Modulation of isocitrate dehydrogenase activity in *Acinetobacter calcoaceticus* by acetate

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The addition of acetate to a culture of *Acinetobacter calcoaceticus* grown in medium containing limiting succinate as the sole carbon and energy source leads to an increase in the specific activity of isocitrate dehydrogenase. This is in contrast to similar studies with several other microorganisms in which acetate induces an ATP-dependent phosphorylation and concomitant decrease in the specific activity of this enzyme.

Isocitrate dehydrogenase Acinetobacter calcoaceticus *Glyoxylate cycle* *Isocitrate lyase* *Phosphorylation*

1. INTRODUCTION

Authors in [1,2] originally described the reversible inactivation of NADP-isocitrate dehydrogenase (EC 1.1.1.42) in several microorganisms – *Escherichia coli, Salmonella typhimurium, Aerobacter aerogenes* and *Serratia marcescens* – following growth of these organisms in medium containing limiting glucose as the sole carbon source. It was proposed that the inactivation of isocitrate dehydrogenase occurred as a result of the accumulation of acetate in the medium, and provided the cells with a regulatory mechanism which would permit isocitrate lyase (EC 4.1.3.1) to compete successfully for isocitrate under conditions where the operation of the glyoxylate cycle was essential for growth [2,3]. It was demonstrated subsequently that in *E. coli* and *S. typhimurium* the reversible inactivation of isocitrate dehydrogenase occurred as a result of the reversible covalent modification of the enzyme by an ATP-dependent phosphorylation/dephosphorylation [4–10]. These studies have been reviewed in [11].

It was recently reported [12] that in *E. coli* mutants devoid of either isocitrate lyase or malate synthase (EC 4.1.3.2) there occurred an acetate-induced phosphorylation and concomitant inactivation of isocitrate dehydrogenase. Thus, the phosphorylation of isocitrate dehydrogenase cannot be envisioned solely as a cellular regulatory mechanism to partition the metabolism of isocitrate through two competing metabolic pathways, and it was suggested that the interrelationship between the glyoxylate cycle and the reversible phosphorylation of isocitrate dehydrogenase should be re-evaluated. Results obtained in [9], which demonstrated the induction of the phosphorylation of the enzyme in *S. typhimurium* by 2-deoxyglucose or α-methylglucoside, also supported this suggestion.

Hitherto, these studies of isocitrate dehydrogenase have concentrated on the facultatively anaerobic bacteria. It therefore seemed worthwhile to examine the behaviour of a strict aerobe and, in view of the earlier demonstration of the novel allosteric properties of the isocitrate dehydrogenase of *Acinetobacter calcoaceticus* [13,14] we selected this organism for study. Here, data are presented which demonstrate that in *A. calcoaceticus* the specific activity of isocitrate dehydrogenase in cells grown in a medium containing limiting succinate as the carbon source in-

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creases concomitant with the induction of the glyoxylate by-pass enzyme, isocitrate lyase, in response to the addition of acetate to the medium. This is in sharp contrast to the marked decrease in isocitrate dehydrogenase activity observed previously in several other microorganisms in response to acetate.

2. EXPERIMENTAL

2.1. *Organism*

The organism employed in these studies was *A. calcoaceticus*, strain 4B, which has been described in [13–16]. A stock culture was maintained on nutrient agar slants at 4°C and, for the experiments to be described, was grown in a mineral salts medium [17] containing limiting succinate (15 mM) as the sole carbon source.

2.2. *Materials and assays*

The materials and reagents used in these experiments have been described [16]. Isocitrate dehydrogenase was assayed by measuring the reduction of NADP at 340 nm as in [6], and isocitrate lyase activity was determined by measuring the formation of glyoxylate phenylhydrazone at 324 nm as in [18]. Protein was determined as in [19], with bovine serum albumin as standard. Specific activities are expressed as μmol of NADPH or glyoxylate phenylhydrazone formed $\cdot$ min^{-1} $\cdot$ mg protein^{-1} at 25°C for isocitrate dehydrogenase and isocitrate lyase, respectively.

2.3. *Modulation of enzyme activities*

The organism was grown in a 500-ml culture flask containing 100 ml of mineral salts medium [17] with 15 mM succinate as the sole carbon source and incubated in a New Brunswick shaker at 300 rev./min for 18 h at 32°C. A 40-ml aliquot was removed from the culture, placed into 200 ml of the same medium and incubated under the same conditions until the cells had entered the stationary phase of growth, when an aliquot was removed for enzyme assay and protein determination. Cell proliferation was measured at 650 nm. One hundred ml of the culture were placed into each of two 500 ml flasks. The experimental flask was supplemented with 25 mM acetate while no addition was made to the control. The two flasks were placed in the incubator at 32°C and shaking continued at 300 rev./min. The initial sample, and those removed at the times indicated in fig.1, were centrifuged at 12000 $\times$ *g* for 10 min at 4°C. All samples were 20 ml aliquots of the culture. The cell pellets were suspended in 3 ml of 20 mM Tris–acetate buffer containing 1 mM EDTA at pH 7.5 and sonicated in an ice-water bath for 3 min. Cell debris was removed by centrifugation at 48000 $\times$ *g* for 30 min at 4°C. The extracts were assayed for enzyme activity and protein as described earlier.

3. RESULTS AND DISCUSSION

The data presented in fig.1 demonstrate the increase in the specific activities of both isocitrate dehydrogenase and isocitrate lyase following the addition of acetate to stationary phase cultures of *A. calcoaceticus* grown in a mineral salts medium with limiting succinate as the sole carbon source.

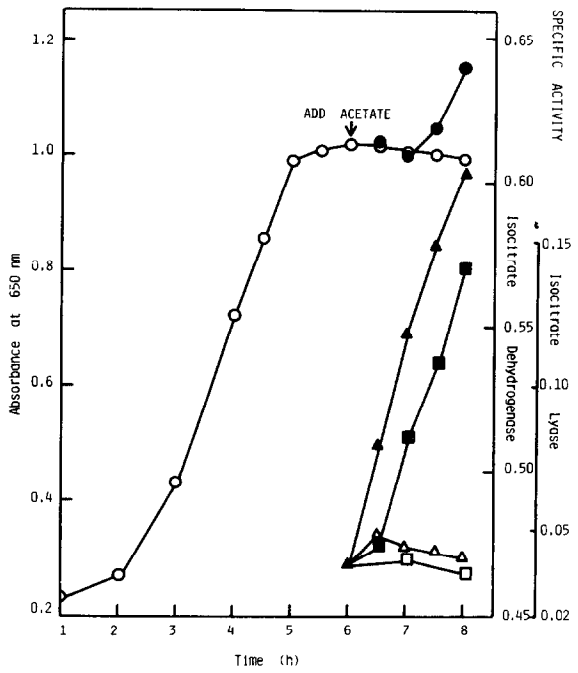

Fig.1. Growth of *A. calcoaceticus* and modulation of enzyme activities. Details are described in text. Cell density in absence (○—○) and presence (●—●) of acetate; specific activity of isocitrate lyase in absence (□—□) and presence (■—■) of acetate and specific activity of isocitrate dehydrogenase in absence (△—△) and presence (▲—▲) of acetate.

The cells are also able to adapt to the utilisation of acetate as a growth substrate as evidenced by the increase in cell density following a short lag period. In the control culture there was no cell proliferation and the specific activities of isocitrate dehydrogenase and isocitrate lyase remained unchanged during the additional incubation period.

In those microorganisms in which it has been studied, the addition of acetate to a culture grown to stationary phase in a mineral salts medium containing a limiting concentration of any one of several different sources of carbon, there occurs a phosphorylation and concomitant decrease in the activity of NADP-isocitrate dehydrogenase (review [11]). Although it was previously suggested that this occurred as a consequence of the induction of the glyoxylate by-pass enzymes, it is now thought that the phosphorylation of isocitrate dehydrogenase may be of much broader physiological significance, and be related to changes in the intracellular levels of one or more of a variety of compounds including phosphoenolpyruvate, adenine and nicotinamide nucleotides and/or isocitrate [11,12].

The results obtained here using *A. calcoaceticus* provide a sharp contrast to similar studies previously conducted with several other microorganisms [11]. Thus, the addition of acetate to a culture of this organism, previously grown in a medium containing limiting succinate as the sole carbon source, results in a marked increase in the specific activity of isocitrate dehydrogenase rather than a dramatic decrease in the activity of the enzyme. It is also of particular interest to note that this increase in isocitrate dehydrogenase activity occurs concomitant with an increase in the activity of isocitrate lyase. The activity of malate synthase was not examined here.

Although further studies are needed, it is known that *Acinetobacter* lacks the enzyme pyruvate kinase and is therefore unable to produce pyruvate from phosphoenolpyruvate [20]. During growth on succinate, pyruvate may be formed from malate through the action of malic enzyme, of which both NAD-linked and NADP-linked forms occur in *Acinetobacter* [21]. However, during growth on acetate the level of NADP-linked malic enzyme is markedly reduced [22]. This must result in an equivalent reduction in the formation of NADPH. We suggest that the increased activity of isocitrate dehydrogenase observed here may be a compensatory mechanism contributing to the maintenance of NADPH production which is essential for biosynthetic processes.

Previous studies have revealed the presence of two distinct NADP-linked isoenzymes of isocitrate dehydrogenase in *Acinetobacter* [13–16]. Current investigations are aimed at elucidating the roles of these two forms of the enzyme during the adaptation to growth on acetate.

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