of sodium citrate buffer (pH 2.2), centrifuged to remove any precipitate, and stored at −14 C until analyzed on the Beckman/Spinco amino acid analyzer.

Incorporation of labeled DL-proline into cellular protein and α-amylase. The procedure used for measuring the incorporation of DL-proline-C^{14} into cellular proteins was that of Neidhardt and Magasanik (1960). Samples of the culture (5 to 10 ml) to be analyzed for proline-C^{14} incorporation were removed and centrifuged, and the supernatant liquid was decanted. The cells were suspended in 5.0 ml of 5% trichloroacetic acid and stored at 4 C for 12 hr.

The cells were removed by centrifugation, and the supernatant fluid was decanted. The pellet was washed once with 5.0 ml of 5% trichloroacetic acid, suspended in exactly 5.0 ml of 5% trichloroacetic acid, and heated in a boiling-water bath for 30 min. The mixture was cooled, centrifuged, and the supernatant liquid decanted. The pellet was dissolved in 0.1 ml of 1.0 N NaOH, diluted to 10 ml with distilled water, and assayed for protein by the method of Lowry et al. (1951). Samples (0.1 ml) for radioactivity measurements were placed on aluminum planchets, mixed with four to five drops of methanol, and dried under an infrared lamp. The planchets were counted in a Nuclear-Chicago end-window gas-flow Geiger counter (model D-47) operating in the proportional range.

For determination of proline-C^{14} incorporation into α-amylase, the enzyme was removed from the culture fluid by the technique of Thayer (1953). The α-amylase was adsorbed on a 2:1 soluble starch-Celite no. 535 (Johns-Manville, New York, N.Y.) mixture. The adsorbent was packed in a column, 2.5 cm in diameter, to a height of 1.5 cm. The adsorbent was washed with 100 ml of 0.1 M sodium acetate buffer (pH 4.6). The enzyme was eluted by pulling through the column, under a vacuum, 5 to 8 ml of an α-amylase digest of a 1% soluble-starch solution. The samples containing 95% of the enzymatic activity were pooled, 0.1 ml was placed on an aluminum planchet, and the radioactivity measured.

Synthesis of methyl-β-D-maltoside. The procedure used for the synthesis of acetylbromomaltose was that of Fischer and Fischer (1910). Pfanstiehl maltose (cp, 10 g) was mixed with acetylbromide (20 ml) in a 1-liter beaker immersed in an ice bath. After 10 to 15 min, the reaction was complete, and 800 ml of ice water were poured into the reaction beaker. The beaker was placed at 4 C for 12 hr. The white powdery material formed was filtered on a Büchner filter and washed four times with 800-ml portions of ice water. The acetylbromomaltose was dried in

TABLE 1. *Effect of pH on the formation of α-amylase by Bacillus stearothermophilus**

pH	α-Amylase formed (units/ml)	Percentage of maximal activity
4.5	0	0
5.0	5	6
5.5	16	18
6.0	57	65
6.3	68	78
6.5	72	81
6.7	87	100
7.0	26	29
7.5	20	23
8.0	5	6
8.5	2	3

* Washed cells were suspended in R buffer at the pH indicated with pure maltose (10^{-3} M) as the inducer. After 60 min of incubation at 55 C in a water-bath shaker, the cells were removed by centrifugation and the supernatant liquid was assayed for α-amylase activity under optimal conditions as described by Welker and Campbell (1963*a*).

a vacuum desiccator over NaOH and stored at −14 C.

The bromo derivative (20 to 50 g) was dissolved in 400 ml of absolute methanol (Schoch, Wilson, and Hudson, 1942). The mixture was dried by adding Na_2SO_4, filtered, and then shaken with Ag_2CO_3 (20 g). The mixture was heated under reflux for 1 hr, treated with charcoal (Darco-60), filtered, and concentrated under reduced pressure. A white powder of heptaacetyl-methyl-maltoside formed and was reprecipitated from ethanol. The reprecipitated product was dried in vacuo over NaOH.

Deacetylation of the heptaacetyl-methyl-maltoside was accomplished by dissolving the compound (20 g) in absolute methanol (20 to 30 ml) and saturating with ammonia at 0 C. The mixture was placed at −14 C for 12 hr, filtered, and concentrated in vacuo to a syrup. The syrup was dissolved in 10 ml of water and further purified by large-scale ascending paper chromatography (Welker and Campbell, 1963*a*).

The methyl-β-D-maltoside had a specific rotation of $[\alpha]_D^{24} = +82.4°$, which agrees with the value reported by Pazur, Marsh, and Ando (1959). The methyl-β-D-maltoside was chromatographically pure. After three ascents in a solvent containing *n*-butanol–ethanol–water (4:1:1 by volume), the synthesized compound had an R_F value identical with that of known methyl-β-D maltoside.

Chemicals. DL-Proline-C^{14} (carboxyl labeled) was obtained from Calbiochem. 8-Azaguanine was purchased from Nutritional Biochemicals Corp., Cleveland, Ohio. *p*-Fluorophenylalanine was obtained from Sigma Chemical Co., St. Louis, Mo. L-Norvaline, 5-methyl-DL-tryptophan, L-norleucine, and D-ethionine were purchased from Mann Research Laboratories, Inc., New York, N.Y.

Mitomycin C, actinomycin D, chloramphenicol, and phenyl-α-D-glucoside were obtained from Sol Spiegelman, Department of Microbiology University of Illinois.

RESULTS

Optimal conditions for α-amylase synthesis. The effect of pH on the synthesis of α-amylase is shown in Table 1. The pH optimum for the induced biosynthesis of α-amylase by washed-cell suspensions was 6.7.

The kinetics of α-amylase formation with pure maltose as the inducer are shown in Fig. 1. α-Amylase synthesis began soon after the addition of the inducer, proceeded at a linear rate for 60 min, and then leveled off.

When the gratuitous inducers methyl-β-D-maltoside or phenyl-α-D-glucoside were used, the same kinetics of α-amylase formation were observed (Fig. 1) as when pure maltose was used as the inducer. Under these conditions, the saturating concentration of inducer (pure maltose, phenyl-α-D-glucoside, or methyl-β-D-maltoside) was 10^{-3} M. Control cells without inducer produced 5.6 units per ml of α-amylase after 150 min of incubation. The addition of glucose (2×10^{-3} M), sucrose (10^{-3} M), or glycerol (4×10^{-3} M) to washed-cell suspensions did not stimulate the formation of α-amylase.

Effect of nitrogen starvation on α-amylase synthesis and amino acid pool. The depletion of the amino acid pool was accomplished by shaking cell suspensions with 2.0% fructose for 2 hr. Table 2 shows that the ability to synthesize α-amylase was lost after 60 min of nitrogen starvation. Replenishment of the amino acid pools with 0.5% casein hydrolysate at this time restored 90% of the enzyme-forming ability. After 120 min of nitrogen starvation, only 60% of the enzyme-forming ability was restored. Replenishment with

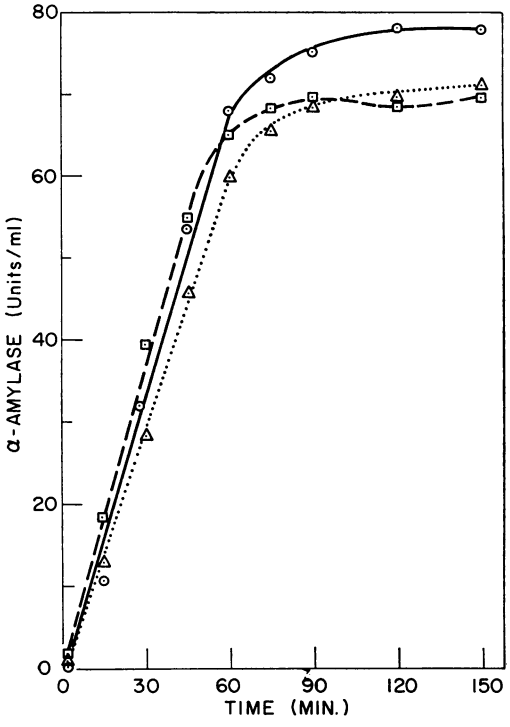

FIG. 1. *Induction of α-amylase in washed-cell suspensions of Bacillus stearothermophilus by pure maltose, phenyl-α-D-glucoside, and methyl-β-D-maltoside. Cells were suspended in 100 ml of R buffer. Inducers were added at zero time. Maltose (10^{-3} M), ⊙; phenyl-α-D-glucoside (10^{-3} M), △; methyl-β-D-maltoside (10^{-3} M), ⊡.*

NH_4Cl (1.0%) did not restore α-amylase synthesis.

The amino acid pool was examined for its amino acid content after 60 and 150 min of starvation. These pools were compared with the nonstarved amino acid pool (Table 3). Examination of Table 3 reveals that the amino acid pool was significantly depleted after 60 min of starvation or induction. Phenylalanine, tyrosine, proline, and valine disappeared from the pool. Starvation for 150 min depleted the amino acid pool to levels where free amino acids could not be detected by the methods used. Depletion of the pools was more complete in the case of induction with maltose than with starvation with fructose.

Effect of chloramphenicol and 8-azaguanine on α-amylase synthesis. Figure 2 shows that the addition of chloramphenicol or 8-azaguanine to pure maltose-induced washed-cell suspensions immediately inhibited the synthesis of α-amylase. The results of the addition of chloramphenicol at various times after addition of the inducer is shown in Fig. 3. It may be seen that the addition of chloramphenicol to induced washed-cell suspensions completely inhibited further synthesis of enzyme regardless of the time of addition.

Effect of amino acid analogues on α-amylase synthesis. Figure 4 shows that 5-methyl-DL-tryptophan (5-MT) did not inhibit α-amylase synthesis,

TABLE 2. *Effect of nitrogen starvation on the ability of Bacillus stearothermophilus to form α-amylase**

Starvation time	Medium†	α-Amylase (units/ml)	Per cent inhibition
min			
0	Buffer	52	—
0	NH_4Cl	50	—
0	Complete	49	—
30	Buffer	20	39.0
30	NH_4Cl	25	—
30	Complete	45	—
60	Buffer	5	96.0
60	NH_4Cl	7	—
60	Complete	44	—
90	Buffer	0	100.0
90	NH_4Cl	0	—
90	Complete	38	—
120	Buffer	0	100.0
120	NH_4Cl	0	—
120	Complete	30	—

* Cells were suspended in a 500-ml flask containing 50 ml of R buffer plus 2.0% fructose and placed on a rotary shaker at 55 C. At the described times, three samples of 5.0 ml each were removed and washed two times with R buffer. One sample was placed in an induction flask containing 30 ml of induction medium (R buffer plus maltose, 10^{-3} M); the other two samples were placed in 20 ml of the appropriate replenishment medium (0.5% casein hydrolysate or 1.0% NH_4Cl). After 20 min, cells were centrifuged, washed two times with R buffer, and placed in induction flasks. Induction was carried out for 60 min.

† Complete = cells which have been replenished by exposure to 0.5% casein hydrolysate. NH_4Cl = cells which have been replenished by exposure to 1.0% NH_4Cl. Buffer = cells receiving no nitrogen replenishment.

while ethionine, norleucine, norvaline, and *p*-fluorophenylalanine inhibited active α-amylase formation by 38, 53, 72, and 80%, respectively.

Effect of p-fluorophenylalanine on the incorporation of DL-*proline-C*^{14} *into α-amylase and cellular proteins.* The data in Table 4 show that *p*-fluorophenylalanine inhibited the synthesis of active α-amylase by 92% and the incorporation of proline-C^{14} into α-amylase and cellular protein by 95 and 74%, respectively.

Effect of mitomycin C and actinomycin D on α-amylase synthesis. Figure 5 shows that both actinomycin D and mitomycin C inhibit the synthesis of active α-amylase.

TABLE 3. *Amino acid pools of starved and nonstarved cells of Bacillus stearothermophilus**

Amino acid	Nondepleted pool (0 min)	Depleted pools	
		Fructose starvation† (60 min)	Maltose induction‡ (60 min)
Tyrosine	1.20	0	0
Phenylalanine	3.20	0	0
Lysine	3.07	1.8	0.093
Histidine	0.23	0.15	0.075
Arginine	1.30	0.44	0.023
Aspartic acid	2.50	1.80	0.095
Threonine	2.00	0.19	0.01
Serine	2.50	0.64	0.035
Glutamic acid	2.80	1.30	0.07
Proline	0.87	0	0
Glycine	2.15	1.20	0.065
Alanine	4.10	0.57	0.03
Valine	0.50	0	0
Methionine	0.30	0.15	0.023
Isoleucine	2.23	0.15	0.0075
Leucine	1.80	0.34	0.018
Tryptophan	0	0	0

* Cells were suspended in 20 ml of deionized water and heated in a boiling-water bath for 20 min. The cell debris was removed by centrifugation, and the supernatant fluid was decanted. The pellet was washed once with deionized water and centrifuged. The supernatant fluids were combined and taken to dryness. The amino acid content of the pools was determined in the Beckman/Spinco amino acid analyzer. Results are expressed as micromoles of amino acid per 100 mg (dry weight) of cells.

† Cells were suspended in 150 ml of R buffer (pH 6.7) containing 2.0% fructose.

‡ Cells were suspended in 150 ml of R buffer (pH 6.7) containing 10^{-3} M pure maltose.

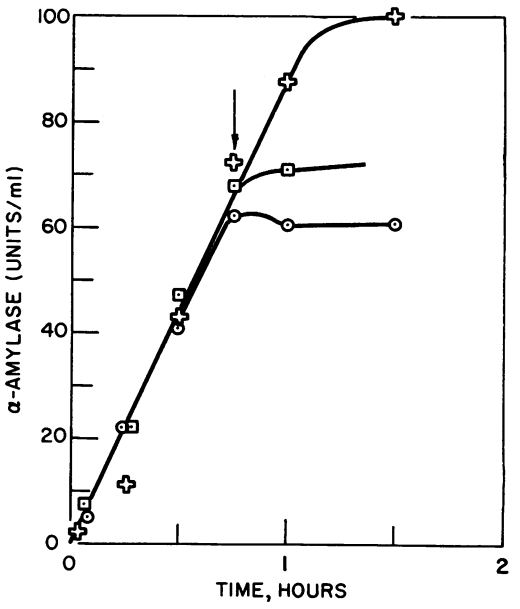

FIG. 2. *Chloramphenicol and 8-azaguanine inhibition of α-amylase synthesis by washed-cell suspensions. Cells were suspended in 100 ml of R buffer containing pure maltose (9 × 10^{-4} M) as an inducer. Chloramphenicol (100 μg/ml) and 8-azaguanine (0.5 μmole/ml) were added 45 min after addition of the inducer. 8-Azaguanine, ⊡; chloramphenicol, ⊙; control, +.*

DISCUSSION

The kinetics of α-amylase formation in washed-cell suspensions were shown to be linear over a 60-min period and then leveled off. The leveling off of α-amylase synthesis presumably results from the depletion of the amino acid pool. Starvation or induction of cells resulted in a general depletion of amino acids and the complete disappearance of phenylalanine, tryosine, proline, and valine from the pool. Replenishment of the amino acid pool with casein hydrolysate restored the ability of the cells to synthesize α-amylase. Similar results were found by Eisenstadt and Klein (1961) where starvation of *Pseudomonas saccharophilia* resulted in the inability of cells to synthesize α-amylase. The cessation of α-amylase synthesis, in this system, was due to the disappearance of aspartate, glutamic acid, and

one unknown compound from the amino pool. Halvorson and Spiegelman (1953) showed that induction of maltozymase in *Saccharomyces cerevisiae* resulted in a marked decrease in cell components of the amino acid pool. Although the experiments with starved cells do not definitely establish the de novo synthesis of α-amylase, they do support this hypothesis.

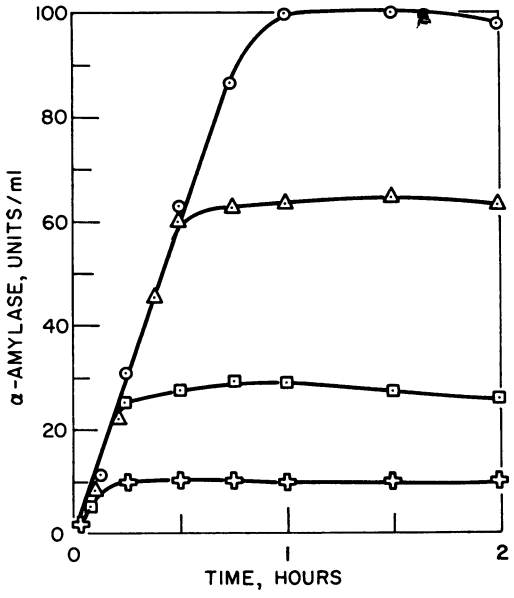

FIG. 3. *Inhibition of α-amylase synthesis by chloramphenicol added at various times after induction. Cells suspended in 50 ml of R buffer containing pure maltose (9 × 10⁻⁴ M) as an inducer. Chloramphenicol (100 μg/ml) was added as follows: at time of inducer, +; 15 min, ⊡; 30 min, △; control, ⊙.*

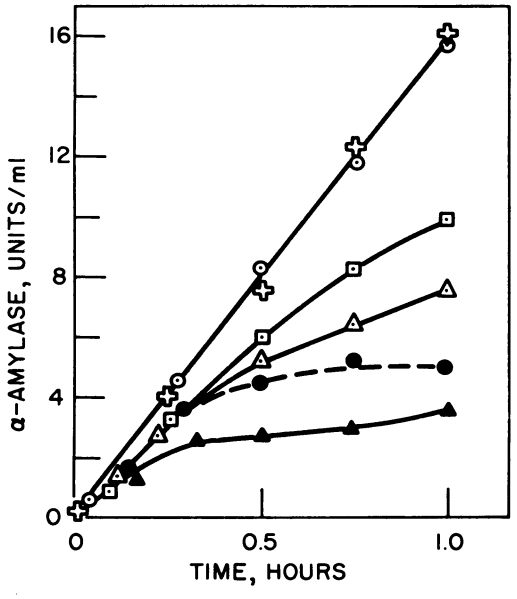

FIG. 4. *Effect of amino acid analogues on α-amylase synthesis. Cells suspended in 15 ml of R buffer containing pure maltose (10⁻³ M) as inducer. The amino acid analogues (10⁻³ M) were added at zero time. Control, +; 5-MT, ⊙; ethionine, ⊡; norleucine, △; norvaline, ●; p-fluorophenylalanine, ▲.*

Mandelstam (1957) demonstrated that the rate of protein turnover in nongrowing cells of *Escherichia coli* is sufficient to permit a differential rate of synthesis of β-galactosidase identical to that observed in rapidly growing cells. If protein turnover occurs in the cell suspensions of *B. stearothermophilus*, it is not sufficient to support the synthe-

TABLE 4. *Effect of p-fluorophenylalanine on the incorporation of DL-proline-C¹⁴ into cellular proteins and the α-amylase of Bacillus stearothermophilus**

Time	Control			*p*-Fluorophenylalanine		
	Cellular protein†	Amt	α-Amylase activity†	Cellular protein†	Amt	α-Amylase activity†
min		*units/ml*			*units/ml*	
5	167	8.4	27	39	3.6	3
15	300	10.8	59	211	4.0	3
30	750	32.0	69	296	4.8	4
45	860	57.0	98	249	5.2	5
60	1290	68.4	145	328	5.6	7

* Cells were suspended in 38 ml of R buffer containing pure maltose (10^{-3} M) as an inducer and proline-C^{14} (2.1×10^4 counts per min per ml). *p*-Fluorophenylalanine (10^{-3} M) was added to one flask at zero time.

† Activity expressed as counts per minute per 10 ml.

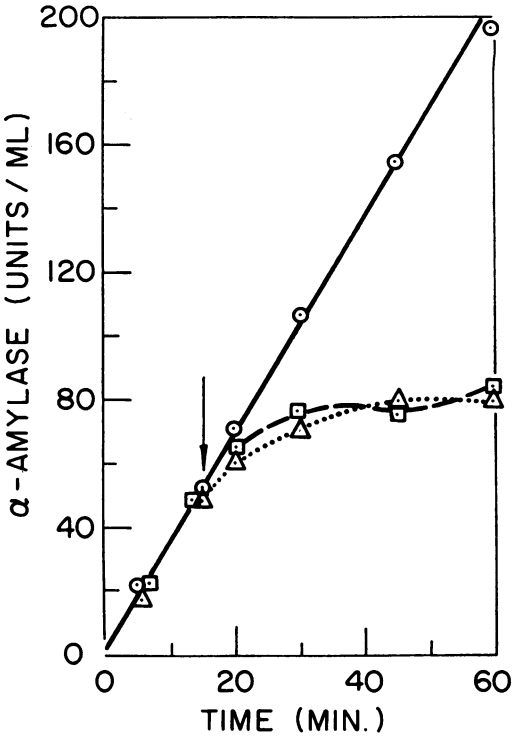

FIG. 5. *Actinomycin D and mitomycin C inhibition of α-amylase synthesis by washed-cell suspensions. Cells were suspended in 100 ml of R buffer containing pure maltose (10^{-3} M) as an inducer. Actinomycin D (10.0 μg/ml) and mitomycin C (0.5 μg/ml) were added 15 min after addition of the inducer. Actinomycin D, △; mitomycin C, ⊡; control, ⊙.*

sis of α-amylase or to prevent the net loss of phenylalanine, tyrosine, proline, or valine from the pool during enzyme induction.

The immediate inhibition of α-amylase formation by chloramphenicol, irrespective of the time of addition to washed-cell suspensions, supports the proposal that the α-amylase of *B. stearothermophilus* is formed de novo. If an α-amylase precursor had accumulated in uninduced cells, a lag in chloramphenicol inhibition, upon induction of the cells, would be expected. No such lag was observed. Chloramphenicol has been shown to inhibit α-amylase formation by washed-cell suspensions of *P. saccharophilia* (Eisenstadt and Klein, 1961) and by various strains of *B. subtilis* (Nomura et al., 1958; Coleman and Elliott, 1962; Oishi, Takahashi, and Maruo, 1962).

The inhibition of enzyme synthesis by amino acid analogues has been reported by several workers [see the reviews of Gale (1962) and Richmond (1962)]. Munier and Cohen (1956, 1959) and Cohen and Munier (1959) have shown that, in some cases, *p*-fluorophenylalanine does not inhibit protein synthesis but gives rise to proteins containing *p*-fluorophenylalanine which are devoid of enzyme activity. The incorporation of amino acid analogues into proteins can also result in the formation of altered structures with reduced enzyme activity. Yoshida (1960) has shown that *p*-fluorophenylalanine replaced 9% of the phenylalanine in *B. subtilis* α-amylase, resulting in a loss of 20 to 30% of its activity. Nomura et al. (1956, 1958) reported that α-amylase synthesis by *B. subtilis* was not affected by *p*-fluorophenylalanine; Coleman and Elliott (1962) showed, with a different strain of *B. subtilis*, that this analogue inhibited α-amylase synthesis by 44%. The inhibition of active α-amylase synthesis by amino acid analogues in our system might be due to the synthesis of a structurally altered protein with reduced activity or one devoid of enzyme activity. However, in the case of *p*-fluorophenylalanine, this seems unlikely in view of the rapidity with which this analogue inhibits active α-amylase synthesis and the concomitant inhibition of proline-C^{14} incorporation into α-amylase. The failure of 5-methyl-tryptophan to inhibit α-amylase formation is consistent with the fact that the α-amylase produced by this strain of *B. stearothermophilus* does not contain tryptophan (Campbell and Manning, 1961).

8-Azaguanine has been shown to inhibit the synthesis of constitutive and inducible enzymes (Creaser, 1956; Richmond, 1959; Chantrenne and Devreux, 1960). Fukumoto, Yamamoto, and Tsuru (1957) and Nomura and Hosoda (1958) have demonstrated that the synthesis of α-amylase by *B. subtilis* is inhibited by 8-azaguanine. Our data show that this analogue inhibits α-amylase formation by *B. stearothermophilus*. Although 8-azaguanine can replace as much as 40% of the guanine in ribonucleic acid [(see review of Davis and Feingold (1962)], the mechanism by which it inhibits protein synthesis is not known.

Mitomycin C and actinomycin D were also found to inhibit α-amylase formation by *B. stearothermophilus*. The mechanism of their inhibition is not known. Actinomycin D combines with deoxyribonucleic acid (Kawamoto and Imanishi,

1961) and thereby prevents the formation of "messenger" ribonucleic acid. Mitomycin C appears to cause the degradation of deoxyribonucleic acid (Kersten, 1962). The net effect of both of these polypeptide antibiotics is to stop ribonucleic acid synthesis [*see* Davis and Feingold (1962)].

It should be mentioned that in every case where a compound was found to inhibit α-amylase synthesis the same level of inhibitor had no effect on the α-amylase activity of the enzyme produced in the control experiments.

The combined results presented in this paper lead us to conclude that induction of resting-cell suspensions of *B. stearothermophilus* results in the de novo synthesis of α-amylase from pre-existing, low molecular weight compounds in the cells.

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