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The Sulfonylurea Herbicide Sulfometuron Methyl Is an Extremely Potent and Selective Inhibitor of Acetolactate Synthase in *Salmonella typhimurium**

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The sulfonylurea herbicide sulfometuron methyl inhibits the growth of several bacterial species. In the presence of L-valine, sulfometuron methyl inhibits *Salmonella typhimurium*, this inhibition can be reversed by L-isoleucine. Reversal of growth retardation by L-isoleucine, accumulation of guanosine 5′-diphosphate 3′-diphosphate (magic spot), and *relA* mutant hypersensitivity suggest sulfometuron methyl interference with branched-chain amino acid biosynthesis. Growth inhibition of *S. typhimurium* is mediated by sulfometuron methyl's inhibition of acetolactate synthase, the first common enzyme in the branched-chain amino acid biosynthetic pathway. Sulfometuron methyl exhibits slow-binding inhibition of acetolactate synthase isozyme II from *S. typhimurium* with an initial K_i of 660 ± 60 nM and a final, steady-state K_i of 65 ± 25 nM. Inhibition of acetolactate synthase by sulfometuron methyl is substantially more rapid (10 times) in the presence of pyruvate with a maximal first-order rate constant for conversion from initial to final steady-state inhibition of 0.25 ± 0.07 min^{-1} (minimal half-time of 2.8 min). Mutants of *S. typhimurium* able to grow in the presence of sulfometuron methyl were obtained. They have acetolactate synthase activity that is insensitive to sulfometuron methyl because of mutations in or near *ilvG*, the structural gene for acetolactate synthase isozyme II.

Sulfometuron methyl,[1] *N*-[(4,6-dimethylpyrimidin-2-yl)-aminocarbonyl]-2-methoxycarbonylbenzenesulfonamide (the active ingredient in DuPont's Oust® Weed Killer; Ref. 34) (Fig. 1), and chlorsulfuron,[1] 2-chloro-*N*-[4-methoxy-6-methyl-1,3,5-triazin-2-yl)aminocarbonyl]benzenesulfonamide (the active ingredient in DuPont's Glean® Weed Killer; Ref. 35), are potent, new sulfonylurea herbicides. At present, their biochemical site of action is unknown (1), although various metabolic consequences of treating plants with chlorsulfuron, including a rapid inhibition of cell division, have been reported (2).

Many herbicides of diverse structure interfere with photosynthetic electron transport (3). That these herbicides affect plants and not animals reflects the difference in energy acquisition between plants and animals. These two kingdoms differ in a second profound way. Metaphytes synthesize all their vitamins and amino acids; metazoans, especially higher animals, usually lack some biosynthetic capabilities. The herbicide glyphosate interferes with aromatic amino acid biosynthesis (4), although its precise target remains uncertain (5–8).

Since bacteria and plants share many common biochemical pathways, bacteria can provide an expedient means of localizing the site of herbicidal action. Bacteria, unlike plants, have the advantage of well-defined biochemistry and genetics. In this report, we establish that growth retardation of *Salmonella typhimurium* induced by sulfometuron methyl is a result of inhibition of the branched-chain amino acid biosynthetic enzyme acetolactate synthase (EC 4.1.3.18) isozyme II.

EXPERIMENTAL PROCEDURES

Materials—[*pyrimidine*-^{14}C]Sulfometuron methyl (153 μCi/mg) or [*phenyl*-^{14}C]sulfometuron methyl (12.0 μCi/mg) and [^{32}P]P_i were obtained from New England Nuclear, the former by custom synthesis. *S. typhimurium* strains LT2 (wild type), TA2439 (*relA2 zga*::Tn*10*), and bacteriophage P22HT int-4 (9) were the gift of Dr. B. N. Ames, University of California. An isogenic *relA*$^+$/*relA1* pair of *S. typhimurium* strains (TR3379=*rel1*$^+$ and TR3381=*relA1*) was obtained from Dr. K. Rudd, University of Utah. The Tn*10*::*relA* insertion mutant, TT7542, of *S. typhimurium* was obtained from the same source. *Escherichia coli* strain DU650 containing pDU9 (10), a plasmid bearing the *ilvG* gene encoding *S. typhimurium* acetolactate synthase isozyme II, was the generous gift of the late Dr. R. O. Burns, Duke University. pDU9 was transformed into *E. coli* strain HB101 by Dr. N. S. Yadav, E. I. du Pont de Nemours & Co. *S. typhimurium* strain CBS501 (*ilvE*::Tn*10*) was obtained from Dr. C. Berg, University of Connecticut. *Citrobacter freundii* was obtained from the American Type Culture Collection. *Acinetobacter* sp. was from Dr. P. Chapman, University of Minnesota.

Microbial Techniques—For growth experiments, Vogel and Bonner medium (11) supplemented with 0.2% glucose was the minimal medium; the rich medium was LB (12).

Antibacterial activity was measured by the disc diffusion method on minimal agar plates (13). Washed, overnight cultures were plated in soft agar overlays; zones of inhibition caused by placing a 6-mm paper disc, impregnated with 40 μg of sulfometuron methyl, upon a bacterial lawn were measured after a 16-h incubation at 30 °C.

Large-scale culture (2000 liters) of *E. coli* HB101/pDU9 was carried out with the minimal salts medium of Omerod *et al.* (14) containing 5.5 g/liter (initially) of dextrose, 1.2 mM L-leucine, 1.2 mM L-proline, 3 mM L-valine (found to be effective in ensuring plasmid maintenance), 20 mg/liter of sodium ampicillin, and 100 mg/liter of thiamine HCl. Additional dextrose (four additions equivalent to the initial)

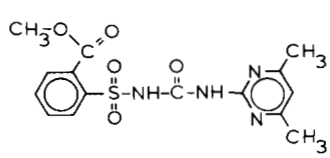

FIG. 1. **Sulfometuron methyl.**

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[1] Proposed common name from the American National Standards Institute.

was added during fermentation as needed. Phosphates used were 2.33 g/liter of K_2HPO_4 and 1 g/liter of KH_2PO_4. Anhydrous ammonia was used to maintain a pH of 7.1 throughout the fermentation and as an additional nitrogen source. Stirring and aeration rates were varied to achieve a dissolved O_2 concentration ≥30% of air saturation. While still in exponential growth, 240 kg of ice were added to the 2000-liter culture to reduce the culture temperature rapidly from 35 to 18 °C. Rapid reduction of the culture temperature ensured maximal preservation of acetolactate synthase activity during harvest of the cells by continuous-flow centrifugation. The final cell paste (16.5 kg; 8.2×10^5 units of acetolactate synthase isozyme II) was frozen (as a finely divided gravel) and stored in liquid nitrogen.

Assay of Acetolactate Synthase—Assays were conducted at 37 °C by a modification of the method of Bauerle *et al.* (15). Each assay (1 ml) contained 0.1 mmol of Tricine[2]/NaOH, pH 7.8, 50 μmol of sodium pyruvate, 10 μmol of $MgCl_2$, 0.1 μmol of thiamine pyrophosphate, and 0.1 μmol of FAD. Enzymic reactions were quenched with 0.25 ml of 12 N H_2SO_4 and subsequently incubated at 80 °C for 5 min. After cooling samples to 37 °C, 0.16 ml of 50% (w/w) NaOH, 0.3 ml of 0.5% (w/v) creatine, and 0.3 ml of 5% α-naphthol in 2.5 N NaOH were added in rapid succession with immediate mixing. Samples were incubated at 37 °C for 1 h with several intervening mixings to ensure efficient aeration (essential to color development). After low-speed centrifugation to remove turbidity, absorbance at 530 nm was determined. Formation of 0.1 mM acetolactate in the assay resulted in 0.65 *A* unit at 530 nm. One unit of activity is the amount required to form 1 μmol of acetolactate (consume 2 μmol of pyruvate) per min under these assay conditions.

Protein was determined by the biuret (16) procedure where practical or by the Coomassie Brilliant Blue binding assay (Bio-Rad) of Bradford (17).

Genetics—Independent mutants were selected on minimal medium supplemented with sulfometuron methyl (33 μg/ml) and L-valine (83 μg/ml). Crosses were mediated via P22 transduction (18). Sulfometuron methyl resistance in the presence of L-valine (83 μg/ml) was an unselected marker in all crosses; the selected marker was either tetracycline resistance associated with Tn*10* or prototrophy conferred by *ilvE*$^+$. The response of strains to sulfometuron methyl can be determined only in the absence of L-isoleucine. Thus, Tn*10*::*ilvE* transductants were converted to IlvE$^+$ derivatives. Excision of Tn*10* from Tn*10*::*ilveE*-bearing strains was accomplished by plating ~10^8 cells from a washed, overnight culture on unsupplemented minimal medium (19). The growth response of these IlvE$^+$ strains to medium containing 33 μg/ml of sulfometuron methyl and 83 μg/ml of L-valine was determined. Standard genetic nomenclature was followed (20).

Preparation of Acetolactate Synthase—Extracts of *E. coli* HB101/pDU9 (35 mg of protein/ml) could be dialyzed at 4 °C (twice, 12 h each) against 0.1 M Taps/KOH, pH 8.8, at 4 °C, containing 1 mM EDTA, 0.1 mM dithiothreitol, and 20% (v/v) glycerol, with little or no loss of enzymic activity. These dialyzed extracts did not require Mg^{2+}, thiamine pyrophosphate, or FAD in short-term (1 min) assays. However, upon extended assay at high dilution (2000-fold), the enzyme lost activity with a half-time of 12 min in the absence of Mg^{2+}, thiamine pyrophosphate, and FAD. After preincubation at high dilution (100-fold) for several hours at 37 °C (assay buffer without thiamine pyrophosphate, FAD, or Mg^{2+}), the enzyme had an absolute dependence on Mg^{2+} or Mn^{2+}, thiamine pyrophosphate, and FAD, with complete restoration of initial levels of enzymic activity by 10 mM $MgCl_2$, 0.1 mM thiamine pyrophosphate, and 0.1 mM FAD (added immediately prior, <1 min, to assay). Dialyzed extracts could be stored at −20 °C for several months without noticeable loss of enzymic activity. Preliminary efforts to purify acetolactate synthase from *E. coli* HB101/pDU9 have established that the enzyme comprises 2.7% of the total extracted protein.[3] A specific activity of 25 units/mg[3] and molecular weight of 59,300 (21) were used to estimate the concentration of acetolactate synthase in extracts.

Nucleotide Levels—A qualitative determination of nucleotide levels was made by the method of Bochner and Ames (22). Two-dimensional thin-layer chromatography on polyethylene imine plates (Brinkmann Instruments) was conducted utilizing solvent Tb in the first dimension and Sb in the second dimension (22).

Data Processing—The MLAB data modeling program (23), available from the National Institutes of Health, was used to fit the following equations to data:

$$v = VA/(K + A) \quad (1)$$

$$P = P_0 + ((v_f - v_0)/k)e^{-kt} + ((v_0 - v_f)/k) + v_f t \quad (2)$$

$$v_i = v/(1 - I/K_i) \quad (3)$$

where v is velocity (the rate of enzymic reaction or the rate of transition between initial and final inhibition for slow-binding inhibition), A is substrate or inhibitor concentration, V is maximal velocity, K is the concentration of substrate or inhibitor which gives half-maximal velocity, P is product, P_0 is product present initially (zero-time absorbancy), v_f is the final, steady-state rate, v_0 is the initial rate, k is the first-order rate constant for transition between initial and final rates, v_i is the inhibited velocity (initial or final for slow binding), I is inhibitor concentration, and K_i is the concentration of inhibitor which gives 50% inhibition.

RESULTS

Initial Observations—Sulfometuron methyl inhibits the growth of *C. freundii* and *Acinetobacter* sp. on minimal medium. Only in the presence of valine, however, is growth inhibition of wild-type *S. typhimurium* by sulfometuron methyl observed. Inhibition in the presence of valine is reversed by isoleucine (but not by the other 18 common amino acids), suggesting sulfometuron methyl inhibits a step in the biosynthesis of branched-chain amino acids (Table I). Thiamine partially and thiamine together with α-ketobutyrate protect against sulfometuron methyl inhibition (data not shown). ppGpp and pppGpp accumulated upon treatment of *S. typhimurium* concomitantly with sulfometuron methyl (100 μg/ml) and L-valine (83 μg/ml). This accumulation is expected if amino acid limitation is a primary event in growth inhibition by sulfometuron methyl.

relA mutants are defective in generalized stimulation of amino acid biosynthetic operons (13); thus, these enzymes (including those involved in the biosynthesis of branched-chain amino acids (24)) exhibit low activities in *relA* mutants on both minimal and valine-supplemented media. In contrast to wild-type *S. typhimurium*, growth of the *relA* mutant TA2439 in the presence of sulfometuron methyl is rescued by inclusion of isoleucine, methionine, or pantothenate. This growth requirement mimics the phenotype of *ilvG* mutants in which auxotrophy is satisfied by isoleucine, methionine, or pantothenate (25). These observations suggest that sulfometuron methyl inhibits the *ilvG* gene product, acetolactate synthase isozyme II.

Partial reversal of sulfometuron methyl inhibition of the *relA* mutant TA2439 was mediated by L-leucine (data not shown). In the presence of sulfometuron methyl and valine,

TABLE I

Reversal and potentiation of sulfometuron methyl growth inhibition

Medium addition (mg/ml)	Inhibition zone[a]	
	LT2 (*relA*$^+$)	TA2439 (*relA2*)
	mm diameter	
None	<6	31
Casamino acids (0.05)	<6	<6
Isoleucine (0.083)	<6	<6
Methionine (0.05)	<6	<6
Pantothenate (0.022)	<6	9
Valine (0.083)	32	44
Valine (0.083) and isoleucine (0.083)	<6	<6

[a] Caused by 40 μg of sulfometuron methyl as described under "Experimental Procedures."

[2] The abbreviations used are: Tricine, *N*-tris(hydroxymethyl)-methylglycine, Taps, 3[tris(hydroxymethyl)methyl]amino-propanesulfonic acid; ppGpp, guanosine 5′-diphosphate 3′-diphosphate; pppGpp, guanosine 5′-triphosphate 3′-diphosphate.

[3] J. V. Schloss, unpublished data.

however, growth of this *relA* mutant is only permitted by inclusion of isoleucine in the medium. The pattern of valine-promoted growth inhibition by sulfometuron methyl and reversal by isoleucine is consistent with selective inhibition of acetolactate synthase isozyme II by sulfometuron methyl, with valine-sensitive (feedback inhibited) isozyme I being resistant.

Analogous results have been obtained for the *relA1* mutant TR3381 and the isogenic *relA*$^+$ strain TR3379. The *relA*::Tn*10* strain TT7542 displays a phenotype indistinguishable from the *relA1* and *relA2* strains in terms of growth inhibition by sulfometuron methyl and reversal of inhibition by pantothenate, methionine, or isoleucine.

Growth inhibition of wild-type *C. freundii* is reversed by methionine, pantothenate, or isoleucine and potentiated by valine (data not shown). Thus, sulfometuron methyl produces an *ilvG* phenocopy in wild-type *C. freundii*. In the presence of valine and sulfometuron methyl, only isoleucine prevents growth inhibition. Thus, the responses to sulfometuron methyl of the *S. typhimurium relA* derivative are quite similar to those of *C. freundii*.

Genetics—Spontaneous mutants of *S. typhimurium* capable of growth in the presence of L-valine and sulfometuron methyl have been selected. These strains were used as donors in transductional crosses with the *ilvE* recipient CBS501. The five sulfometuron methyl-resistant mutations were all cotransduced with *ilvE*$^+$ at frequencies ranging from 0.5 to 1.00 (Table II). This cotransduction suggests that these sulfometuron methyl-resistant mutations lie within the *ilv* operon. To substantiate this conclusion, a reciprocal cross was performed. The donor was strain CBS501; the recipients were the sulfometuron methyl-resistant mutants. The *ilvE* mutation in CBS501 is due to an insertion of Tn*10*; thus, transduction of the *ilv* region is mediated by selection of tetracycline resistance. The growth response to sulfometuron methyl of the resulting *ilvE*::Tn*10* transductants cannot be directly analyzed because of the isoleucine requirement of *ilvE* mutants. Ilv$^+$ derivatives (possibly the product of precise excision events) of at least 19 such tetracycline-resistant, Ilv$^-$ transductants arising from each cross were obtained. That the responses to sulfometuron methyl of all derivatives from a single transductant were identical allowed deduction of its sulfometuron methyl phenotype. The sulfometuron methyl-sensitive phenotype arose at a frequency of 0.72 to 1.00 (Table II). The cotransduction indicates close linkage of *ilvE* and mutations resulting in sulfometuron methyl resistance. Assay of acetolactate synthase isozyme II (in the presence of 1 mM L-valine) in extracts from these mutants showed the enzyme to be far less sensitive to sulfometuron methyl (negligible inhibition by 100 μM sulfometuron methyl) than the wild-type enzyme.

TABLE II

Sulfometuron methyl resistance maps to the ilvGEDA operon

In Experiment 1, the donors were those strains listed in the far left column; the recipient in each cross is the *ilvE*::Tn*10* strain CBS501. In Experiment 2, donors and recipients are reversed. The selected marker is the tetracycline resistance mediated by *ilvE*::Tn*20*. Cotransduction frequencies are in parentheses.

Strain	Experiment 1: fraction of sulfometuron methyl resistant/No. of *ilvE*$^+$	Experiment 2: fraction of sulfometuron methyl sensitive/No. of *ilvE*::Tn*10*
SM3	24:24 (1.00)	23:26 (0.88)
SM18	80:111 (0.73)	21:29 (0.72)
SM38	40:80 (0.50)	29:29 (1.00)
SM48	38:48 (0.80)	29:29 (1.00)
SM55	62:82 (0.76)	19:19 (1.00)
LT2	0:141 (<0.01)	5:5 (1.00)

Interaction of Sulfometuron Methyl with Acetolactate Synthase—Acetolactate synthase activity in extracts from wild-type *S. typhimurium* LT2 was completely inhibited by 1 mM sulfometuron methyl. A detailed characterization of the mode of inhibition was conducted with extracts from *E. coli* HB101/pDU9. Assay time courses were markedly biphasic in the presence of sulfometuron methyl (Fig. 2). Although long-term enzymic instability under assay conditions (half-time = 3 h) somewhat distorts the results shown in Fig. 2, it is clear that a final steady-state rate is achieved. Thus, despite the time-dependent nature of the inhibition of acetolactate synthase by sulfometuron methyl, it is not irreversible. To determine the kinetic constants which define the interaction of sulfometuron methyl with acetolactate synthase, a higher concentration of enzyme (10 nM) and a shorter-time interval (10 min) were used. Under these conditions, instability of acetolactate synthase was negligible. Assay progress curves could be adequately defined by first-order transients in which there were both an initial (weak) level and a final, steady-state (more potent) level of inhibition (Equation 2). Initial inhibition, final inhibition, and the first-order rate constant for slow binding were all dependent on the concentration of sulfometuron methyl. Analyses of initial and final inhibitions (Equation 3) gave K_i values of 660 ± 60 nM and 65 ± 25 nM, respectively. Examination of the first-order transient rate constant revealed it was saturable (Equation 1), with a maximal rate (high sulfometuron methyl) of 0.25 ± 0.07 min^{-1}. From these values (initial K_i, final steady-state K_i, and maximal tight-binding rate), the maximum reversal rate for inhibition under standard assay conditions (see "Experimental Procedures") is 0.02 min^{-1} (half-time = 0.5 h). While not examined in detail, inhibition of acetolactate synthase by sulfometuron methyl developed far more slowly (10-fold) in

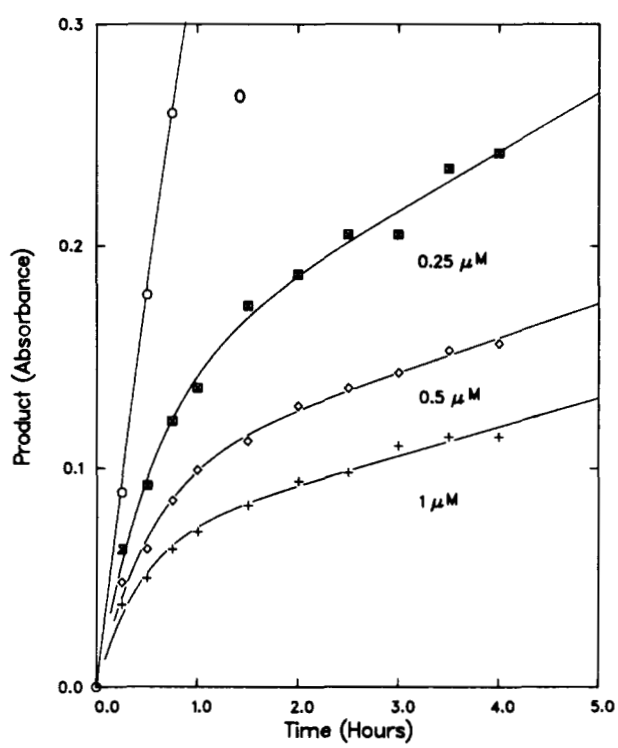

FIG. 2. **Assay time courses for *S. typhimurium* acetolactate synthase isozyme II (*E. coli* HB101/pDU9) in the absence (○) and presence of 1 (+), 0.5 (◇), or 0.25 (⊠) μM sulfometuron methyl.** The enzyme concentration in these assays was 0.6 nM.

the absence of pyruvate. Examination of the degree of initial inhibition, final inhibition, and the first-order transient rate constant at various pyruvate concentrations (1 μM sulfometuron methyl) has given similar results over the concentration range of 1–50 mM. In this context, the enzyme displays hyperbolic saturation by pyruvate (0.25–50 mM) with a Michaelis constant of 2.7 ± 0.4 mM. Similarly, the concentration of thiamine pyrophosphate (2–200 μM) or FAD (20–100 μM) used in the assay did not affect the degree of inhibition of acetolactate synthase by sulfometuron methyl.

Incubation of 26 μM acetolactate synthase with 20 μM sulfometuron methyl in 0.1 M Taps/KOH, pH 8.8 (at 4 °C), 1 mM EDTA, 0.1 mM dithiothreitol, 20% glycerol on ice resulted in a decline of the enzymic activity to 31% of its initial value (within 21 h). Gel filtration (Sephacryl S-200; room temperature; 0.1 M Tricine/KOH, pH 8, as eluant) of incubation mixtures that contained [*phenyl*-^{14}C]- or [*pyrimidine*-^{14}C]sulfometuron methyl resulted in complete reactivation of the enzyme and resolution of the enzyme from radiolabel. However, elution profiles of ^{14}C-labeled sulfometuron methyl from enzymic incubation mixtures were skewed to an earlier elution position than was observed for sulfometuron methyl passed through the gel filtration column alone. It would appear that sulfometuron methyl was initially bound by the enzyme, but was slowly released during gel filtration. When the rapid, centrifugal gel filtration technique of Penefsky (26) was employed to resolve enzyme and unbound [^{14}C]sulfometuron methyl, 0.8 mol of radiolabel/mol of enzyme eluted coincident with the enzyme (with or without 50 mM pyruvate present in the incubation mixture).

Inhibition could be completely reversed (94%) by incubation of the diluted (100-fold) enzyme at 37 °C (0.1 M Tricine/KOH, pH 7.8, 1 mM EDTA) for several hours prior to addition of 10 mM $MgCl_2$, 0.1 mM thiamine pyrophosphate, 0.1 mM FAD, and 50 mM sodium pyruvate to initiate the assay. Similarly, dilution (4000-fold) of the inhibited enzyme (26 μM acetolactate synthase, 20 μM sulfometuron methyl as above) directly into an assay mixture results in substantial reversal (45%) of inhibition over the course of a 1-h assay.

DISCUSSION

Clearly, sulfometuron methyl inhibits growth of *S. typhimurium* via acetolactate synthase isozyme II. Sulfometuron methyl is an exceptionally potent inhibitor of acetolactate synthase isozyme II. Sulfometuron methyl does not inhibit the growth of *E. coli*, which lacks a functional acetolactate synthase isozyme II, and only potently inhibits *S. typhimurium* in the presence of L-valine, which selectively blocks other acetolactate synthase isozymes. Mutations that confer resistance to sulfometuron methyl are in the *ilvG* region and result in an acetolactate synthase isozyme II resistant to sulfometuron methyl.

An *S. typhimurium ilvG* mutant (lacking acetolactate synthase isozyme II) is an isoleucine auxotroph since the remaining acetolactate synthase isozyme inefficiently catalyzes α-aceto-α-hydroxybutyrate formation (25). This mutant accumulates high levels of α-ketobutyrate, which is toxic due to its interference with pantothenate formation. This toxicity is overcome by supplementation of the growth medium with either pantothenate or methionine. Thus, the *ilvG* auxotrophy is satisfied by isoleucine, methionine, or pantothenate (25). In both *relA* mutants of *S. typhimurium* and wild-type *C. freundii*, sulfometuron methyl inhibition is prevented by inclusion of pantothenate, methionine, or isoleucine in the growth medium. This suggests that in both organisms, α-aceto-α-hydroxybutyrate synthesis (isoleucine pathway) is impeded, while acetolactate formation (valine-leucine pathway) proceeds. That valine potentiates growth inhibition by sulfometuron methyl in both organisms implies a comparable division of function between acetolactate synthase isozymes in *S. typhimurium* and *C. freundii*.

Lack of inhibition of acetolactate synthase isozyme I by sulfometuron methyl[4] would seem to suggest the lack of a mechanistic (active site directed) basis for the inhibition. The absence of marked protection of acetolactate synthase by thiamine pyrophosphate (2–200 μM), FAD (20–100 μM), or pyruvate (1–50 mM) is inconsistent with the interaction of sulfometuron methyl at the active site. The complementary inhibitors, L-valine and sulfometuron methyl, of isozymes I and II, respectively, may both inhibit acetolactate synthase via allosteric sites. Unlike isozyme I, however, no allosteric regulation of isozyme II has been reported.

Slow-binding inhibition (27), which is seen with sulfometuron methyl, is becoming recognized as a typical feature of potent, reversible inhibitors (28). The unusually slow onset of potent inhibition of acetolactate synthase by sulfometuron methyl is more characteristic of allosteric inhibitors (29) than the slow-binding inhibition associated with reaction-intermediate analogs (28). The slow phase of inhibition could be due to a slow change in the oligomeric state of acetolactate synthase, as is induced in isozyme I by FAD (30), or it could simply be due to a "tightening" of the interaction between the enzyme and sulfometuron methyl.

Since there is no net oxidation or reduction in the acetolactate synthase reaction, the absolute dependence on FAD is unusual. Two similar examples of carbon-carbon lyases that require FAD but catalyze reactions that involve no net oxidation have been reported, tartronate-semialdehyde synthase (EC 4.1.1.47) (31) and mandelonitrile lyase (EC 4.1.2.10) (32). Presumably, the FAD serves as a transient carbanion trap (electron sink) during the acetolactate synthase reaction, as has been proposed for a number of flavoproteins (33), but without concomitant oxidation of the carbanion intermediate.

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