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PHOSPHORYLATION OF ACINETOBACTER ISOCITRATE LYASE

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During growth on succinate, Acinetobacter calcoaceticus contains two forms of the enzyme isocitrate dehydrogenase. Addition of acetate to a lag-phase culture grown on succinate causes a dramatic increase in activity of form II of isocitrate dehydrogenase and in isocitrate lyase. Form II of isocitrate dehydrogenase may be responsible for the partition of isocitrate between the TCA cycle and the glyoxylate by-pass. This report describes the phosphorylation of the enzyme isocitrate lyase from A. calcoaceticus. This phosphorylation may be a regulatory mechanism for the glyoxylate by-pass.

Bacteria, in general, are capable of utilizing a variety of compounds as carbon and energy sources for growth and reproduction. This diversity is accomplished by the use of several different metabolic pathways. Since not all pathways, or all parts of a given pathway, need be present at the same time, some form of regulation is needed by the bacteria. This regulation of metabolic pathways is often accomplished by reversible post-translational modification of selected enzymes. A common method of regulation by post-translational modification is phosphorylation of specific amino acid residues of an enzyme (1,2). Phosphorylation of serine, threonine, tyrosine, histidine and aspartate residues are known (3).

$NADP^+$-dependent isocitrate dehydrogenase (IDH, EC 1.1.1.42) from Escherichia coli was the first known prokaryotic enzyme whose activity was regulated by phosphorylation (4). In response to growth on acetate, or compounds that are

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metabolized to acetate, E. coli phosphorylates and inactivates IDH. This inactivation process enables isocitrate to enter the glyoxylate by-pass, via the enzyme isocitrate lyase (EC 4.1.3.1), thus circumventing the TCA cycle and the steps that are responsible for loss of incorporated carbon as carbon dioxide (5). Concomitant with IDH phosphorylation and inactivation during growth on acetate, the enzymes of the glyoxylate by-pass are induced. Isocitrate lyase (ICL), the first enzyme of the glyoxylate by-pass, has also been shown to be phosphorylated in E. coli (6) and in Saccharomyces cerevisiae (7). In E. coli the phosphorylated form of ICL is believed to be active (8). However, in Saccharomyces the phosphorylated form is inactive (7).

In the strict aerobe Acinetobacter calcoaceticus, the relationship between IDH and ICL is more complex than in E. coli. A. calcoaceticus contains two distinct forms of IDH which differ in molecular weight and kinetic characteristics (9). During growth on succinate both IDH forms are present and active (9). However, when acetate is added to a lag-phase culture, IDH form II as well as ICL activity increase dramatically (9,10). IDH form II is an allosteric enzyme that is believed to regulate isocitrate metabolism between the competing TCA cycle and glyoxylate by-pass during growth on acetate (9).

However, possible regulatory functions of A. calcoaceticus ICL have not yet been examined. In this communication we demonstrate that ICL from A. calcoaceticus is phosphorylated in vivo.

MATERIALS AND METHODS

Materials: [^{32}P]-Sodium monophosphate (900-1100 mCi/mmol) was obtained from DuPont NEN Research Products. Nitrocellulose transfer membrane was obtained from BioRad. Goat anti-rabbit

alkaline phosphatase conjugate, naphthol AS-Bi phosphate, fast red violet, DNase I and RNase A were obtained from Sigma Chemical Co. All other chemicals were of analytical reagent grade. Distilled deionized water was used to prepare all aqueous solutions.

Bacterial Strain and Growth Conditions: Acinetobacter calcoaceticus B4 was a gift from PDJ Weitzman (Cardiff Institute of Higher Education, U.K.). To study in vivo phosphorylation, this organism was grown in 50 ml of low phosphate media (4) containing 0.5% sodium acetate trihydrate. [^{32}P]-sodium phosphate, 1.4 mCi, was added immediately after inoculation. The culture was harvested at late log-phase (14 hr) by centrifugation. Cell pellets were used immediately.

Cell Lysis and Analysis of Cell Extract: Cells were lysed with lysozyme according to Schwinghamer (11) using Triton X-100. After cell lysis, 0.1 ml of DNase I, RNase A (1mg/ml in 10 mM tris hydrochloride, 1 mM EDTA pH 8) and 4.5 M $MgCl_2$ were added to degrade nucleic acids. After 45 min incubation at 25°C, cell debris was removed by centrifugation at 20,000xg for 30 min at 2-6°C. Supernatant volume was about 3 ml. The supernatant was subjected to SDS polyacrylamide gel electrophoresis on 1.5 mm thick, 16 cm long gel (BioRad Protean II) using a 4% stacking gel and a 10% resolving gel according to Laemmli (12). After electrophoresis at 150 V, constant voltage, the gel was transfered onto nitrocellulose according to Towbin (13). After transfer was complete, a portion of the nitrocellulose membrane was developed using antibody, raised against A. calcoaceticus ICL in New Zealand White rabbits, and anti-rabbit alkaline phosphatase conjugate according to Kunce (14). A second portion was stained for total protein using Ponceau S (15). An additional nitrocellulose transfer was treated for 1 hr at 25°C with 6 M urea and 1 M sodium thiocyanate to remove nonspecifically bound material prior to staining with Ponceau S and autoradiography. Developed transfers were subjected to autoradiography using Kodak X-Omat x-ray film at -20°C for 24 hr using intensifying screens.

RESULTS

Analysis of proteins on developed Western transfers (Fig. 1) clearly shows that the enzyme, isocitrate lyase (ICL), is phosphorylated in the bacteria Acinetobacter calcoaceticus. Detection of ICL protein by the general protein stain Ponceau S (Fig. 1A) or by use of anti-ICL antibody (Fig. 1B) indicates that the ICL band, indicated by arrows, aligns with a radioactive band on the corresponding autoradiogram (Fig. 1C).

Treatment of a nitrocellulose Western transfer with dissociating agents, urea and sodium thiocyanate, prior to staining with Ponceau S and autoradiography did not remove

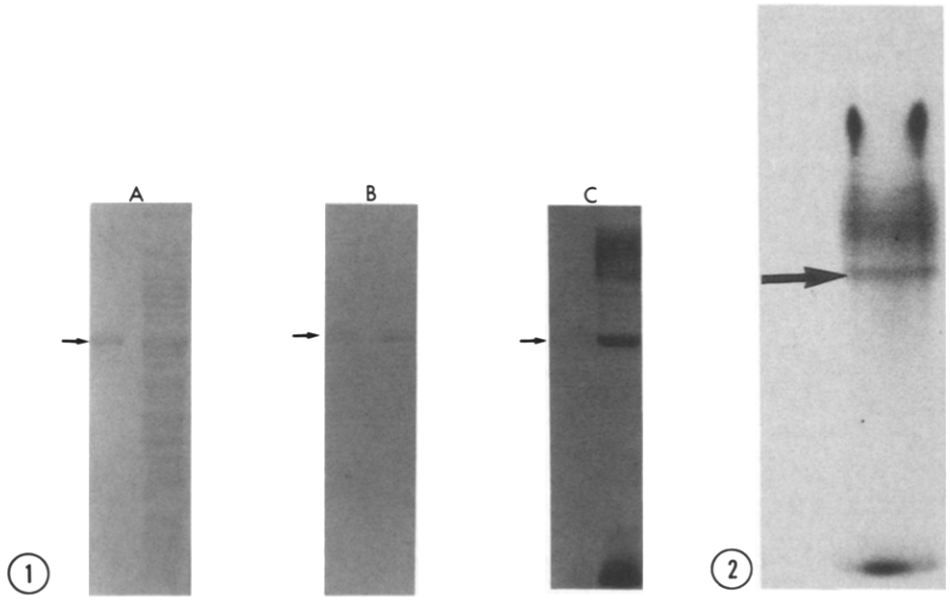

Figure 1. Western transfer of ^{32}P-labeled Acinetobacter cell extract. Figure 1A is stained for total protein using Ponceau S. Figure 1B is developed using anti-ICL antibody. Figure 1C is the autoradiogram corresponding to Fig 1A and 1B. Left hand lane in each pannel is purified Acinetobacter ICL and the right hand lane is cell extract. The arrow marks the location of ICL.

Figure 2. Autoradiogram of Western transfer treated with 6 M urea and 1 M sodium thiocyanate prior to staining with Ponceau S and autoradiography. Arrow marks the location of ICL.

^{32}P-labeled material from the ICL protein band (Fig. 2). This suggests that ^{32}P-labeled material is covalently associated with ICL protein.

DISCUSSION

In E. coli, the activity of the enzymes isocitrate dehydrogenase and isocitrate lyase are controlled by phosphorylation. These phosphorylation events are believed to partition isocitrate between the TCA cycle and the glyoxylate by-pass as required. During growth on acetate, IDH is inactivated by phosphorylation (4) and ICL is activated by phosphorylation (6). This allows isocitrate to enter the glyoxylate by-pass and circumvent the carbon dioxide loosing steps of the TCA cycle.

The relationship between IDH and ICL in A. calcoaceticus during growth on acetate is quite different than the situation in E. coli. Activity of IDH-form II and ICL are high during growth on acetate while activity of IDH-form I decreases (9,10). There have been no reports that have examined the possibility that ICL may function in the regulation of isocitrate flow into the glyoxylate by-pass. The demonstration that ICL from A. calcoaceticus is indeed phosphorylated in vivo suggests such a function for this enzyme.

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