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# Effect of Succinate on Isocitrate Lyase Synthesis in *Mima polymorpha*

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It seems well established that in *Escherichia coli*, at least, isocitrate lyase (*threo*-Ds-isocitrate glyoxylate-lyase, EC 4.1.3.1) synthesis is under the control of intracellular levels of phosphoenol pyruvate (PEP). With a variety of metabolic mutants, H. L. Kornberg (Biochem. J. **99**:1, 1966) has presented evidence which suggests that this compound not only controls the synthesis of the enzyme ("coarse" control) but also its activity ("fine" control). Intracellular levels of PEP during growth on precursors such as C-6 and C-4 substrates appear sufficient to repress the synthesis of the enzyme. However, during growth on C-2 substrates, a derepression of synthesis apparently occurs and the enzyme is "induced." This may result from decreased PEP levels occasioned by insufficient production or by the demands for saccharide and other biosyntheses. The addition of C-4 intermediates (such as succinate) to cells growing in the presence of C-2 compounds (such as acetate) causes, in most systems, the immediate repression of enzyme synthesis (H. L. Kornberg and S. R. Elsden, Advan. Enzymol. **23**:401, 1961). R. F. Rosenberger (Biochim. Biophys. Acta **64**:168, 1962), however, reported that enzyme synthesis in a species of *Achromobacter* is not repressed by the presence of succinate in an acetate growth medium and that acetate will induce in a succinate growth medium. He suggested that an altered metabolism of succinate might be responsible, and this may well be true. However, our similar results with *Mima polymorpha* ATCC 9957 suggest that, possibly, this "altered metabolism" may not be unique in this related group of bacteria, or that in certain organisms PEP might not represent the key coarse control mechanism. An "induction," to a slight extent, of isocitrate lyase by succinate has also been indicated by the data of B. A. McFadden and W. V. Howes (J. Bacteriol. **84**:72, 1962).

Experimental details concerning culture maintenance, basal salts growth medium, incubation, preparation of extracts, enzyme assay, and protein determination have been published (E. J. Bell and A. Marus, J. Bacteriol. **91**:2223, 1966). Acetate and succinate were added to a concentration of 0.03 M each. Specific activity is expressed as micromoles of glyoxylate formed per milligram of protein per hour.

Time study experiments were designed to compare the induction rate of cells grown in the presence, as sole carbon source, of acetate or succinate, or in the presence of both. The washed inocula were prepared from cells grown for 24 hr in a noninducing Trypticase Soy Agar medium. The results (Fig. 1) show a normal induction

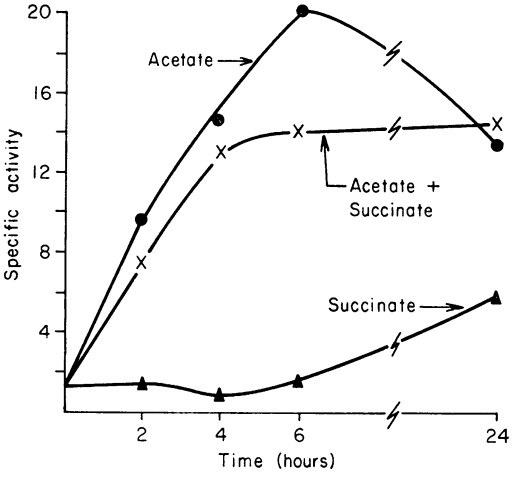


FIG. 1. *Isocitrate lyase formation in basal salts medium containing either 0.03 M acetate, 0.03 M succinate, or 0.03 M acetate plus 0.03 M succinate.*

pattern for acetate and, similar to Rosenberger's observations, good induction although to a lesser degree when succinate is included as a second substrate. More surprising is the fact that, after 24 hr of incubation, succinate itself allowed an increased level of enzyme activity. Growth was essentially equivalent in all three media (Table 1), although there was a slight lag in the acetate-containing media, presumably the time necessary for the induction of sufficient levels of

TABLE 1. *Growth (expressed as optical density) of Mima polymorpha in the presence of various carbon sources*

| Carbon source | Time (hr) | | | | |
|---|---|---|---|---|---|
| | 0 | 2 | 4 | 6 | 24 |
| Acetate..... | 0.12 | 0.15 | 0.20 | 0.29 | 0.45 |
| Acetate plus succinate..... | 0.11 | 0.11 | 0.17 | 0.22 | 0.50 |
| Succinate..... | 0.06 | 0.12 | 0.22 | 0.26 | 0.58 |

one or both of the bypass enzymes. No lag in growth initiation was observed in the succinate medium.

A second set of experiments was designed to show the effect of adding either acetate or succinate to cells after 4 hr of "induction" in the presence of the opposite substrate. To obviate a dilution effect, sterile saline was added to the control flask. The results (Fig. 2) indicate that succinate, when added to cells synthesizing the enzyme in the presence of acetate, allows induction to continue for at least 2 hr, although at a slightly decreased rate. Furthermore, acetate added to cells in the succinate medium immediately stimulates the rate of enzyme formation a great deal. The results obtained upon the addition of acetate to cells in a succinate-containing medium are strikingly similar to those of Rosenberger.

The results clearly show then that succinate, in the presence of acetate, will allow significant induction of isocitrate lyase and, as sole carbon source, will induce synthesis to some extent after

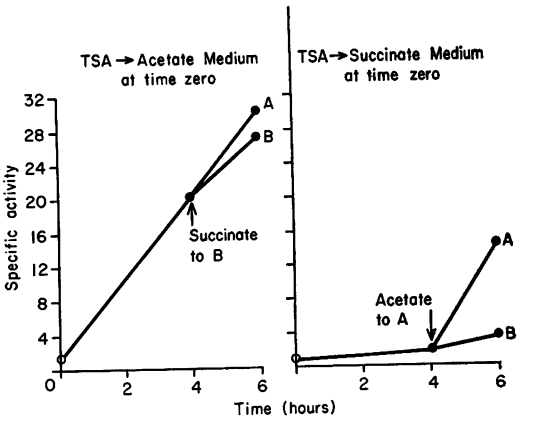


FIG. 2. *Effect of a supplemental substrate, added after 4 hr of induction, on the subsequent rate of synthesis of isocitrate lyase. All concentrations are 0.03 M. TSA = Trypticase Soy Agar.*

a lag period. The reasons for this are not clear, but, until evidence for a defective metabolism of succinate is forthcoming, it might be well to look for a coarse control mechanism other than, or in addition to, PEP levels in these organisms. Evidence has been presented (L. A. Jacobson, R. C. Bartholomaus, and I. C. Gunsalus, Biochem. Biophys. Res. Commun. 24:955, 1966) that acetate itself exerts (in *Pseudomonas putida*) a "coarse" control via the repression of synthesis of the malic enzyme.

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