Inhibition of Isocitrate Lyase: the Basis for Inhibition of Growth of Two *Arthrobacter* Species by Pyruvate

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Growth of *Arthrobacter atrocyaneus* and *A. pyridinolis* on certain growth substrates was found to be inhibited by pyruvate and compounds which can be converted to pyruvate. Growth of *A. atrocyaneus* on acetate, for example, was completely inhibited by 5 mM pyruvate; growth of this organism on glucose was less sensitive and growth on succinate was insensitive to inhibition by pyruvate. Growth of a third *Arthrobacter* species, *A. crystallopoietes*, on acetate and other substrates was not inhibited by pyruvate. The site of pyruvate inhibition was shown to be the isocitrate lyase reaction. Glyoxylate, which affords a bypass of this reaction, restored the ability of *A. atrocyaneus* to evolve $^{14}CO_2$ from acetate in the presence of pyruvate. The isocitrate lyases from *A. atrocyaneus* and *A. pyridinolis* were competitively inhibited by concentrations of pyruvate as low as 1 mM, whereas the enzyme from *A. crystallopoietes* was unaffected by this concentration of pyruvate. Comparable levels of phosphoenolpyruvate did not inhibit the isocitrate lyases from any of the species. A mutant strain of *A. atrocyaneus*, PW11, which is deficient in isocitrate lyase activity, grew on glucose at a reduced rate that was comparable to the rate of growth of the wild-type strain on glucose plus lactate. Addition of lactate to PW11 did not further reduce its rate of growth on glucose. Thus, the glyoxylate pathway appears to be used as an anaplerotic pathway during growth of *A. atrocyaneus* on glucose. Two other considerations suggest that *A. atrocyaneus* and *A. pyridinolis*, but not *A. crystallopoietes*, may be deficient in the ability to convert pyruvate to 4-carbon acids. First, the former two species accumulate intracellular pyruvate from exogenous L-alanine to a much greater extent than does *A. crystallopoietes*. Moreover, *A. atrocyaneus* and *A. pyridinolis* are incapable of growth on lactate as sole source of carbon whereas *A. crystallopoietes* can grow on lactate.

The metabolism of three *Arthrobacter* species is presently being studied in our laboratory. Two of these species, *A. atrocyaneus* and *A. pyridinolis*, are unable to grow on sodium lactate as sole source of carbon. The third species, *A. crystallopoietes*, can use this substrate for growth. During the course of experiments with multiple carbon sources, it was observed that inclusion of lactate in media containing other carbon sources caused a reduction in the cell yield of *A. atrocyaneus* or *A. pyridinolis*. No such effect was observed with *A. crystallopoietes*. Inhibition by lactate suggested the possible existence of some regulatory control exerted by lactate itself, or by pyruvate, reduced pyridine nucleotides, or some other product of lactate metabolism. The inhibition has therefore been examined. We now report evidence that pyruvate is the actual inhibitor and that the site of inhibition is the isocitrate lyase (EC 4.1.3.1) of the two sensitive species.

MATERIALS AND METHODS

Organisms and growth conditions. *A. atrocyaneus* (ATCC 13752), *A. crystallopoietes* (ATCC 15481), and *A. pyridinolis* (obtained from J. C. Ensign, University of Wisconsin) were used for all studies. *A. atrocyaneus* and *A. crystallopoietes* were grown in the mineral salts (MS) described previously (5). Carbon sources were added separately from sterile solutions. Unless indicated, all carbon sources were employed at 0.05 M. *A. pyridinolis* was grown in

the same medium with the trace salts of Hegeman (1) substituted for the normal trace salts. Cultures were grown, with shaking at 200 rev/min in a New Brunswick G25 shaker, at 30 C. Growth experiments were conducted as previously described (4) in 300-ml side-arm flasks (Bellco Glass, Inc.). Cell densities were determined by use of a Klett-Summerson colorimeter with a no. 42 filter blanked against a side-arm flask containing uninoculated medium. Under these conditions, 50 Klett units are equivalent to a cell concentration of 2.5×10^8 cells/ml.

Chemicals. The lactate employed was the sodium salt of DL-lactic acid. The other organic acids used were also neutralized with sodium hydroxide. Lysozyme, sodium glyoxylate, phosphoenolpyruvate (PEP), oxalacetic acid, and DL-isocitrate (trisodium salt) were obtained from Sigma Chemical Co. Chloramphenicol and penicillin G were purchased from Calbiochem, and ethylmethane sulfonate, from Eastman Chemicals. Uniformly labeled ^{14}C-sodium acetate and ^{14}C-glucose were purchased from Amersham-Searle. Pyruvate-*1*-^{14}C was obtained from New England Nuclear Corp. All other chemicals were obtained from commercial sources at the highest purity available.

Determination of $^{14}CO_2$ evolution during growth on labeled substrates. Cells were grown in a culture tube containing 5 ml of MS plus 0.05 M uniformly labeled ^{14}C-acetate (10 μCi/mmole) and the additions indicated. The culture was aerated through a sintered-glass sparger. The CO_2 evolved during respiration was collected at intervals, and the amount of $^{14}CO_2$ evolved was determined by scintillation counting as previously described (4).

Enzyme assays and chemical determinations. For preparation of cell extracts, cells were harvested by centrifugation at $15,000 \times g$ for 10 min. The cells were washed by resuspending the pellets in 25 ml of the buffer to be used in the assay, followed by a second centrifugation. The cells were resuspended in 4 ml of the same buffer and broken by sonic disruption for 2 min in a Heat Systems-Ultrasonics Inc. model W185D Sonifier. During sonic treatment, the cells were kept below 10 C. Finally, unbroken cells and large debris were removed from the extracts by centrifugation at $16,000 \times g$ for 20 min.

Isocitrate lyase was assayed by the method of McFadden (7) with the use of extracts prepared from cells growing logarithmically on acetate. When additional compounds were included in the assay for determination of their inhibitory activity, separate blanks and standards were prepared. Enzyme units are given as micromoles of glyoxylate formed per minute. Specific activities are expressed as units per milligram of extract protein. Proteins were determined by the method of Lowry et al. (6), with lysozyme as a standard. Pyruvate was assayed enzymatically in extracts deproteinized with trichloroacetic acid by use of lactic dehydrogenase and reduced nicotinamide adenine dinucleotide (8).

Uptake experiments. Exponentially growing cells were washed with and resuspended in MS. The radioactive compound (0.05 M; 0.06 μCi/ml) was added, and 1-ml samples were filtered, at intervals, through 0.45 μm HA filters (Millipore Corp.). The filters were

washed with 10 ml of MS containing acetate, pyruvate, or glucose (for acetate, pyruvate, and glucose uptake experiments, respectively). The filters were dried and the radioactivity was measured by scintillation counting. Uptake is expressed as counts per minute per milligram of cell protein.

Mutant isolation and characterization. For production of mutants unable to grow in acetate, 10 ml of logarithmically growing cells of *A. atrocyaneus* were harvested by centrifugation at $15,000 \times g$ for 10 min and were resuspended in 10 ml of MS. Ethylmethane sulfonate (0.15 ml) was added. The cells were incubated for 90 min, with shaking, at 30 C. The cells were then washed with MS and incubated overnight in MS plus 0.05 M acetate and 80 μg of penicillin G per ml. After being washed with MS, the cells were inoculated on plates of MS plus succinate. The colonies formed were replicated onto plates of MS plus acetate. A series of mutant strains was isolated which fail to grow on acetate as sole source of carbon, but grow normally on Krebs cycle intermediates. One such strain, PW11, was used in one of the experiments. This strain evolves less than 5% of the amount of $^{14}CO_2$ evolved by the wild type during a 10-hr period of incubation in ^{14}C-acetate, as described above. Addition of glyoxylate (0.025 M) to the culture tube containing PW11 results in $^{14}CO_2$ evolution from labeled acetate reaching greater than 40% of that evolved by the wild-type strain under the same conditions. PW11 is presumed to be a mutant which is deficient in isocitrate lyase activity. Direct determination of the deficiency by enzyme assay is difficult to achieve because the mutant cannot be grown on acetate alone and the inclusion of other carbon sources strongly represses isocitrate lyase formation.

RESULTS

Sodium lactate, at concentrations as low as 0.005 M, inhibited the growth of *A. atrocyaneus* on glucose, glycerol, and acetate (Fig. 1). The inhibition of growth on acetate was virtually complete. Growth on glucose and glycerol was less severely affected. Growth of *A. atrocyaneus* on succinate was not inhibited by lactate even at concentrations of 0.05 M. When cells were grown to the exponential phase on acetate before lactate was added, the addition of 0.05 M lactate resulted in the immediate cessation of growth (Fig. 2). Addition of 0.005 M lactate caused cessation of growth approximately 2 hr after the lactate was added. Growth of *A. pyridinolis* on acetate was also sensitive to inhibition by lactate (Fig. 3). *A. pyridinolis* grew on acetate as sole carbon source after a long lag period. Growth was almost completely inhibited by 0.05 M lactate; 0.005 M lactate inhibited only slightly. Addition of 0.05 M lactate to cells of *A. pyridinolis* growing exponentially on acetate caused a rapid cessation of growth similar to that seen with *A. atrocyaneus*. In contrast, growth of *A.*
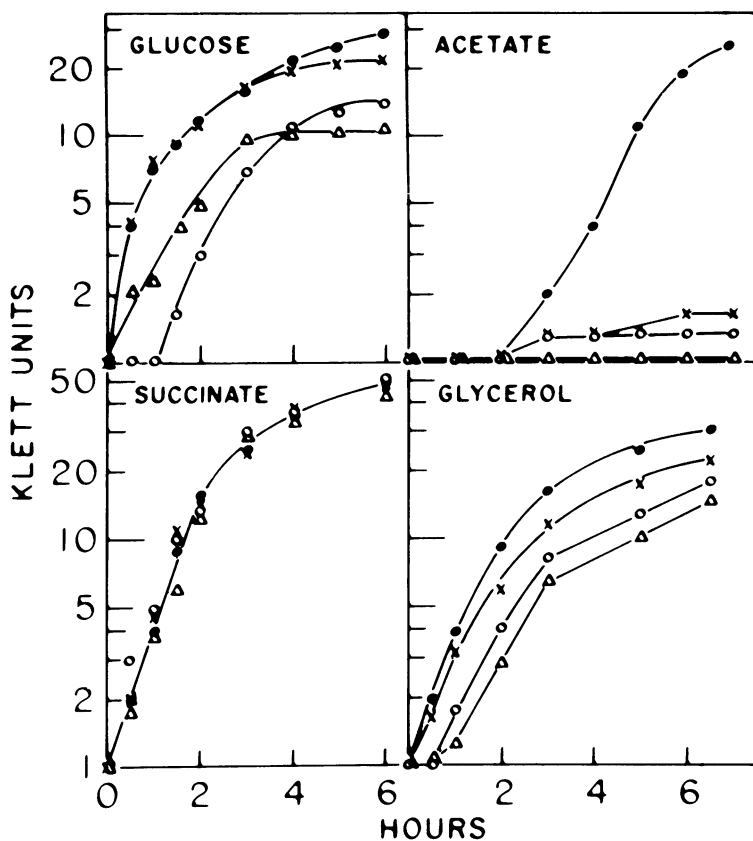
FIG. 1. *Inhibition of the growth of Arthrobacter atrocyaneus on various substrates by lactate. Cells were grown on the indicated carbon sources (0.05 M) plus:* ●, *no addition;* ×, *0.005 M lactate;* O, *0.01 M lactate; and* △, *0.05 M lactate.*

crystallopoietes on acetate was not inhibited by lactate added either at the time of inoculation (Fig. 4) or after exponential growth on acetate had commenced.

The inhibition caused by lactate might occur via the production of pyruvate or some product thereof, or operate via the formation of reduced pyridine nucleotide concomitant with lactate oxidation. Pyruvate and compounds which can be metabolized to produce pyruvate, i.e., glycine, L-serine, and L-alanine, caused inhibition of the growth of A. atrocyaneus and A. pyridinolis on acetate. All of these compounds also failed to support growth of these two species when present as sole carbon source. The effect of glycine on growth of A. atrocyaneus in media containing acetate is shown in Fig. 5. Malate, sorbitol, and glycerol, compounds which would be expected to lead to the formation of reduced pyridine nucleotides, all supported growth of A. atrocyaneus; these compounds did not produce the inhibition of growth on acetate observed with

lactate, glycine, etc., when added at the time of inoculation. The effects of glycerol and sorbitol, and those of compounds related to pyruvate, were further examined with the use of cells of A. atrocyaneus growing exponentially on acetate. As shown in Fig. 6, alanine, serine, pyruvate, and lactate (at 0.05 M) all caused rapid cessation of growth. Glycine also inhibited growth, but less rapidly. Glycerol did not alter the growth pattern at all, but sorbitol caused complete cessation of growth 2 hr after it was added.

The possibility that inhibition of growth is caused by inhibition of the transport of the growth substrate was investigated. As shown in Fig. 7, succinate-grown cells did not take up radioactive acetate, whereas acetate-grown cells exhibited appreciable uptake. Addition of either 0.05 M lactate or pyruvate at the same time as the acetate caused a marked decrease in the amount and rate of acetate uptake. However, acetate was still transported at appreciable rates in such cells. When PW11, a
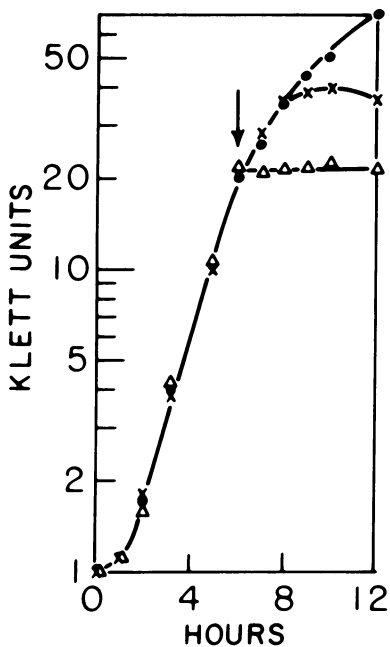
FIG. 2. *Inhibition of A. atrocyaneus growing exponentially on acetate by addition of lactate. Cells were grown in 0.05 M acetate for 6 hr before no additions (●), 0.005 M lactate (×), or 0.05 M lactate (△) was added to the flasks.*

FIG. 3. *Inhibition of the growth of A. pyridinolis on acetate by lactate. A. pyridinolis was grown on 0.05 M acetate (●) plus 0.005 M lactate (×) or 0.05 M lactate (△).*

FIG. 4. *Growth of A. crystallopoietes on acetate in the presence of lactate. A. crystallopoietes was grown on 0.05 M acetate. At the time indicated by the arrow, no additions (●), 0.005 M lactate (×), or 0.05 M lactate (△) was added.*

mutant deficient in isocitrate lyase, was used in the same experiment, essentially the same results were obtained. The effect of various compounds on the uptake of acetate by acetate-grown *A. atrocyaneus* was further examined. Two experimental conditions were employed: (i) washed acetate-grown cells were incubated with chloramphenicol for 30 min before the compound tested and the radioactive acetate were added; and (ii) the cells were incubated for 30 min with the compound tested, followed by the incubation with chloramphenicol and then the determination of uptake. Presumably, the first condition only allows detection of inhibition that is independent of the synthesis of any new proteins, whereas the second condition allows protein synthesis during the exposure to the compound tested for inhibition. As shown in Table 1, sorbitol, lactate, and pyruvate caused 36 to 45% inhibition of acetate uptake when added after chloramphenicol. There was no appreciable increase in the amount of inhibition by these compounds when they were added 30 min before the chloramphenicol. In contrast, alanine, glycine, and serine caused essentially no inhibition of acetate uptake unless added before chloramphenicol. This may reflect a require-
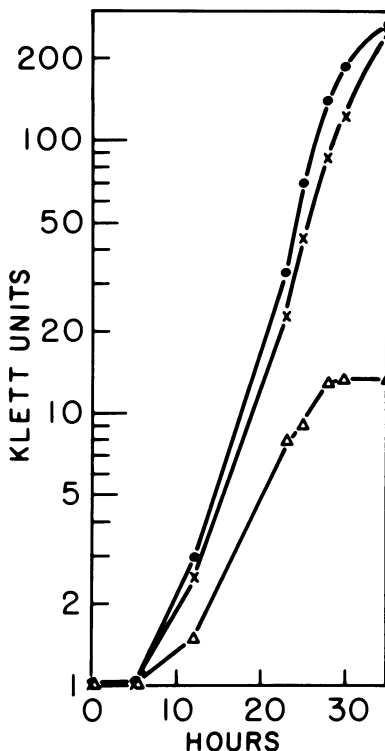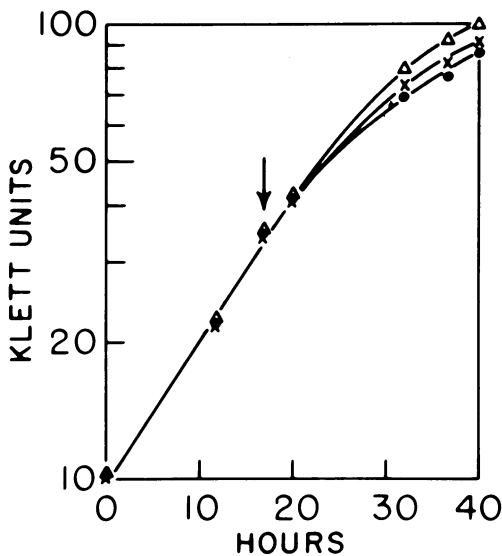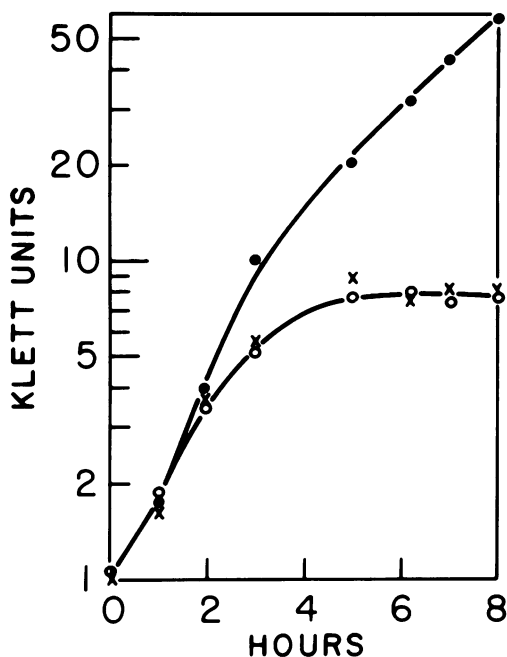
FIG. 5. *Inhibition of the growth of A. atrocyaneus on acetate by glycine. Cells were grown on 0.05 M acetate plus no additions (●), 0.005 M glycine (×), or 0.05 M glycine (○).*
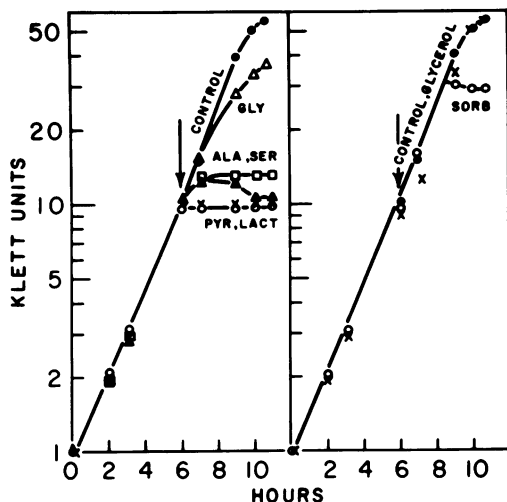

FIG. 6. *Effects of various additions on A. atrocyaneus growing exponentially on acetate. Cells grown on 0.05 M acetate for 6.5 hr. Pyruvate, lactate, serine, alanine, glycine, glycerol, or sorbitol (0.05 M) was added as indicated.*

FIG. 7. *Effects of pyruvate and lactate on acetate uptake by A. atrocyaneus. Uptake of ¹⁴C-acetate by washed succinate-grown cells (×) and by washed acetate-grown cells with no additions (●), 0.05 M pyruvate (△), or 0.05 M lactate (○) was measured. The washed cells were incubated in mineral-salts with chloramphenicol (80 μg/ml) for 30 min. Then acetate (0.05 M; 0.06 μCi/ml) was added together with the added compounds.*

ment for inducible enzyme synthesis for conversion of these compounds to pyruvate. One such enzyme, L-serine dehydratase, is present in very low amounts unless induced by glycine
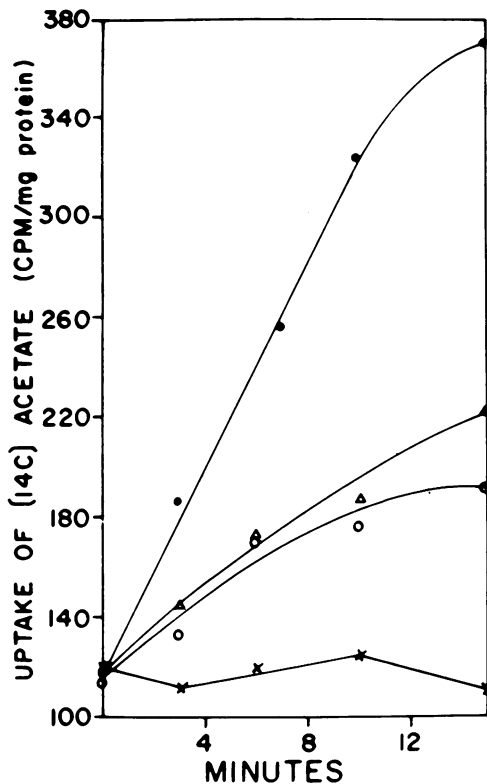
(S. Schechter and T. Krulwich, *unpublished results*). Glycerol caused no inhibition of acetate uptake, whether added before or after the chloramphenicol.

Pyruvate, alanine, and glycine inhibited growth of *A. atrocyaneus* on glucose to the same extent as lactate did (see Fig. 1). In uptake experiments similar to those conducted with acetate, these compounds caused less than 15% inhibition of glucose uptake.

The observations above suggested that pyruvate or some product derived from pyruvate inhibits the growth of *A. atrocyaneus* and *A. pyridinolis* on certain growth substrates. Inhibition of substrate transport does not appear to be the mechanism of growth inhibition, since transport of acetate and glucose is not inhibited to nearly the same extent as growth on these compounds. The extreme sensitivity of

TABLE 1. *Effect of various compounds on* [14]*C-acetate uptake by acetate-grown A. atrocyaneus*[a]

Compound added	Percent inhibition of uptake when added	
	30 min after chloramphenicol	30 min before chloramphenicol
Glycerol	0	4
Sorbitol	36	41
L-Alanine	0	37
Glycine	0	37
L-Serine	6	22
Sodium lactate	42	30
Sodium pyruvate	45	47

[a] Chloramphenicol (80 μg/ml) was added to washed acetate-grown cells. After 30 min, ^{14}C-acetate (0.05 M; 0.06 μCi/ml) was added and uptake was determined. The compounds indicated were added at the same time as ^{14}C-acetate (30 min after chloramphenicol) or 30 min before chloramphenicol. Percent inhibition is calculated from uptake by control and treated cells over a 10-min period.

growth on acetate to inhibition by pyruvate, and the insensitivity of cells growing on succinate, suggested that the glyoxylate pathway could be the site of inhibition. Cells of A. atrocyaneus, A. pyridinolis, and A. crystallopoietes were grown in acetate as sole carbon source to induce fully the enzymes of the glyoxylate pathway. The kinetics of the isocitrate lyase reaction in extracts of these cells, and the effects of various compounds on the enzyme activity, were then studied. As shown in Fig. 8, the isocitrate lyases from A. atrocyaneus and A. pyridinolis were inhibited competitively by pyruvate (at 1 mM). The enzyme from A. crystallopoietes, on the other hand, was unaffected by pyruvate at this concentration. The effects of PEP and oxalacetate on the isocitrate lyase activities were also determined. PEP had no inhibitory effect at 1 mM concentration, and had only a slight inhibitory effect at 10 mM. Oxalacetate, at 1 mM, had an inhibitory effect which appeared to be noncompetitive in nature.

The inhibition by pyruvate of the isocitrate lyases from the two pyruvate-sensitive species but not of that from the pyruvate-insensitive species was consistent with the suggestion that isocitrate lyase is the site of inhibition. To prove that this is in fact so, cells of A. atrocyaneus were grown to the mid-exponential phase of growth on acetate. Volumes of 5 ml of the culture were incubated in each of four culture tubes containing ^{14}C-acetate plus (i) no other additions, (ii) 0.025 M glyoxylate, (iii)
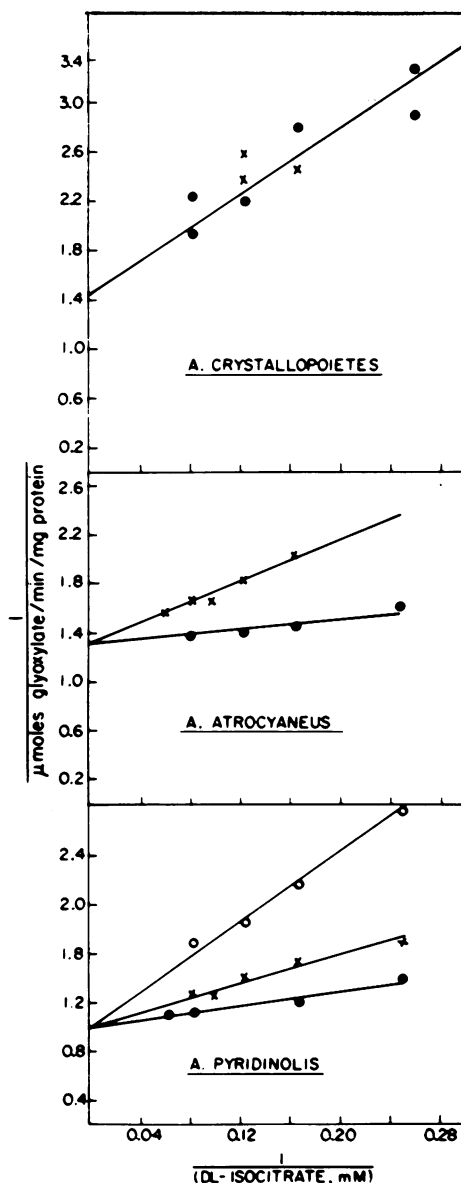

FIG. 8. *Lineweaver-Burk plots of the isocitrate lyase activities from A. crystallopoietes, A. atrocyaneus, and A. pyridinolis showing the effect of pyruvate. Initial enzyme reaction velocities were determined at various concentrations of D L-isocitrate in the absence (●) and presence of 1 mM pyruvate (×) or 4 mM pyruvate (○).*

0.05 M pyruvate, and (iv) 0.05 M pyruvate and 0.025 M glyoxylate. The [14]CO_2 evolved from the radioactive substrate was determined at intervals. As shown in Fig. 9, the addition of glyoxylate caused no change in the initial rate of [14]CO_2 evolution from acetate and allowed
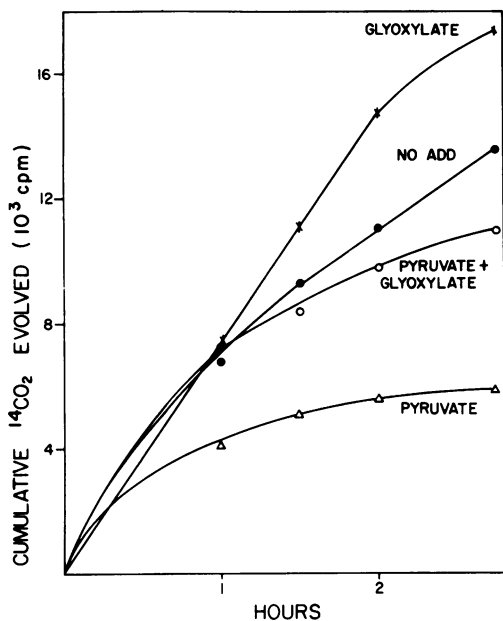
FIG. 9. *Effects of pyruvate and glyoxylate on $^{14}CO_2$ evolution from labeled acetate by A. atrocyaneus. Cells growing exponentially on acetate were transferred to culture tubes containing ^{14}C-acetate (0.05 M, 0.5 μCi/ml) plus: no additions (●), 0.025 M glyoxylate (×), 0.05 M pyruvate (△), or 0.05 M pyruvate and 0.025 M glyoxylate (○). $^{14}CO_2$ was collected at intervals and counted by scintillation counting.*

maintenance of this rate of evolution for some time after the control culture began to decrease in its rate of $^{14}CO_2$ evolution. Addition of pyruvate caused a rapid inhibition of $^{14}CO_2$ evolution from labeled acetate. In the presence of glyoxylate plus pyruvate, the initial rate of $^{14}CO_2$ evolution was restored to the control rate, after which it began to decrease somewhat. Thus, the inhibitory effect of pyruvate in vivo appeared to be overcome by glyoxylate, which can provide a bypass of the isocitrate lyase reaction. The failure of glyoxylate to restore $^{14}CO_2$ evolution to the control rate for more than 1 to 2 hr probably represents depletion of glyoxylate; it was desirable to use relatively low glyoxylate concentrations to avoid effects on transport. The possibility that in this experiment glyoxylate interfered with pyruvate transport was eliminated. Cells growing exponentially on acetate were allowed to take up ^{14}C-pyruvate (0.05 M, 0.06 μCi/ml) in the presence and absence of 0.025 M glyoxylate. No difference in the rate of uptake was observed under the two conditions.

The site of inhibition by pyruvate of growth of the sensitive species on acetate appeared to

be the isocitrate lyase reaction. It was of interest to determine whether an effect on this enzyme is also responsible for the inhibition of growth on other substrates, e.g., glucose. If so, it was predicted that a mutant which lacks a functional glyoxylate pathway would grow on glucose alone at a rate similar to that of the wild-type strain in the presence of lactate or pyruvate. Moreover, growth of such a mutant on glucose should not be further inhibited by addition of lactate. PW11 is a strain of *A. atrocyaneus* which cannot grow on acetate as sole carbon source as a result of a deficiency in isocitrate lyase activity (see Materials and Methods). As shown in Fig. 10, this strain did not grow as well as the wild-type strain on glucose. Growth of PW11 on glucose alone was very similar to growth of the wild-type strain in the presence of 0.05 M lactate. In the presence of both glucose and lactate (both at 0.05 M), PW11 was not further inhibited; rather, there
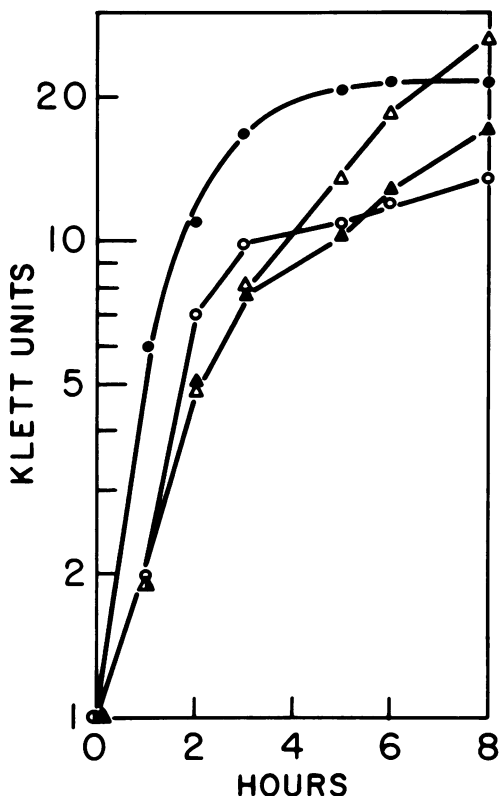

FIG. 10. *Growth of wild-type and mutant PW11 strains of A. atrocyaneus on glucose in the presence and absence of lactate. Wild type strain: ●, growing on 0.05 M glucose; ○, growing on 0.05 M glucose plus 0.05 M lactate. PW11: ▲, growing on 0.05 M glucose; △, growing on 0.05 M glucose plus 0.05 M lactate.*

appeared to be a slight augmentation of the growth of PW11 over its growth on glucose alone.

For the occurrence of the inhibition by pyruvate in vivo, sufficiently high concentrations of intracellular pyruvate must accumulate from exogenous pyruvate or substrates which would be converted to pyruvate. To obtain an estimate of whether the two sensitive species accumulate pyruvate, cells of *A. atrocyaneus* and *A. pyridinolis*, and also of *A. crystallopoietes*, were grown to mid-exponential phase on acetate. L-Alanine was added to a final concentration of 0.05 M. L-Alanine is transported by all three species without an appreciable lag. After 2 more hr of incubation, the cells were harvested, washed, and subjected to sonic disruption. A sample of each extract was used to determine the milligrams of protein per milliliter. The remainder of the extracts was deproteinized with trichloroacetic acid and used for spectrophotometric determination of pyruvate concentration. By this method, the amount of pyruvate per milligram of extract protein was found to be 0.11 μmole for *A. crystallopoietes*, 0.36 μmole for *A. pyridinolis*, and 3.4 μmoles for *A. atrocyaneus*.

DISCUSSION

Severe inhibition of the growth of *A. atrocyaneus* and *A. pyridinolis* on acetate is caused by pyruvate and compounds which can be converted to pyruvate. Even the inhibition of *A. atrocyaneus* by sorbitol could operate via conversion of the compound to pyruvate, since this species may possess an Entner-Doudoroff pathway (9). The inhibitory effect of pyruvate is probably not due entirely to inhibition of acetate or other substrate transport. Addition of pyruvate completely inhibits growth on acetate, whereas acetate uptake is inhibited only 22 to 50%. Moreover, growth on glucose is inhibited to a significant extent by the same compounds, but uptake of glucose is inhibited less than 15% by pyruvate, lactate, glycine, or alanine. It should be pointed out that the values given here for percent inhibition of uptake are maximum estimates, since these values may also reflect inhibition of acetate incorporation into cellular material. Acetate is metabolizable, and measurements of its uptake, even over short time periods, may be influenced by incorporation. However, uptake of acetate by PW11, a strain with reduced ability to incorporate acetate, was inhibited by pyruvate to the same extent as was acetate by the wild-type strain. In any event, if part of the inhibition of uptake is actually an inhibition of

incorporation, it only strengthens the conclusion that inhibition of growth by pyruvate is not primarily caused by an effect on acetate transport.

The site of pyruvate inhibition is apparently the isocitrate lyase reaction. The restoration by glyoxylate of $^{14}CO_2$ evolution from acetate in the presence of pyruvate is strong positive evidence for this site of inhibition. The in vitro studies of isocitrate lyase are also consistent with this conclusion. Since these studies were conducted on crude extracts, they are cited only as evidence for the possible site of pyruvate inhibition. The enzyme from the two sensitive species is competitively inhibited by pyruvate, whereas that from *A. crystallopoietes* is not inhibited by pyruvate. Pyruvate has also been found to inhibit the isocitrate lyases from *Chlorella pyrenoidosa* (2) and from *Escherichia coli* (3). The enzyme from *E. coli*, however, was more strongly inhibited by PEP than by pyruvate. The failure of PEP to inhibit the enzymes from *A. atrocyaneus* and *A. pyridinolis* at concentrations comparable to strongly inhibitory levels of pyruvate supports the conclusion that pyruvate itself is the inhibitory metabolite. Moreover, the competitive nature of the inhibition by pyruvate is consistent with our repeated failure to isolate pyruvate- and lactate-resistant mutants of *A. atrocyaneus* or *A. pyridinolis*, i.e., mutants that grow on acetate in the presence of lactate and pyruvate. Such a mutation would presumably have to involve a change in the active site of the isocitrate lyase and would therefore be likely to cause a defect in the enzyme.

The extreme sensitivity to pyruvate of cells of *A. atrocyaneus* growing on acetate and the insensitivity of cells growing on succinate is understandable in terms of the inhibition of the glyoxylate pathway. It further appears that this organism uses the glyoxylate pathway as an anaplerotic route during growth on glucose. The inhibition of growth on glucose by pyruvate and the growth properties of mutant strain PW11 indicate that growth of *A. atrocyaneus* on glucose is impaired in the absence of isocitrate lyase activity. The use of the glyoxylate pathway during growth of *A. atrocyaneus* on glucose is one of several observations suggesting that this species and *A. pyridinolis* are naturally "deficient" in their ability to convert pyruvate to 4-carbon acids as compared to other organisms, including *A. crystallopoietes*. Also supporting this suggestion is the intracellular accumulation of pyruvate from exogenous pyruvate and compounds which are converted to pyruvate in *A. atrocyaneus* and *A. pyridi-*

nolis to a much greater extent than in *A. crystallopoietes*. This accumulation may indicate an inability of the former two species to dissimilate large amounts of pyruvate effectively through routes other than pyruvate dehydrogenase. The accumulation of pyruvate is most pronounced in *A. atrocyaneus*, which is also more sensitive than *A. pyridinolis* to inhibition by exogenous lactate, etc. A limited capacity to convert pyruvate into 4-carbon acids (either directly or via PEP) would also explain the inability of *A. atrocyaneus* and *A. pyridinolis* to grow on lactate. *A. crystallopoietes*, on the other hand, which seems to have no such limitation, can grow on lactate. It is of interest in connection with these suggestions that mutants of *E. coli* which lack PEP synthase or PEP carboxylase, or both, whereby pyruvate is converted to 4-carbon acids, are sensitive to inhibition by pyruvate during growth on acetate (3). We do not believe that *A. atrocyaneus* or *A. pyridinolis* will prove to be completely devoid of enzymes (such as pyruvate carboxylase or PEP carboxylase and PEP synthase) whose activities result in the conversion of pyruvate to 4-carbon acids. If that were true, growth of obligately aerobic *A. atrocyaneus* on glucose would be expected to be even more sensitive to inhibition by pyruvate, and PW11 would be expected to be negative for growth on glucose as well as on acetate. Moreover, there is an indication that PW11, which lacks an operative glyoxylate pathway, has gained some ability to use lactate (see Fig. 10). This capacity could result from an amplification (by induction, release from repression, or release from inhibition) of the existing routes for conversion of pyruvate to 4-carbon acids. We are now beginning a study of the anaplerotic routes employed by *A. atrocyaneus*, *A. pyridinolis*, and *A. crystallopoietes* with the expectation that this study will clarify the basis for many of the observations reported here.

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