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Involvement of *ack-pta* operon products in α-ketobutyrate metabolism by *Salmonella typhimurium*

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Summary. The herbicide sulfometuron methyl inhibits acetolactate synthase II of *Salmonella typhimurium*, resulting in toxic accumulation of α-ketobutyrate. Four mutants, containing Tn*10* insertions in the acetate kinase (*ack*) or phosphotransacetylase (*pta*) genes, were found among a collection of mutants hypersensitive to sulfometuron methyl. The genetic map location of these four Tn*10* insertions at 46 min was identical to that of *ack* and *pta* point mutants. The insertion and point mutants shared the following phenotypes: resistance to fluoroacetate, sensitivity to alizarin yellow, inability to utilize inositol as a sole carbon source, and hypersensitivity to sulfometuron methyl. Three of the four Tn*10* insertion mutants were deficient in phosphotransacetylase but not in acetate kinase activities, indicating insertion of Tn*10* in the *pta* gene. The fourth mutant contained an insertion in the *ack* gene and was deficient in both acetate kinase and phosphotransacetylase activities. This polarity is consistent with cotranscription of *ack* and *pta*. All *ack* and *pta* mutants tested were defective in α-ketobutyrate turnover. Acetate kinase and phosphotransacetylase are proposed to be part of a pathway for α-ketobutyrate metabolism. Propionyl-CoA, an intermediate of that pathway, and propionate, the product of the pathway, accumulated upon inhibition of acetolactate synthase.

Key words: α-Ketobutyrate – Acetate kinase – Phosphotransacetylase – Acetolactate synthase – Sulfometuron methyl

Introduction

The formation of α-aceto-α-hydroxybutyrate from α-ketobutyrate and pyruvate by the enzyme acetolactate synthase is the second step in isoleucine biosynthesis. Loss of acetolactate synthase isozyme II activity in *Salmonella typhimurium* by mutation (Primerano and Burns 1982) or by inhibition with sulfometuron methyl (Van Dyk and LaRossa 1986) leads to accumulation of α-ketobutyrate. Higher levels of α-ketobutyrate accumulate when the other isozyme, acetolactate synthase I, is simultaneously inhibited by valine (LaRossa et al. 1987). Accumulation of this normal cellular metabolite results in growth inhibition and contributes to the toxicity of sulfometuron methyl towards *S. typhimurium* (LaRossa et al. 1987). Accumulation of α-ketobutyrate is also likely to play a role in the potent herbicidal activity of sulfometuron methyl (LaRossa and Falco 1984) and in an inborn error of human metabolism (Yang and Roth 1985).

Further information about the role of α-ketobutyrate accumulation in sulfometuron methyl toxicity has been obtained through analysis of a collection of Tn*10* insertion mutations that confer a sulfometuron methyl hypersensitive phenotype upon *S. typhimurium* (Van Dyk and LaRossa 1986). These 15 mutants display a range of sensitivities to sulfometuron methyl and α-ketobutyrate and can be divided into several phenotypic classes. For example, seven mutants are deficient in α-ketobutyrate turnover (LaRossa et al. 1987). Two of these have an almost absolute α-ketobutyrate turnover defect. The other five have intermediate turnover rates; α-ketobutyrate is metabolized by these mutants at slower rates than by the parental strain. One mutant in this class contains a Tn*10* insertion in *poxA* (Van Dyk et al., manuscript in preparation), a gene that controls the activity of pyruvate oxidase (Chang and Cronan 1982). In this report, we show that the other four mutants displaying an intermediate rate of α-ketobutyrate turnover contain Tn*10* insertions in *ack* or *pta*. We suggest that the products of these genes, acetate kinase and phosphotransacetylase, are components of a pathway for α-ketobutyrate metabolism.

Materials and methods

Bacterial strains. The *S. typhimurium* strains used in this study are listed in Table 1. Known *ack* and *pta* mutations were transferred by P22-mediated generalized transduction into the *S. typhimurium* LT2 strain previously used in the isolation of sulfometuron methyl hypersensitive mutants. A genetic marker (*hisJ8908*::Tn*10*) near the *ack-pta* region was introduced into strains TA3501 (*ack-1*) and TA3492 (*pta-1*) using P22 phage grown on TA3178 as the donor in transductional crosses. Screening tetracycline-resistant recombinants for fluoroacetate resistance and alizarin yellow sensitivity yielded strains TV074 and TV075. Phage P22 grown on these two transductants were used as donors in crosses with our standard strain LT2, giving strains TV076, TV077, and TV078.

Media and phenotypic tests. Green indicator plates, rich LB medium, and minimal E and M9 media were prepared as described (Davis et al. 1980). Tetracycline at 12.5 μg/ml and EGTA at 10 mM were added to LB plates for transduction experiments.

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Table 1. *Salmonella typhimurium* strains

Strain	Genotype	Source or reference
LT2		Laboratory collection
SMS209	*pta-209*::Tn*10*	Van Dyk and LaRossa (1986), this study
SMS406	*pta-406*::Tn*10*	Van Dyk and LaRossa (1986), this study
SMS408	*ack-408*::Tn*10*	Van Dyk and LaRossa (1986), this study
SMS419	*pta-419*::Tn*10*	Van Dyk and LaRossa (1986), this study
TA3178	*hisJ8908*::Tn*10*, *hisF645*, *dhuAl*	G. Ames
TA3492	*pta-1*	LeVine et al. (1980)
TA3501	*ack-1*	LeVine et al. (1980)
TR5663	*purF145*, *ypsLl*	Davis et al. (1980)
TV074	*hisJ8908*::Tn*10*, *ack-1*	P22(TA3178) × TA3501 → Tet^r[FlAc^r, AY^s]
TV075	*hisJ8908*::Tn*10*, *pta-1*	P22(TA3178) × TA3492 → Tet^r[FlAc^r, AY^s]
TV076	*hisJ8908*::Tn*10*	P22(TV074) × LT2 → Tet^r[FlAc^s, AY^r, Ino$^+$]
TV077	*hisJ8908*::Tn*10*, *ack-1*	P22(TV074) × LT2 → Tet^r[FlAc^r, AY^s, Ino$^-$]
TV078	*hisJ8908*::Tn*10*, *pta-1*	P22(TV075) × LT2 → Tet^r[FlAc^r, AY^s, Ino$^-$]

Tet^r, tetracycline resistance; FlAc, Fluoroacetate; AY, alizarin yellow; Ino, inositol utilization

Inositol utilization was tested on M9 plates containing 0.4% inositol incubated for three days at 37° C. Fluoroacetate resistance was scored on M9 plates containing 0.3% lactate and 10 mM fluoroacetate (Fluka AG) incubated for two days at 37° C. Tests for alizarin yellow sensitivity were done by measuring the diameter of isolated single colonies after overnight growth on LB plates with and without 0.25% alizarin yellow (Aldrich); *ack* and *pta* mutants were reduced in diameter by 20% in the presence of alizarin yellow, while the parental strains were unaffected. Routine screening for the *ack* and *pta* markers was done by comparing growth on green indicator plates, which contain alizarin yellow; on this medium, the mutants had smaller and yellower single colonies than did controls. Tests for minimum inhibitory concentration of sulfometuron methyl(N-[(4,6-dimethylpyrimidin-2-yl)aminocarbonyl]-2-methoxycarbonylbenzenesulfonamide, Agricultural Products Department, E. I. du Pont de Nemours & Co., Inc.) were performed as described (Van Dyk and LaRossa 1986).

Genetic methods. For preliminary mapping, Hfr derivatives formed by Tn*10*-directed insertion of F′*ts114lac*$^+$ plasmids (Chumley et al. 1979) were used as donors in conjugational crosses to a series of auxotrophic mutants, as previously described (Van Dyk and LaRossa 1986). In contransduction experiments, donor phage, obtained by growing P22Ht*Δint-4* (from B.N. Ames) on the Tn*10*-containing strains (Davis et al. 1980), were used at a multiplicity of infection of 0.8. A 0.10 ml aliquot containing 5×10^8 auxotrophic cells was preincubated with the phage lysate at 37° C for 30 min before plating. After overnight incubation at 37° C, plates with the tetracycline-resistant transductants were replica-plated to minimal E plates containing 0.33% glucose. The number of prototrophic cotransductants was determined after incubation for 24 h at 37° C.

Acetate kinase and phosphotransacetylase assays. Cells were grown at 37° C in LB medium to $2–3 \times 10^9$ cells/ml; washed once with a solution of 10 mM potassium phosphate, pH 7.5, 10 mM $MgCl_2$, 1 mM EDTA; and stored as frozen pellets at −20° C. Cell-free extracts were prepared by sonication of the thawed pellets resuspended in a small volume of the washing solution, followed by centrifugation at $32800 \times g$ at 4° C for 1 h. The protein content of the clarified extracts was determined by the coomassie brilliant blue binding assay (Biorad Laboratories) of Bradford (1976).

The 1.0 ml acetate kinase assay mixture containing 100 mM Tapso (Research Organics Inc.), pH 7.6, 6.0 mM ATP, 1.0 mM phospho(enol)pyruvate, 0.2 mM NADH, 3.0 mM $MgCl_2$, 10 units pyruvate kinase (Sigma Chemical Company) and 20 units lactic dehydrogenase (type XXIX-S, Sigma Chemical Company). Following addition of the cell-free extract (25–65 μg protein) to the above assay mixture at 25° C, the background absorbance at 340 nm was continuously recorded for approximately 1 min. The substrate, sodium acetate, was added to a final concentration of 0.30 *M* and the absorbance at 340 nm continuously monitored. The difference between the rates in the presence and absence of acetate, and the NADH molar extinction coefficient of 6200 were used to calculate units of acetate kinase activity. One unit was defined as that amount of enzyme required to oxidize one μmole of NADH per minute.

Phosphotransacetylase activity was measured in a coupled assay as described by Brown et al. (1977). The 1.0 ml assay mixture contained 100 mM Tris-HCl, pH 8.0, 5 mM $MgCl_2$, 0.5 mM NAD, 0.5 mM coenzyme A, 5 mM malic acid, 10 mM acetyl phosphate (Li salt), 45 units malate dehydrogenase (Sigma Chemical Company) and 3 units citrate synthase (Sigma Chemical Company). The reaction was initiated by addition of cell extract (50–125 μg protein) and production of NADH at 25° C was monitored at 340 nm. Phosphotransacetylase activity was expressed in terms of μmoles NADH produced per minute. Both phosphotransacetylase and acetate kinase activities were measured in the linear range.

Propionyl-CoA accumulation. S. typhimurium LT2 cells growing in low phosphate medium (Bochner and Ames 1982) supplemented with L-valine (710 μM) were labelled with ^{32}P orthophosphate (0.2 mM, 200 mCi/mmol), at 37° C for at least four generations. The culture was split and one-half was treated with 100 μM sulfometuron methyl for twenty minutes. Nucleotides, extracted from cells with 1 *M* formic acid at 4° C, were separated by two dimensional thin layer chromatography (1st dimension, 0.9 *M* Guanidine HCl; 2nd dimension, 3 *M* ammonium sulfate; Bochner and Ames 1982). The propionyl-CoA spot was identified by comparison with previous two-dimensional thin layer chromatographic identificaiton of propionyl-CoA (Danchin et al. 1984), by comigration with a non-radioactive standard, and by comparison with two dimensional thin layer chromatographs of nucleotides prepared from *S. typhimurium* cells treated for 20 min with 100 μM sulfometuron methyl after labelling for at least four generations with ^{35}S-sulfuric acid in low sulfate medium (Bochner et al. 1984) supplemented with L-valine (710 μM).

Propionate and α-aminobutyrate analyses. Cells grown in E medium with 0.33% glucose to approximately 5×10^8 cells/ml were treated with 100 μM sulfometuron methyl

and 1.4 mM L-valine at 37° C for 18 hours. Culture supernatants were collected by filtration through a 0.2 μm filter. Propionate was analyzed on a Dionex 4000i ion chromatograph, with conductivity detection, using a Dionex HPICE AS1 column with ion suppression column attached. The eluant was 0.9 mM HCl at a flow rate of 0.7 ml/min. Propionate was identified and quantified by comparison to a standard propionate solution. α-Aminobutyrate was identified and quantified by automated amino acid analysis.

Results

Genetic map position of Tn10 in strains SMS209, SMS406, SMS408, and SMS419

The Tn*10* insertion mutation of the sulfometuron methyl hypersensitive mutant SMS419 was localized to the region between *his* (42 min) and *purF* (47 min) on the *S. typhimurium* chromosome by conjugation using Hfr donor strains formed by the integration of an F′ factor at the chromosomal Tn*10* site (data not shown). More refined genetic mapping used P22 phage grown on various Tn*10* insertion mutants as the donors and strain TR5663 (*purF*) as the recipient in cotransduction experiments. The Tn*10* insertions of SMS419, SMS209, SMS406, and SMS408 each had a cotransduction frequency of 0.2%–0.3% with *purF*. These four mutants have identical minimum inhibitory concentrations for sulfometuron methyl (Van Dyk and LaRossa 1986) and were the only Tn*10*-induced sulfometuron methyl hypersensitive mutations that were cotransduced with $purF^+$. These Tn*10* insertions were estimated by the formula of Wu (1966) to be located 0.7 min counterclockwise from *purF*. This map location at 46 min closely corresponds with those of *ack* and *pta* (Sanderson and Roth 1983), the genes encoding acetate kinase and phosphotransacetylase, respectively.

Phenotypic comparison with pta *and* ack *mutants*

S. typhimurium point mutants lacking either *ack* or *pta* function are resistant to fluoroacetate, sensitive to the dye alizarin yellow, and unable to ferment inositol (LeVine et al. 1980). Previously isolated *ack* and *pta* point mutations were transferred into the LT2 strain used for Tn*10* mutant isolation (Materials and methods) because *S. typhimurium* LT2 strains obtained from various sources displayed a range of sensitivities to sulfometuron methyl (unpublished observations). The *ack-1* and *pta-1* mutations in this genetic background conferred the same phenotypes as originally described (LeVine et al. 1980), and had minimum inhibitory concentrations of 210 μM for sulfometuron methyl, identical to that for the four strains with Tn*10* insertion mutations at 46 min. Strains SMS209, SMS406, SMS408, and SMS419 were also resistant to fluoroacetate, sensitive to alizarin yellow, and unable to ferment inositol. The four Tn*10*-induced mutations, *ack-1*, and *pta-1* thus conferred identical phenotypes, suggesting strongly that these four insertions inactivated either *ack* or *pta*.

Acetate kinase and phosphotransacetylase levels in ack *and* pta *mutants*

In order to fully characterize these mutants, the activities of phosphotransacetylase and acetate kinase were assayed in crude extracts. The *ack-1* mutant, TV077, lacked detectable acetate kinase activity and the *pta-1* mutant, TV078, was deficient in phosphotransacetylase activity (Table 2). Three of the mutants with Tn*10* insertions at minute 46 had normal acetate kinase activity but were deficient in phosphotransacetylase activity (Table 2). These three strains, SMS209, SMS406, and SMS419, thus contained a Tn*10* insertion that destroyed *pta* function. Strain SMS408 had no detectable acetate kinase activity and tenfold reduced phosphotransacetylase activity (Table 2). This mutant, therefore, contained a Tn*10* insertion that inactivated *ack* and was polar on *pta*.

Table 2. Biochemical characterization of *ack* and *pta* mutants

Strain	Relevant genotype	ACK units[a, c]	PTA units[b, c]	AKB Turnover[d]
LT2	ack^+pta^+	0.78	2.0	2.6
TV076	ack^+pta^+	0.76	1.6	2.6
TV077	ack^-pta^+	<0.2	2.3	0.7
TV078	ack^+pta^-	0.65	<0.01	0.6
SMS209	ack^+pta^-	0.76	<0.01	1.3
SMS406	ack^+pta^-	0.87	<0.01	1.4
SMS408	ack^-pta^+	<0.2	0.18	0.8
SMS419	ack^+pta^-	0.86	<0.01	1.0

[a] Acetate kinase units in μmol NADH/min per mg
[b] Phosphotransacetylase units in μmol NADH/min per mg
[c] These data represent the average of two independent experiments
[d] α-Ketobutyrate turnover rate in nmol/min per 10^9 cells; derived from Fig. 1 and LaRossa et al. (1987)

α-Ketobutyrate turnover in ack *and* pta *mutants*

Each of these four Tn*10* insertion mutants is defective in α-ketobutyrate turnover (LaRossa et al. 1987). The α-ketobutyrate turnover kinetics of the *ack-1* and *pta-1* point mutants were also reduced relative to the control strain (Fig. 1), and were comparable with the turnover rates of

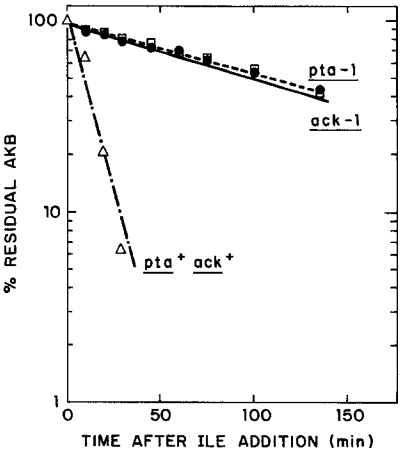

Fig. 1. α-Ketobutyrate turnover kinetics. △, Strain TV076 (ack^+, pta^+); ●, strain TV077 (*ack-1*); and □, strain TV078 (*pta-1*). α-Ketobutyrate, produced by threonine deaminase in vivo from radiolabelled threonine, was chased with isoleucine, an inhibitor of threonine deaminase. This assay, which was done in the presence of the acetolactate synthase isozymes I and II inhibitors, valine and sulfometuron methyl, assesses acetolactate synthase-independent metabolism of α-ketobutyrate (LaRossa et al. 1987)

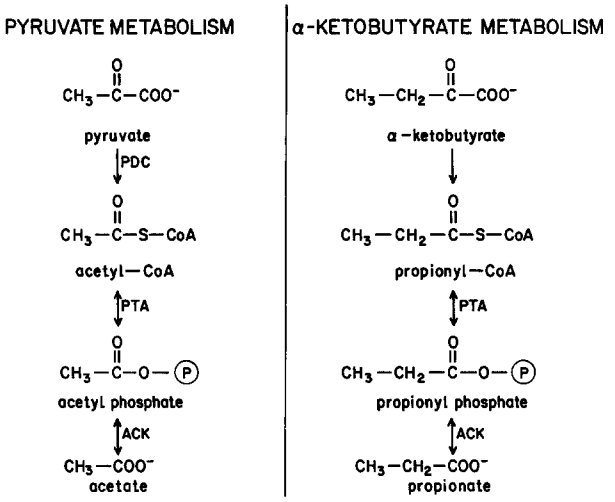

Fig. 2. Proposed pathway for α-ketobutyrate metabolism. Shown on the *left* are known reactions in the metabolism of pyruvate. On the *right* are the proposed reactions which result in the conversion of α-ketobutyrate to propionate. PDC, pyruvate dehydrogenase complex; PTA, phosphotransacetylase; ACK, acetate kinase

the four Tn*10* insertion mutants (Table 2). Thus, deficiency in α-ketobutyrate turnover correlated with the lack of functional acetate kinase or phosphotransacetylase, suggesting that these enzymes are involved in α-ketobutyrate metabolism.

Analyses of metabolites

The proposed role of phosphotransacetylase and acetate kinase in α-ketobutyrate metabolism is outlined in Fig. 2. An essential feature is the conversion of α-ketobutyrate to propionyl-CoA. In growing *S. typhimurium* cultures, propionyl-CoA was not detected. In contrast, propionyl-CoA was easily observed by two-dimensional thin layer chromatography of radiolabelled nucleotides when α-ketobutyrate levels were elevated (Fig. 3). Nearly equivalent concentrations of propionyl-CoA and acetyl-CoA were found when *S. typhimurium* cultures were treated with sulfometuron methyl and valine to inhibit acetolactate synthase.

Propionate, the end product of this proposed pathway, was analyzed by HPLC. The culture supernatant of LT2 (wild type) cells treated for 18 h with sulfometuron methyl and valine contained 0.22 mM propionate. No propionate was detected in culture supernatants of untreated LT2 cells. Likewise, culture supernatants of SMS406 and SMS408 cells treated for 18 h with sulfometuron methyl and valine contained no detectable propionate. Each of these three sulfometuron methyl and valine treated cultures contained 1.1–1.2 mM α-aminobutyrate.

Discussion

The phenotype of one sulfometuron methyl-hypersensitive mutant obtained after Tn*10* mutagenesis of *S. typhimurium* is due to an *aspC* insertion that limits the cell's ability to form aspartate during periods of α-ketobutyrate accumulation (Van Dyk and LaRossa 1986). Analysis of other classes of sulfometuron methyl hypersensitive *S. typhimurium* mutants has provided further insights into the physiological consequences of acetolactate synthase inhibition by this important agrichemical. This work identified a new class of sulfometuron methyl hypersensitive mutations as mutations in either *ack* or *pta*, the genes for acetate kinase and phosphotransacetylase, respectively. Both insertion and point mutants in these genes were defective in α-ketobutyrate turnover by enzymes other than acetolactate synthase. The likely cause of these mutants' sulfometuron methyl hypersensitivity is their reduced ability to metabolize α-ketobutyrate. These results suggest that loss of an α-ketobutyrate degradation route leads to greater accumulation of this toxic metabolite in the presence of sulfometuron methyl. The sulfometuron methyl hypersensitivity of strains containing mutations in a putative α-ketobutyrate turnover pathway supports the importance of α-ketobutyrate accumulation in the mechanism of sulfometuron methyl action.

The most plausible explanation for the α-ketobutyrate turnover deficiency of strains lacking acetate kinase or phosphotransacetylase activity is that these strains have lost a pathway for α-ketobutyrate metabolism (Fig. 2). The first step in this proposed pathway is the conversion of α-ketobutyrate to propionyl-CoA. *Escherichia coli* cells treated with exogenous α-ketobutyrate accumulate propionyl-CoA (Danchin et al. 1984). Here, it was shown that high endogenous levels of α-ketobutyrate lead to propionyl-CoA forma-

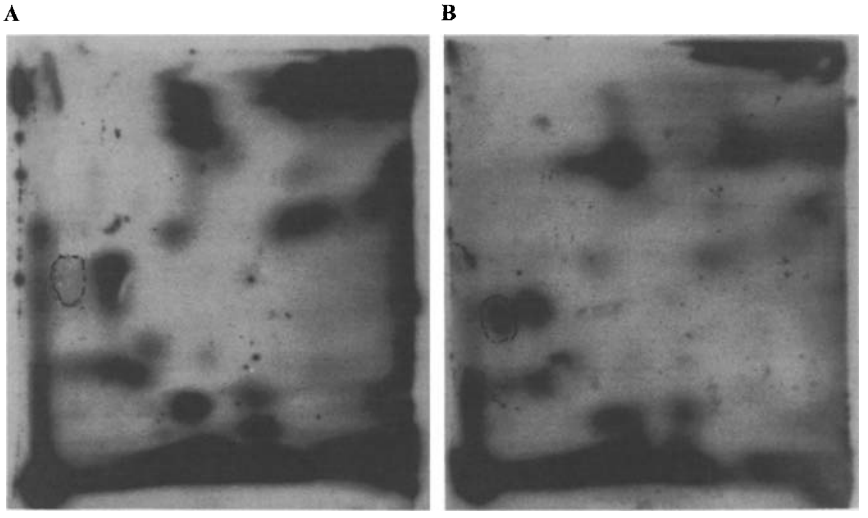

Fig. 3A, B. Accumulation of propionyl-CoA upon inhibition of acetolactate synthase. **A** Autoradiograph of separated nucleotides isolated from exponentially growing *S. typhimurium* cells. **B** Nucleotides isolated from cells that had been treated for 20 min with 100 μM sulfometuron methyl in the presence of 710 μM L-valine, conditions where α-ketobutyrate accumulates (LaRossa et al. 1987). The first dimension was the vertical; the second dimension was the horizontal. The position of propionyl-CoA is *circled* on each autoradiograph

tion in *S. typhimurium* cells. This reaction is likely to be catalyzed by the pyruvate dehydrogenase complex because the *E. coli* complex readily condenses α-ketobutyrate with CoA in vitro, forming propionyl-CoA (Bisswanger 1981). Other α-keto acid dehydrogenases may also contribute to the formation of propionyl-CoA from α-ketobutyrate.

Phosphotransacetylase and acetate kinase catalyze the subsequent steps in the proposed degradative pathway for α-ketobutyrate (Fig. 2). These enzymes, which are seemingly restricted to bacteria, are thought to be involved in the activation of acetate to acetyl-CoA (Brown et al. 1977). Both of these enzymes catalyze reversible reactions. In fact, the equilibrium for acetate kinase favors acetate formation (Brown et al. 1977). Phosphotransacetylase from *Clostridium kluyveri* uses propionyl-CoA at a rate that is one-tenth to one-half that seen with acetyl-CoA (Stadtman 1955). Likewise, it has been reported that acetate kinase from *E. coli* can phosphorylate propionate at one-tenth the rate of acetate (Rose et al. 1954). However, recent studies reported that highly purified acetate kinases from both *E. coli* and *S. typhimurium* are inactive in the phosphorylation of propionate (Fox and Roseman 1986). The reason for this discrepancy is not clear. The evidence from this work that mutants lacking acetate kinase activity are deficient in α-ketobutyrate metabolism argues that acetate kinase can interconvert propionyl phosphate and propionate in vivo. Acetate kinase and phosphotransacetylase have also been proposed to convert propionyl-CoA to propionate in *Desulfobulbus propionicus* (Stams et al. 1984). Thus, it is likely that the acetate kinase and phosphotransacetylase of *S. typhimurium* will react with the compounds that contain an extra methylene group in comparison to their normal substrates, as shown in Fig. 2.

Propionate, the product of the proposed α-ketobutyrate degradation pathway, was found in culture supernatants of sulfometuron methyl and valine treated wild-type cells but was absent in *ack* and *pta* insertion mutants. This is further evidence that the pathway shown in Fig. 2 is functioning in *S. typhimurium* when endogenous levels of α-ketobutyrate are elevated. The relatively low concentrations of propionate found may indicate that this pathway is a minor one or that propionate is further metabolized (Wegener et al. 1968).

Several other routes of α-ketobutyrate metabolism in enterobacteria are known, such as the conversion of α-ketobutyrate to α-aminobutyrate by transamination. Both α-ketobutyrate and α-aminobutyrate accumulated upon sulfometuron methyl and valine treatment of *S. typhimurium* cells. Culture supernatants of an *S. typhimurium ilvG* mutant also contain α-ketobutyrate and α-aminobutyrate (Primerano and Burns 1982). Conversion of propionyl-CoA to succinyl-CoA via methylmalonyl-CoA occurs in many microorganisms (Wegener et al. 1968). However, the methyl malonyl-CoA mutase component of this pathway requires vitamin B_{12} (Gottschalk 1986) which is not synthesized in aerobically grown *S. typhimurium* (Jeter et al. 1984). Likewise, condensation of glyoxylate with propionyl-CoA to form α-hydroxyglutarate is unlikely to occur under the conditions used in this work because growth on short chain fatty acids is required to induce α-hydroxyglutarate synthase (Wegener et al. 1968). The oxidation of propionyl-CoA to pyruvate via acrylyl-CoA and lactyl-CoA occurs in *E. coli* grown on propionate (Wegener et al. 1967; Kay 1972). It is unknown if this pathway exists in *S. typhimurium* grown on glucose. One or more routes of α-ketobutyrate degradation other than that shown in Fig. 2 must be present in *S. typhimurium* because *ack* and *pta* mutants retained a reduced ability to metabolize α-ketobutyrate by enzymes other than acetolactate synthase.

Analysis of acetate kinase and phosphotransacetylase activity in the Tn*10*-induced mutants provided strong evidence for cotranscription of *ack* and *pta*. The three mutants lacking phosphotransacetylase activity had normal acetate kinase activity; however, the mutant that lacked detectable acetate kinase activity was also deficient in phosphotransacetylase activity. Thus, in *S. typhimurium*, *ack* and *pta* are present in a single operon, with *ack* upstream of *pta*. Other bacteria may also have an operon structure of *ack* and *pta*. A class of *E. coli* mutants (*fac-A*) lacking both acetate kinase and phosphotransacetylase activities has been reported (Guest 1979). A single mutational event that destroys both acetate kinase and phosphotransacetylase activity in *Azotobacter vinelandii* has also been described (McKenney and Melton 1986).

The precise nature of α-ketobutyrate toxicity is unclear. Pantothenate biosynthesis (Primerano and Burns 1982), valine biosynthesis (Shaw and Berg 1980), aspartate biosynthesis (Daniel et al. 1983, Van Dyk and LaRossa 1986), isoleucine catabolism (Primerano and Burns 1982), aminoacylation of $tRNA^{Ile}$ and $tRNA^{Val}$ (Singer et al. 1984), *pts*-mediated sugar transport (Daniel et al. 1983), and acetyl-CoA formation (Primerano and Burns 1982; Danchin et al. 1984) have all been proposed as in vivo targets of α-ketobutyrate. This metabolite has also been suggested to act as an alarmone signalling a shift from an anaerobic environment to an aerobic one (Daniel et al. 1983). We suggest, however, that the many manifestations of α-ketobutyrate accumulation are readily explained by invoking α-ketobutyrate as a competitive inhibitor of several enzymes that utilize other α-keto acids as their principle substrates.

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