REPRESSION OF MALIC ENZYME BY ACETATE IN PSEUDOMONAS[1]

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The choice between alternate routes of central metabolism may be regulated by the levels of cellular metabolites. In the glyoxylate shunt both the synthesis and the activity of isocitrate lyase are controlled by the intracellular level of phosphoenolpyruvate (Kornberg, 1965). Thus in cells growing on two-carbon compounds such as acetate, the level of glyoxylate shunt enzymes is increased and a net synthesis of C-4 compounds occurs; in cells which have a ready supply of C-3 or C-4 compounds the formation of these enzymes is repressed.

Kogut and Podoski (1953) have reported that acetate-grown cells of Pseudomonas fluorescens strain KB1 do not oxidize succinate or other tricarboxylic acid cycle intermediates at appreciable rates.

We have now found that the "malic enzyme" (Ochoa et al., 1948) is present at high levels in Pseudomonas putida during growth on succinate when acetyl CoA production is required for oxidation by the tricarboxylic acid cycle. This enzyme is repressed in the presence of acetate with consequent conservation of C-4 compounds.

Materials and Methods

Organism and Medium: Pseudomonas putida strain C1B (Bradshaw et al., 1959) was grown in a basal medium containing potassium phosphate buffer, pH 6.8, 37 mM; NH_4Cl, 40 mM; $MgCl_2$, 1.6 mM; $MnCl_2$, 0.3 mM; and trace amounts of Fe^{++}, Ca^{++}, NaCl and $NaMoO_4$. Carbon sources were added as sterile aqueous solutions.

Cell Extracts and Enzyme Assays: Cells were harvested by centrifugation for 15 minutes at 37K x g, resuspended in 0.05 M phosphate buffer, pH 7.0 and treated for 3 minutes in a Mullard sonic oscillator (Measuring and Scientific Equipment Ltd., London). Cell debris was removed by centrifugation as above.

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For oxidation by sonic extracts the cells were broken and the preparations used without centrifugation. The unbroken cells remaining were shown to be less than 1%.

The malic enzyme was measured by following the rate of TPN reduction at 340 mμ in a 1 ml reaction mixture containing in μmoles: Tris-Cl pH 9, 100; dipotassium malate, 10; TPN, 1; $MgCl_2$, 25; and 0.1 ml of extract diluted to give an optical density change of 0.5 per minute or less. One unit of enzyme activity is defined as that amount required to produce one micromole of TPNH per minute at 25°C. Conventional manometric techniques were used throughout (Umbreit, Burris and Stauffer, 1957).

Results and Discussion

Cells grown on acetate oxidize succinate at a rate about 6% of the rate shown by succinate-grown cells, Table I. With sonicated suspensions the rates are more similar; acetate-grown cells show about 40% of the rate for succinate grown cells. Since much of the disparity in oxidative capacity is no longer

Table I

Influence of Growth Substrate on Succinate Oxidation

Carbon Source for Growth	Succinate Q_{O_2}	
	Cells	Sonicated
Succinate, 30 mM	380	56
Acetate, 25 mM	25	24

Warburg cups contained 0.1 ml 20% KOH in the center well and in the main compartment 2.9 ml 0.05 M KPO_4 pH 7 containing 10 μmoles succinate, 5 mg (dry wt.) washed cells and 1.5 mg chloramphenicol. When whole cells were replaced by 10 mg sonicated cells, 0.5 μmoles each DPN and TPN, 50 μmoles $MgCl_2$, 10 μmoles succinate and 0.02 mg phenazine methosulfate were present in addition to the above components.

observed after the cells are broken there must be some difference in the permeability of the cells to succinate. Experiments with C^{14}-labeled succinate have shown that succinate-grown cells take up this substrate 5-6 times more rapidly than acetate-grown cells. The residual 2.3-fold difference in oxidation rates by extracts is postulated to represent a difference in the enzyme levels within the cells.

An investigation of the "malic enzyme" (malate: TPN oxidoreductase (decarboxylating); E.C. 1.1.1.40) as a possible source of this difference was prompted by the observation that its specific activity in extracts of acetate-grown cells was lower than in extracts of succinate-grown cells. The malate-

dependent reduction of TPN was inferred to be due exclusively to "malic enzyme" on the basis of requirement for Mg^{++} ions, identification of pyruvate (as its 2,4-dinitrophenylhydrazone) as the sole product of the action of extracts on malate, competitive inhibition by oxaloacetate (K_i = 1.7 mM $\pm$ 0.3) and magnesium-reversible substrate inhibition by malate (K_s' = 7.3 mM). These properties compare point for point with the properties of purified malic enzyme from pigeon liver (Stickland, 1959).

The repression of malic enzyme formation by acetate was measured in two ways. In the first experiment, 5 mM acetate was added to a culture growing logarithmically on 30 mM succinate. Samples were withdrawn from the culture at intervals preceding and following the addition of acetate, chloramphenicol was added to a final concentration of 500 μg/ml and the activity of malic enzyme determined in sonic extracts. The results are shown in Figure 1. It may be seen that the increase in total activity (about 0.15 units per mg increase in cell mass) which is observed during logarithmic growth is abruptly retarded by the addition of acetate. Upon exhaustion of acetate from the medium synthesis of the enzyme resumes at the original rate. The results of this experiment suggest that the repression of succinate-oxidizing capacity may derive from repression of the malic enzyme.

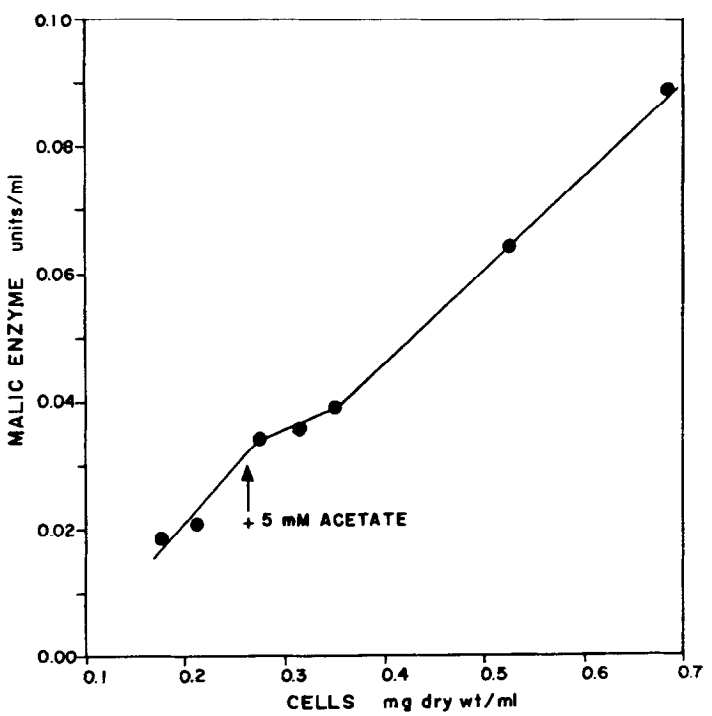

Figure 1. Effect of acetate addition on synthesis of malic enzyme during growth on succinate.

To determine whether the degree of repression of the malic enzyme in the presence of acetate was sufficient to account for the 2.3-fold difference in rates of oxidation by cell extracts, cells growing in early logarithmic phase on succinate were harvested and resuspended in medium containing 20 mM acetate. Samples were withdrawn at zero time, and at intervals thereafter, and the activity of malic enzyme measured in sonic extracts. It may be seen from Figure 2(a) that the specific activity drops in the presence of acetate to one-third of its initial value. Only a small part of the decrease in specific activity takes place before the initiation of growth on acetate and it does not represent a net loss of enzyme. In a control experiment, Figure 2(b), cells resuspended in succinate medium show a very slight increase in the specific activity of malic enzyme. The drop in specific activity during growth on acetate may be attributed to an adjustment in the differential rate of enzyme synthesis (Figure 3), similar to that seen in Figure 1.

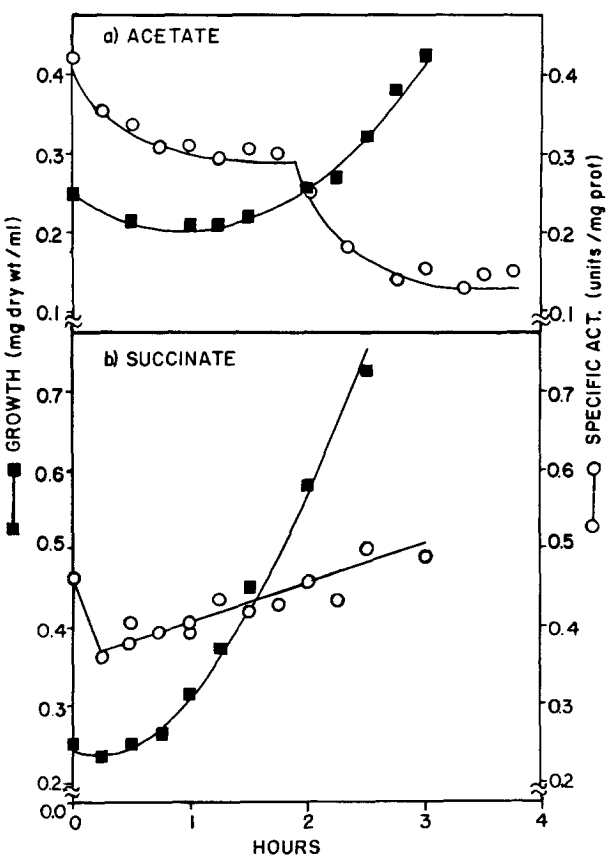

Figure 2. Growth and specific activity of malic enzyme after transfer from succinate medium to (a) acetate or (b) succinate.

The results of a brief survey of enzyme levels in cells grown on a number of carbon sources (Table II) suggest that the malic enzyme is repressed by acetate or a product formed from acetate (possibly acetyl CoA) rather than induced by a metabolic product of succinate. The fact that growth on succinate,

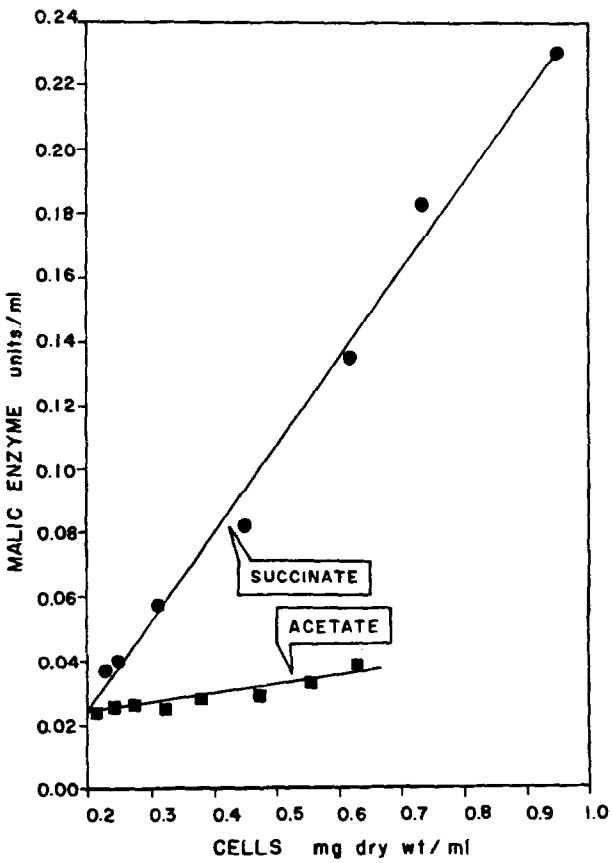

Figure 3. Differential synthesis of malic enzyme during growth on acetate or succinate.

malate, glutamate, asparagine or glucose, all of which are at some metabolic distance from acetate, leads to approximately equal enzyme levels suggests that none of these is an inducer nor metabolically closer to some inducer than any of the others. Lactate or pyruvate, which are metabolically close to acetate, give considerable repression though much less than acetate. While these results are suggestive, they do not rigorously exclude the possibility of induction by some metabolite whose intracellular concentration is lowered in the presence of acetate.

Table II

Effect of Carbon Source on Malic Enzyme Activity

Carbon Source for Growth (20 mM)	Malic Enzyme Specific Activity	Malic Enzyme Relative Activity
Succinate	0.65	1.0
Malate	0.63	0.97
Asparagine	0.64	0.99
Glutamate	0.64	0.99
Glucose	0.65	1.0
Pyruvate	0.48	0.74
Lactate	0.48	0.74
Acetate	0.25	0.38

The observed difference in succinate oxidation by acetate-grown and succinate-grown cells may be attributed at least in part to repression of the malic enzyme in the presence of acetate. The oxidation of succinate via the tricarboxylic acid cycle necessitates the conversion of stoichiometric amounts of succinate to acetyl CoA, with only catalytic amounts of C-4 compounds preserved to act as acceptors. Thus the decarboxylation of C-4 compounds by the malic enzyme can be a key step in catabolism. In contrast, cells growing on C-2 compounds must effect a continuous synthesis of C-4 compounds to compensate for the withdrawal of tricarboxylic acid cycle intermediates for the biosynthesis of amino acids and porphyrins (Kornberg, 1965). This required synthesis of C-4 compounds appears to occur via the glyoxylate shunt with "coarse control" exerted by acetate through repression of the malic enzyme resulting in decreased loss of C-4 compounds through decarboxylation. It is of interest to note that we have found no inhibition of the malic enzyme by acetate, pyruvate, acetyl CoA or phosphoenolpyruvate. Thus the mechanism of "fine control" of the enzyme, if indeed any exists, remains obscure.

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