In Vivo Phosphorylation of Isocitrate Lyase in Escherichia coli and Acinetobacter calcoaceticus

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Abstract. Isocitrate lyase in Escherichia coli and in Acinetobacter calcoaceticus is phosphorylated when the cells are grown with acetate as the sole carbon source in low-phosphate mineral salts medium containing $^{32}$P inorganic phosphate. The level of $^{32}$P incorporation into the enzyme in both microorganisms appears to be constant throughout the entire growth cycle. Further, the results of immunoblots and rocket immunoelectrophoresis suggest that the amount of isocitrate lyase protein, although at different levels in each microorganism, also remains constant throughout the growth cycle.

Microbial growth on acetate or compounds such as long-chain fatty acids or ethanol, which are metabolized to acetate, requires expression of the genes of the ace operon. The genes of this operon code for the enzymes of the glyoxylate bypass, isocitrate lyase (ICL) and malate synthase. These two enzymes produce a pathway that circumvents the two carbon dioxide losing steps in the tricarboxylic acid cycle and permit the net incorporation of carbon into cellular structures during growth on C2 compounds such as acetate or ethanol.

The facultative anaerobic bacterium Escherichia coli and the strict aerobic bacterium Acinetobacter calcoaceticus are able to adapt to growth on acetate, or similar compounds, in strikingly different manners [1, 6, 7]. E. coli responds to the presence of acetate by inducing transcription of the ace operon genes and by phosphorylating and inactivating the TCA cycle enzyme, isocitrate dehydrogenase (IDH) [1]. This inactivation of IDH directs isocitrate flux into the glyoxylate bypass, avoiding the two carbon dioxide losing steps of the TCA cycle. The situation in A. calcoaceticus is more complex [6, 7], because A. calcoaceticus contains two isozymes of IDH [7]. One IDH isozyme decreases in activity during growth on acetate, while the second isozyme, and ICL, increases in activity [6]. It is not known whether IDH activity in A. calcoaceticus is controlled by a post-translational modification event as it is in E. coli.

Isocitrate lyase enzymes from both E. coli and A. calcoaceticus have been shown to be phosphorylated in vivo, and these enzyme phosphorylations are believed to be required for enzymatic activity in each case [2, 3]. These studies were performed with bacterial cultures that had reached the late-exponential growth phase. Since the metabolic capacity of microorganisms in liquid shake culture substantially varies with growth phase, it was deemed important to investigate and degree of ICL phosphorylation as a function of the age of the culture, and the results of these studies are presented in this report.

Materials and Methods

Materials. The $^{32}$P monosodium phosphate (900–1000 mCi/mm) was purchased from DuPont NEN Research Products (Boston, Massachusetts). Immunoelectrophoresis agarose and nitrocellulose sheets were purchased from BioRad (Richmond, California). Goat anti-rabbit alkaline phosphatase conjugate, naphthol AS-biphosphate, and fast red violet were purchased from Sigma Chemical Co. (St. Louis, Missouri). All other chemicals were of analytical reagent grade or higher. Distilled, deionized water was used to prepare all aqueous solutions.

Bacterial strains and growth conditions. Starter cultures of Acinetobacter calcoaceticus B4 and Escherichia coli D3H6G7 were grown in low-phosphate medium to the stationary phase in the presence of acetate as previously described [2, 4]. At this time, fresh low-phosphate medium cultures were inoculated, to an absorbance of 0.2 AU (650 nm), with the respective starter cultures.

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Fig. 1. Isocitrate lyase from A. calcoaceticus. Figure 1A is the autoradiogram of Western immunoblots of cell extracts from (1) early exponential, (2) mid-exponential, and (3) stationary phases of growth. Each lane contained 120 μg protein. Figure 1B is the rocket immunoelectrophoresis gel of cell extracts from (1) early exponential, (2) mid-exponential, and (3) stationary phases of growth. Each lane contained 40 μg of protein. Arrow indicates the position of ICL.

Sterile sodium acetate trihydrate (3.7 M) was added to each culture to a final concentration of 25 mM. At this time, 0.5 mCi [32P]monosodium phosphate was added to each culture. Cultures were grown in a gyrorotatory shaker (275 rpm) at 30°C for A. calcoaceticus and at 37°C for E. coli. At various times, 12 to 20-ml aliquots were aseptically removed from each culture. These aliquots were centrifuged at 1000 g for 10 min and the cell pellets stored at -20°C overnight.

Preparation and analysis of cell extracts. Frozen cell pellets were resuspended in 0.8 ml of sonication buffer (50 mM MOPS, pH 7.5 containing 10% [v/v] glycerol, 1 mM Na₂EDTA, and 1 mM benzamidine hydrochloride). The resuspended pellets were disrupted using a Mini-Bead Beater (BioSpec Products, Bartlesville, Oklahoma) for 8 min at 4°C. Cell debris was removed by centrifugation at 11,000 g for 15 min. The resultant cell extracts were subjected to SDS polyacrylamide gel electrophoresis and Western transfer and to rocket immunoelectrophoresis. SDS-PAGE was performed in a 1.5-mm-thick vertical slab mini gel utilizing a 4% stacking gel and a 10% resolving gel according to Laemmli [5]. After electrophoresis at 150 V, the gel was transferred to a nitrocellulose membrane according to Towbin [8]. Western transfers were immunochemically developed with the appropriate anti-ICL antiserum, produced in New Zealand white rabbits [2, 3], and goat anti-rabbit alkaline phosphatase conjugate as previously described [2-4] prior to autoradiography at -20°C [2, 3]. Rocket immunoelectrophoresis was performed at 100 V for 16 h at 4°C according to Zarembinski [9]. Immunoprecipitating lines were visualized by staining dried gels with Coomassie brilliant blue G-250 [9].

Results

Isocitrate lyase from A. calcoaceticus appears to be phosphorylated throughout the growth curve, i.e., early exponential, mid-exponential, and stationary phases (Fig. 1A). The amount of [32P]phosphate incorporated appears to be similar at all three stages of the growth cycle. Rocket immunoelectrophoresis suggests that the amount of ICL protein is constant during growth (Fig. 1B).

In E. coli, ICL appears to be phosphorylated in a similar, if not identical manner to A. calcoaceticus (Fig. 2). The amount of isocitrate lyase protein does not appear to vary with growth time (Fig. 2A), and ICL appears to phosphorylate to a similar degree at the three times examined (Fig. 2B).

Discussion

Even though E. coli and A. calcoaceticus are strikingly different in their metabolism and physiology, both microorganisms are capable of utilizing acetate as the sole carbon source for growth. However, even in this similarity these microorganisms deal with the problem of net carbon incorporation in different manners [1, 6, 7]. In E. coli, IDH activity decreases [1], whereas in A. calcoaceticus the activity of one IDH isozyme decreases and the activity of the other increases [6, 7]. Nevertheless, in both microorgan-