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ENZYMATIC ACTIVITIES OF STREPTOMYCIN-DEPENDENT *ESCHERICHIA COLI* IN RELATION TO VALINE FORMATION

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ABSTRACT

BRAGG, P. D. (University of British Columbia, Vancouver, B.C., Canada), AND W. J. POLGLASE. Enzymatic activities of streptomycin-dependent *Escherichia coli* in relation to valine formation. J. Bacteriol. **88**:1399–1402. 1964.—The activities of several enzymes were compared in antibiotic-depleted and antibiotic-supplemented streptomycin-dependent *Escherichia coli*. Depleted cells were somewhat lower than supplemented cells in several oxidase activities. Isocitric dehydrogenase was very much lower in depleted cells than in supplemented cells. The lactic dehydrogenase activity of depleted and supplemented cells was similar. The balance of enzymatic activities in depleted and supplemented cells was thus found to correlate well with the observed extracellular products. Thus, depleted cells excreted lactic acid and were deficient in oxidase activity (although normal in lactic dehydrogenase activity), whereas supplemented cells excreted valine and were rich in the reduced nicotinamide adenine dinucleotide phosphate-producing enzyme, isocitric dehydrogenase. Since antibiotic-depleted cells were not deficient in lactic dehydrogenase, it appeared probable that the failure of depleted cells to metabolize lactate was related to a deficiency in the electron-transport system.

The formation of the amino acid L-valine by streptomycin-dependent microorganisms has been reported from two laboratories (Tirunarayanan, Vishcher, and Renner, 1962; Bragg and Polglase, 1962). Subsequent work (Bragg and Polglase, 1964) showed that, whereas valine was produced by dependent *Escherichia coli* growing aerobically in the presence of antibiotic, lactic acid was formed instead of valine under conditions of either oxygen or antibiotic deprivation. It was proposed (Bragg and Polglase, 1964) that valine (aerobically) and lactate (anaerobically) represented secondary products of hydrogen transfer from reduced coenzymes. It was suggested, further, that the antibiotic (dihydrostreptomycin) participated, in some way, in the transfer of hydrogen, with the concomitant formation of valine, under aerobic conditions.

Spotts and Stanier (1961) and Spotts (1961) concluded that the effects of antibiotic deprivation on the enzyme balance of streptomycin-dependent *E. coli* were essentially random when the enzyme activities were classified according to metabolic function.

In contrast, we have noted marked preferential effects of antibiotic depletion on respiratory components (Bragg and Polglase, 1963) and on extracellular metabolites (Bragg and Polglase, 1964). We have therefore extended our comparative studies on antibiotic-depleted and antibiotic-supplemented *E. coli* with the objective of correlating activities of enzymes with the streptomycin-controlled formation of valine.

MATERIALS AND METHODS

The streptomycin-dependent *E. coli* was depleted of antibiotic by growth in salts-glucose medium as described previously (Bragg and Polglase, 1963). These were referred to as "depleted-DA" cells. The depleted-DA cells were collected by centrifugation, resuspended in fresh medium, and divided into equal samples. To one of these samples, dihydrostreptomycin (1,000 μg/ml) was added. Cells treated in this way were called "supplemented-DA" cells. Both the depleted-DA and supplemented-DA cells were aerated vigorously at 37 C over a period of 3 hr, and then harvested by centrifugation. The cells were suspended in a suitable volume of tris(hydroxymethyl)aminomethane (tris) buffer (0.05 M, pH 7.5)—usually 5 to 15 times the volume of the packed cells—and were treated for 4 min in a 20-kc Bronwill sonic oscillator. The disrupted cell suspensions were then centrifugued for 15 min at 10,000 $\times$ g. The supernatant layers were then dialyzed for 16 hr at 4 C against this buffer. Protein analyses were performed by the method of Lowry et al. (1951). Enzyme

activities were then determined as described below.

Valine-glutamate transaminase. The system contained the following: 0.25 ml (50 μmoles) of sodium L-glutamate (pH 8.0); 1.0 ml of sodium α-keto isovalerate (20 μmoles); 1.0 ml of 0.1 M phosphate (pH 8.0); 0.02 ml (0.2 mg) of pyridoxal phosphate; 0.1 ml of 0.05 M $MgCl_2$; and 0.5 ml of cell extract. The mixture was incubated for 1 hr at 30 C, and the reaction was stopped by heating for 3 min at 100 C. The mixture was centrifuged, the supernatant solution was concentrated to 0.5 ml, and amino acids were determined by the method of Bode (1955).

Alanine-glutamate transaminase. Alanine-glutamate transaminase was determined in a manner similar to that used for valine-glutamate transaminase, with sodium pyruvate and sodium glutamate as substrates.

Acetokinase. Acetokinase activity was measured at 35 C by the procedure of Lipmann and Tuttle (1945). The standard curve for the determination of acetohydroxamic acid was constructed by use of succinic anhydride.

The procedures used for assaying hexokinase, phosphofructokinase, aldolase, phosphohexoisomerase, glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, and triose phosphate dehydrogenase were described previously (Bragg and Reeves, 1962). Lactic dehydrogenase was determined by reduction of methylene blue as described by Dixon (1955).

Isocitric dehydrogenase. This activity was determined spectrophotometrically at 30 C by observation of the change in optical density at 340 mμ in the following system: 1.0 ml of tris buffer (0.04 M, pH 7.5) containing 0.01 M $MgCl_2$, 0.3 ml (0.3 μmoles) of nicotinamide adenine dinucleotide phosphate (NADP), 0.25 ml (5 μmoles) of sodium isocitrate, and 0.1 ml of bacterial extract.

Glutamic dehydrogenase. Glutamic dehydrogenase was determined by measurement of the change in optical density at 340 mμ of a solution in 0.05 M phosphate buffer (pH 7.0) of the following: 0.2 μmoles of reduced NADP ($NADPH_2$), 5 μmoles of α-ketoglutarate, and 0.5% ammonium sulfate in a final volume of 1.8 ml.

Reduced pyridine nucleotide oxidases. These activities were determined at 35 C by measuring the change in optical density at 340 mμ in the following system: 1.5 ml of tris buffer (0.05 M, pH 7.5) containing reduced nicotinamide adenine dinucleotide ($NADH_2$) (or its phosphate, $NADPH_2$) and 0.15 ml of bacterial extract.

Pyruvate oxidase. This activity was determined manometrically at 35 C with the following system: 0.5 ml of sodium pyruvate (25 μmoles), 1 ml of 0.3 M phosphate buffer (pH 7.6) containing 0.5 μmole of nicotinamide adenine dinucleotide, 0.25 μmole of NADP, 2.2 μmoles of cysteine, 1.7 μmoles of $MgCl_2$, 0.1 μmole of thiamine pyrophosphate, and 0.5 ml of bacterial extract.

Isocitric, succinic, and malic oxidases. These activities were determined as described for pyruvic oxidase, by substituting the appropriate substrates and omitting thiamine pyrophosphate.

RESULTS AND DISCUSSION

For most enzyme activities tested, depleted and supplemented cells were approximately equivalent (Table 1). Exceptions were isocitric dehydrogenase and a number of oxidases, which were of higher activity in dependent cells after supplementation with dihydrostreptomycin. In previous work (Bragg and Polglase, 1963), it was shown that depleted dependent *E. coli* cells were deficient in cytochromes a_1 and a_2. It has been reported also (Bragg and Polglase, 1964) that depleted cells, under aerobic conditions, produced lactic acid from glucose. In contrast, supplemented cells produced valine under aerobic conditions but produced lactic acid instead in a nitrogen atmosphere. Depleted cells were equivalent to supplemented cells in lactic dehydrogenase activity in an assay with methylene blue as hydrogen acceptor (Table 1). Thus, the most plausible explanation for the accumulation of lactic acid by depleted cells would be that these cells had a respiratory chain deficiency. Presumably, the diminished oxidase activity of depleted cells is also a reflection of the same deficiency.

Engelberg and Artman (1961) observed that streptomycin-dependent cells grown with a low concentration of antibiotic had lower oxidase activity toward a number of substrates than did cells grown with an adequate quantity of streptomycin. These workers also observed a deficient cytochrome system in cells grown with a suboptimal concentration of antibiotic. A similar observation had been reported by Schaeffer (1952) for antibiotic-starved streptomycin-dependent *Bacillus cereus*. Thus, all observations

TABLE 1. *Enzyme activities of antibiotic-depleted and antibiotic-supplemented streptomycin-dependent Escherichia coli-DA**

Enzyme	Depleted	Supplemented	Ratio†
Transaminases			
Valine-glutamate	49	39	0.8
Alanine-glutamate	90	69	0.8
Kinases			
Acetokinase	18	14	0.8
Hexokinase	59	63	1.1
Phosphofructokinase	58	43	0.7
Aldolase	57	62	1.1
Phosphohexoisomerase	86	103	1.2
Dehydrogenases			
Glucose-6-phosphate	165	210	1.3
6-Phosphogluconate	27	26	1.0
Triose phosphate	86	72	0.8
Lactic	168	158	0.9
Isocitric	130	667	5.1
Glutamic	133	123	0.9
Oxidases			
$NADH_2$	140	260	1.9
$NADPH_2$	8	11	1.4
Pyruvate	6	13	2.2
Isocitric	4	7	1.8
Succinic	20	25	1.3
Malic	14	19	1.4

* Activity expressed as millimicromoles per minute per milligram of protein.

† Ratio of enzyme activities, supplemented-DA to depleted-DA.

point to a primary respiratory deficiency in depleted cells.

In contrast, depleted cells that were supplemented with dihydrostreptomycin were shown to have recovered from a deficiency in cytochromes a_1 and a_2 (Bragg and Polglase, 1963). These cells were found to form valine instead of lactic acid (Bragg and Polglase, 1964). The production of L-valine from glucose as a major metabolite would require that adequate quantities of $NADPH_2$ be generated. Although other pathways for the generation of this reduced coenzyme might be available, we observed that the isocitric dehydrogenase level of supplemented cells was several-fold greater than that of depleted cells (Table 1). Thus, the coenzyme requirement for valine biosynthesis could have been satisfied through the elevated activity in supplemented-DA cells of this NADP-requiring enzyme. In contrast, the NADP-requiring enzymes of the hexose monophosphate pathway showed equivalent activities in both depleted and supplemented dependent cells.

The results thus support the conclusion reached earlier (Bragg and Polglase, 1963) that streptomycin-dependent *E. coli* cells require the antibiotic for maintenance of the integrity of the electron-transport system. This conclusion contradicts that of Spotts (1961) and Spotts and Stanier (1961), who concluded that the loss of enzyme activities resulting from antibiotic depletion was essentially random.

None of the observations so far reported provides an explanation for the production of L-valine as a major end product of the aerobic metabolism of glucose in streptomycin-dependent *E. coli*. The biosynthesis of valine in streptomycin-dependent *E. coli* is, therefore, now being studied in detail in this laboratory and will be the subject of a future report.

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