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Sensitivity of a *Salmonella typhimurium aspC* Mutant to Sulfometuron Methyl, a Potent Inhibitor of Acetolactate Synthase II

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Sulfometuron methyl is a potent and specific inhibitor of acetolactate synthase II in *Salmonella typhimurium*. Mutant strains sensitive to sulfometuron methyl on minimal medium were isolated following mutagenesis with Tn*10*. A conditionally auxotrophic insertion mutant, strain SMS409, which required aspartate at high temperatures or in the presence of tyrosine, was found among the 15 mutants isolated. The Tn*10* insertion in strain SMS409 was mapped by conjugation and transduction to the region between *aroA* and *pncB* at 20 min on the chromosome of *S. typhimurium*; this location is similar to the genetic location of *aspC* in *Escherichia coli*. The specific activity of the *aspC* product, aspartate aminotransferase, was severely reduced in strain SMS409. This indicated that the Tn*10* insertion in strain SMS409 inactivated *aspC*. An *aspC* mutant of *E. coli* was also inhibited by either sulfometuron methyl or tyrosine. We present a hypothesis which relates the observed α-ketobutyrate accumulation in sulfometuron methyl-inhibited cultures of strain SMS409 to aspartate starvation.

Bacterial cells respond in a multifaceted manner to the inhibition of an enzyme within a biosynthetic pathway. When the end product is depleted, the synthesis of pathway enzymes increases due to mechanisms such as derepression, relief from attenuation, and response to global signals. Allosteric inhibition of the first enzyme of the pathway by the end product also ceases. The flux of intermediates through the truncated pathway is thereby increased, leading to accumulation of the substrate of the blocked enzyme, with possible toxic effects (17, 25, 33).

Acetolactate synthase (ALS) is an enzyme in the biosynthetic pathway for branched-chain amino acids. ALS catalyzes the following parallel reactions in the biosynthesis of isoleucine and valine: (i) 2 pyruvate → α-acetolactate + CO_2; and (ii) pyruvate + α-ketobutyrate → α-aceto-α-hydroxybutyrate + CO_2. (Fig. 1). Isozymes of ALS in *Salmonella typhimurium* and *Escherichia coli* have similar cofactor requirements (13), subunit compositions (12, 14, 27), and primary structures (16, 22, 27, 31, 32).

The herbicide sulfometuron methyl (SM) {*N*-[(4,6-dimethylpyrimidin-2-yl)aminocarbonyl]-2-methoxycarbonylbenzenesulfonamide} is a potent inhibitor of ALS in bacteria (20), yeasts (15), and plants (6). In addition to depleting cells of branched-chain amino acids and pantothenate, another potential toxic effect of SM inhibition of ALS is the accumulation of α-ketobutyrate (AKB). Exogenously added AKB is toxic to *S. typhimurium* (25, 28), although the precise nature of toxicity is unclear. It has been proposed that AKB interferes with valine biosynthesis (28), formation of valyl-tRNA^{Val} and isoleucyl-tRNA^{Ile} (30), pantothenate biosynthesis (25), *pts*-mediated sugar transport (9, 10), flux to and through the tricarboxylic acid cycle (8), and formation of aspartate (9). It has also been suggested that AKB functions as an alarmone which signals a shift from an anaerobic environment to an aerobic environment (9).

In *S. typhimurium*, the two ALS isozymes differ in SM sensitivity and catalytic activity. SM inhibits ALS II (20), while ALS I is not affected by this inhibitor (21). Thus, *S. typhimurium* is able to grow in the presence of SM unless valine, the feedback inhibitor of ALS I, is also present (20). In contrast to ALS II, ALS I is inefficient in the utilization of AKB (25, 29). Therefore, the inhibition of ALS II by SM should result in accumulation of AKB in *S. typhimurium*.

To understand the roles of both end product depletion and substrate accumulation in SM toxicity, insertion mutants of *S. typhimurium* sensitive to SM in the absence of valine were isolated and characterized. One such mutation inactivated *aspC*, the gene encoding one of transaminases capable of catalyzing aspartate formation (19). We present a hypothesis which suggests that AKB accumulation is the cause of the aspartate requirement in the *aspC* mutant.

MATERIALS AND METHODS

Strains, media, and chemicals. The bacterial strains which we used are listed in Table 1. The chemicals were reagent grade. SM is a product of E.I. du Pont de Nemours & Co., Inc. L-[U-^{14}C]threonine (228 mCi/mmol; 44 μM) and Aquasol-2 were purchased from New England Nuclear Corp. Green indicator plates, rich LB medium, and minimal medium E containing 0.2% glucose as a carbon source were prepared as described previously (11). Tetracycline hydrochloride was added to media at a concentration of 20 μg/ml unless otherwise stated. Additions to minimal medium E were L-aspartate (560 μM), L-tyrosine (180 μM), and thiamine (0.50 μM), as required. Auxanography was performed as described previously (11). Plates used for selection of tetracycline-sensitive mutants were prepared with 12 μg of fusaric acid per ml, as described by Bochner et al. (3) and modified by Maloy and Nunn (23).

Tn*10* mutagenesis. Defective P22 phage carrying Tn*10* were prepared from *S. typhimurium* lysogen NK337 by thermal induction (11). P22 tail protein from *E. coli* strain MM294 bearing tail-producing plasmid pPB13 (2) was attached to the phage particles (11). Transposition mutagenesis of strain LT2 was done as described previously (11). After 2 days of incubation at 41°C, transductant colonies from LB plates containing 12.5 μg of tetracycline per ml and 10 mM EGTA [ethylene glycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid] were transferred by using toothpicks to grid patterns on LB plates containing 12.5 μg of

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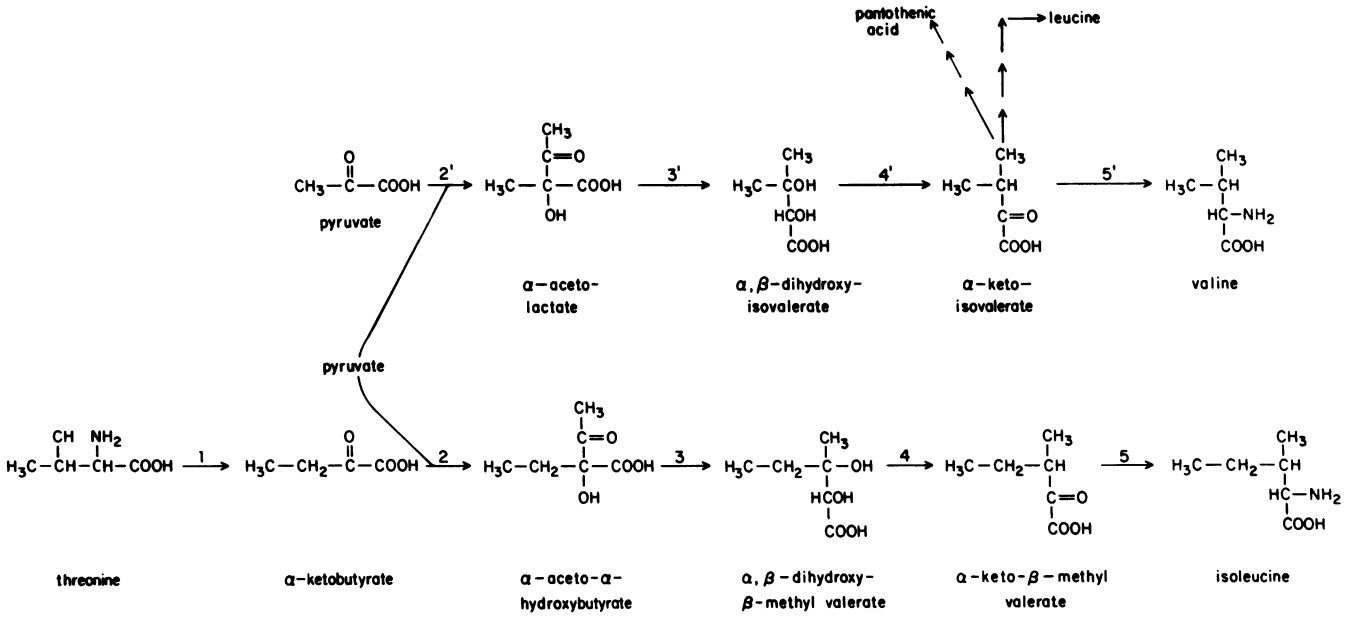

FIG. 1. Branched-chain amino acid and pantothenate biosynthesis. Threonine deaminase catalyses step 1. Acetolactate synthase catalyzes parallel reactions 2 and 2'. ALS II efficiently catalyzes both reaction 2 and reaction 2'. Catalysis of reaction 2 by ALS I is inefficient both in vivo (25) and in vitro (29). SM inhibits ALS II (20) without affecting the catalysis mediated by ALS I (21). Single enzymes catalyze reactions 3 and 3', reactions 4 and 4', and reactions 5 and 5'.

tetracycline per ml and 10 mM EGTA, which were incubated at 37°C overnight. These preparations were replica-plated to minimal medium E plates supplemented with tetracycline, minimal medium E plates supplemented with tetracycline and 140 μM SM, and LB plates containing tetracycline. After overnight incubation at 37°C, the plates were scored for transductants that grew on minimal medium E but did not grow on the SM-supplemented minimal medium E plates. Putative SM-sensitive mutants were purified on green indicator plates.

Genetic methods. P22 generalized transduction of *S. typhimurium* was performed with P22HTΔ*int-4* (obtained from B. N. Ames) as described previously (11) by using a multiplicity of infection of 0.8. P1 generalized transduction of *E. coli* was done by using P1clr100Cm as described previously (24). The method of Chumley et al. (7, 11) was used for Tn*10*-directed insertion of F' ts114 *lac*$^+$ plasmids to form Hfr derivatives of strain SMS409. The Hfr donor strains (10^8 cells) were mated with a series of streptomycin-resistant recipients (10^8 cells) and plated on minimal medium E plates containing 1.0 mg of streptomycin per ml. After incubation at 37°C for 2 days, the recombinant colonies were counted.

Determination of MICs. Fresh overnight cultures of the strains to be tested were collected by centrifugation and suspended at 10-fold dilutions in unsupplemented minimal medium E. Then 5 μl (2×10^6 cells) was spotted onto minimal medium E plates containing 0.1 to 20 mM AKB or 55 to 2,750 μM SM. Thiamine was added for tests of *E. coli* strains. The plates were scored after overnight growth at 37°C. The MIC of AKB was defined as the concentration at which the cells showed no growth. The MIC of SM was defined as the concentration at which the cells showed only very weak growth.

Aminotransferase assays. Cells were grown in minimal medium E at 34°C to a density of 2×10^8 cells per ml, chilled on ice, harvested, washed with 0.85% NaCl in phosphate buffer (pH 7.0), and stored as frozen pellets at −20°C. Cell-free extracts were made by sonication, and the conversion of oxaloacetate (OAA) to aspartate was measured in a glutamate dehydrogenase-coupled assay as described by Gelfand and Steinberg (19). The concentration of freshly prepared OAA used was 0.4 mM. Initial velocities were corrected for the low background rate present prior to enzyme addition. Protein concentrations were determined by the Coomassie brillant blue binding assay (Bio-Rad Laboratories) of Bradford (4).

Accumulation of AKB. The accumulation of threonine-derived AKB was monitored by using a minor modification of the radiochemical method of Daniel et al. (9). Portions (2 ml) of exponentially grown cultures shaken at 34°C in minimal medium E supplemented with 0.2% glucose were labeled with L-[U-^{14}C]threonine in the presence or absence of 100 μM SM. At various times samples from the cultures were reacted to form dinitrophenylhydrazone derivatives and extracted with toluene, and the radioactivity of the organic phase was determined.

RESULTS

Isolation of SM-sensitive mutants. A collection of 5,000 random Tn*10* insertion mutants of *S. typhimurium* LT2 was screened for growth on minimal medium in the presence and absence of 140 μM SM. Auxotrophic mutants were found at a frequency of 0.8%. Mutants whose growth was slowed on SM-containing medium compared with their growth on minimal medium were found at a frequency of 0.4%. P22 phage stocks grown on 19 SM-sensitive mutants were used to transduce *S. typhimurium* LT2 to tetracycline resistance. In 15 of the 19 cases, the transductants were SM sensitive, indicating that the mutation in these isolates was due to insertion of Tn*10*. These 15 SM-sensitive transductants which were used for further study were designated SMS strains.

The 15 strains bearing Tn*10*-linked SM-sensitive mutations were characterized by determining the MICs of SM and AKB. Many different phenotypes were present. As Table 2

TABLE 1. Bacterial strains used

Strain	Genotype	Source or reference
S. typhimurium		
LT2		Laboratory collection
NK337	*hisC527 leu-414 supE* (P22 *c*2ts29 12⁻amN11 13⁻amH101 *int-3*, Tn*10*)	11
SA2676	Δ*pyrD121*	K. E. Sanderson
SA2978	*aroA124*	K. E. Sanderson
SGSC 163	*galE503*	G. Ames via K. E. Sanderson
SMR102	chr::Tn*10*[a]	Tn*10* mutagenesis of LT2
SMS409	*aspC409*::Tn*10*	Tn*10* mutagenesis of LT2
SMS409/20a	*aspC409*::Tn*10*/F' ts114 *lac*⁺ *zzf-20*::Tn*10* (A)	TT627 × SMS409 → Lac⁺
SMS409/21b	*aspC409*::Tn*10*/ F'ts114 *lac*⁺ *zzf-21*:: Tn*10* (B)	TT628 × SMS409 → Lac⁺
SMS409/22a	*aspC409*::Tn*10*/F' ts114 *lac*⁺ *zzf-22*::Tn*10* (A)	TT629 × SMS409 → Lac⁺
TR5654	*rpsL1 thrA9*	11
TR5655	*rpsL1 leu-485*	11
TR5655	*rpsL1 proA36*	11
TR5657	*rpsL1 purE8*	11
TR5658	*rpsL1 pyrC7*	11
TR5660	*rpsL1 pyrF146*	11
TR5662	*rpsL1 his-2236*	11
TR5663	*rpsL1 purF145*	11
TR5664	*rpsL1 cysA533*	11
TR5665	*rpsL1 cysC519*	11
TR5666	*rpsL1 serA13*	11
TR5667	*rpsL1 cysG439*	11
TR5668	*rpsL1 cysE396*	11
TR5669	*rpsL1 ilv-508*	11
TR5670	*rpsL1 metA53*	11
TR5671	*rpsL1 pyrB64*	11
TT627	*pyrC7 rpsL1*/F' ts114 *lac*⁺ *zzf-20*:: Tn*10* (A)	11
TT628	*pyrC7 rpsL1*/F' ts114 *lac*⁺ *zzf-21*::Tn*10* (B)	11
TT629	*pyrC7 rpsL1*/F' ts114 *lac*⁺ *zzf-22*::Tn*10* (A)	11
TT6197	*pncB150*::Tn*10*	26
TV041	*aspC409*::Tn*10 relA*::IE[b]	P22(SMS409) × TV7542.3 → Tc^r
TV062	*aspC409*::IE[b]	Spontaneous Tc^s mutant of SMS409
TV063	*aspC409*::IE[b]	Spontaneous Tc^s mutant of SMS409
TV064	*aspC409*::IE[b]	Spontaneous Tc^s mutant of SMS409
TV065	*aspC409*::IE[b]	Spontaneous Tc^s mutant of SMS409
TV066	*aspC409*::IE[b]	Spontaneous Tc^s mutant of SMS409
TV7542.3	*relA*::IE[b]	21
E. coli		
CY307	*zcb-222*::Tn*10 pyrD34 relA1 spoT1 metB1*	J. Cronan via B. Bachmann
DG30	*proA2 aspC13 hisG4 ilvE12 argE3 thi-1 tyrB507 hsdS14 hppT29 lacY1 galK2 xyl-5 mtl-1 rpsL31 tsx-33* λ⁻ *supE44 recB21 recC22 sbcB15*	19
MC4100	F⁻ *araD139* Δ(*lacIPOZYA*)*U169 rpsL thi*	5
MM294(pPB13)	*endA hsdR thi pro* (pPB13)	2
TV1000	Same as DG30, *zcb-222*::Tn*10 pyrD34*	P1(CY307) × DG30 → Tc^r (AspC⁻ Ura⁻)
TV1002	Same as MC4100, *zcb-222*::Tn*10*	P1(TV1000) × MC4100 → Tc^r (AspC⁺ Ura⁺)
TV1006	Same as MC4100, *zcb-222*::Tn*10 aspC13*	P1(TV1000) × MC4100 → Tc^r (AspC⁻ Ura⁺)

[a] Chromosomal insertion of Tn*10*.
[b] IE, Imprecise exision of Tn*10*.

shows, the SM MICs ranged from 60 μM to 1.4 mM for the SMS mutants, compared with a value of more than 2.8 mM for the parent strain (strain LT2) and a strain containing a random insertion of Tn*10* into the chromosome (strain SMR102). The AKB MICs for the mutants were both lower (0.4 mM) and higher (10 mM) than the MIC for the parent strain (2.5 mM).

Phenotypes of strain SMS409. Strain SMS409 was hypersensitive to AKB as well as SM, having an SM MIC of 210 μM and an AKB MIC of 0.4 mM. In contrast to the other insertion mutants, the growth of this mutant was severely inhibited on minimal medium at 42°C. When the Tn*10*-linked mutation in this strain was transduced into a *relA* mutant, strain TV7542.3, the resulting tetracycline-resistant transductant, strain TV041, did not grow on minimal medium at any temperature tested (30 to 42°C). Of the 20 amino acids, addition of only aspartic acid allowed growth of both strain SMS409 at 42°C and strain TV041 at any temperature.

Biosynthesis of aspartate occurs by transamination of OAA, a Kreb's cycle intermediate (Fig. 2). In *E. coli*, the product of *aspC* (aspartate aminotransferase; EC 2.6.1.1) and the product of *tyrB* (tyrosine aminotransferase; EC 2.6.1.5) both catalyze the conversion of OAA to aspartate (19). Since in *E. coli tyrB* is repressed by tyrosine (19), we expected that tyrosine would be inhibitory if strain SMS409 contained an insertion of Tn*10* in the *S. typhimurium aspC* gene. In fact, the presence of tyrosine in minimal medium completely inhibited the growth of strain SMS409, and this

TABLE 2. MICs of SM and AKB for SMS mutants and other strains

Strain	MIC of SM (μM)	MIC of AKB (mM)
SMS429	60	0.4
SMS103	100	0.4
SMS401	140	0.4
SMS409	210	0.4
SMS209	210	1.0
SMS406	210	1.0
SMS408	210	1.0
SMS419	210	1.0
SMS211	410	1.0
SMS421	550	0.4
SMS301	410	10
SMS307	410	10
SMS404	820	10
SMS424	1,400	0.4
SMS001	1,400	5
LT2	>2,800	2.5
SMR102	>2,800	2.5

inhibition was reversed when aspartate was added to the medium. Both aspartate and isoleucine reversed the SM sensitivity of strain SMS409.

The observed phenotypes of strain SMS409 were shown to be genetically linked by cotransduction. When strain SMS409 was transduced with phage P22 grown on *S. typhimurium* LT2 to aspartate prototrophy in the presence of tyrosine, all 50 transductants tested were SM resistant and tetracycline sensitive. Likewise, the SM sensitivity and the tyrosine-dependent aspartate auxotrophy of strain SMS409 were 98% cotransduced to strain LT2 with tetracyline resistance. The single tetracycline-resistant transductant which grew in the presence of tyrosine and was SM resistant may have been due to transposition of Tn*10* to another chromosomal location. Therefore, the determinants for the tetracycline-resistant, SM-sensitive, and tyrosine-dependent aspartate-requiring phenotypes were limited to a small region of the chromosome and were most likely due to a single mutational event.

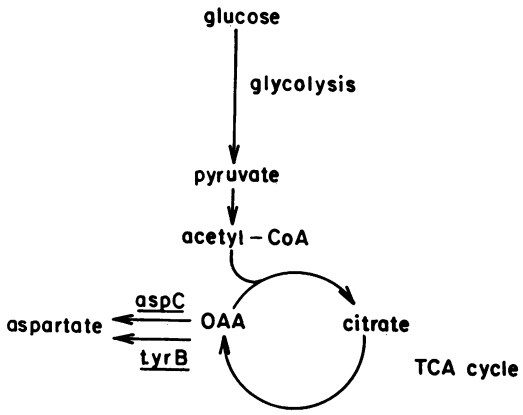

FIG. 2. Biosynthesis of aspartate in relation to glycolysis and the tricarboxylic acid (TCA) cycle. The *aspC* product, aspartate aminotransferase, catalyzes the formation of aspartate from OAA with a greater V_{max} than the *tyrB* product, tyrosine aminotransferase, does.

TABLE 3. Aspartate aminotransferase activities of *aspC* mutants of *S. typhimurium* and *E. coli*

Strain	Sp act (μmol of NADPH oxidized per min per mg of extract protein)[a]		
	With glutamic acid	Without glutamic acid	Difference
S. typhimurium			
SMS409	13	4	9
SMR102	171	9	162
E. coli			
TV1006(*aspC13*)	26	<1	26
TV1002(*aspC*$^+$)	145	<1	145

[a] Specific activities were determined by using a glutamate dehydrogenase-coupled assay with OAA as the substrate as described in Materials and Methods. The differences in the results of assays with and without glutamic acid represent the aminotransferase activities.

Aspartate aminotransferase activity in strain SMS409. In a glutamate dehydrogenase-coupled assay which measured the conversion of OAA to aspartate, extracts from *S. typhimurium* SMS409 had 6% of the aspartate aminotransferase specific activity of extracts from the isogenic control strain, strain SMR102 (Table 3). These results are consistent with the hypothesis that strain SMS409 contains a Tn*10* insertion in the *aspC* locus of *S. typhimurium*. The low level of aspartate aminotransferase activity remaining in strain SMS409 may have been due to the activities of tyrosine aminotransferase, the *tyrB* gene product, and other transaminases.

Genetic mapping of the Tn*10* insertion in strain SMS409. Tn*10*-mediated Hfr formation yielded three donors for the conjugation experiments designed to map the Tn*10* insertion in strain SMS409. Table 4 shows that the Tn*10* insertion was between *purE* (11 min) and *pyrC* (23 min) on the *S.*

TABLE 4. Hfr mapping of *aspC409*::Tn*10*[a]

Recipient	Selected marker	Map position (min)	No. of prototrophic recombinant colonies with:		
			Donor strain SMS409/20a	Donor strain SMS409/22a	Donor strain SMS409/21b
TR5654	*thrA*	0	29	36	0
TR5655	*leu*	2	362	650	8
TR5656	*proA*	7	49	220	0
TR5657	*purE*	11	517	768	4
TR5658	*pyrC*	23	2	21	149
TR5660	*pyrF*	33	5	25	67
TR5662	*his*	42	87	222	526
TR5663	*purF*	47	5	1	2
TR5664	*cysA*	49	0	0	0
TR5665	*cysC*	60	0	0	2
TR5666	*serA*	62	0	0	1
TR5667	*cysG*	72	0	1	0
TR5668	*cysE*	79	6	2	2
TR5669	*ilv*	83	10	23	3
TR5670	*metA*	89	73	181	12
TR5671	*pyrB*	98	12	18	2

[a] Homologous recombination between the F′ ts114 plasmids and Tn*10* in the chromosome resulted in Hfr strains. The direction of transfer for strains SMS409/20a and SMS409/22a (direction A) was opposite the direction of transfer for SMS409/21b (direction B). Matings with strain TR5657 (*purE8*; 11 min) gave large numbers of recombinants for transfer direction A, white matings with strain TR5658 (*pyrC7*; 23 min) gave few recombinants. The opposite result was obtained for transfer direction B. This indicates that Tn*10* lies between *purE* and *pyrC* on the chromosome.

TABLE 5. Cotransduction of markers near 20 min on the *S. typhimurium* chromosome

Donor (marker)	Recipient (marker)	Selected marker (No. scored)	Unselected marker (no.)	% Cotransduction
SMS409 (*aspC409*::Tn*10*)	SGSC 163 (*galE503*)	Tc^r (1,732)	Gal$^+$ (0)	<0.05
SMS409	SA2676 (Δ*pyrD121*)	Tc^r (3,304)	Ura$^+$ (0)	<0.03
SMS409	SA2978 (*aroA124*)	Tc^r (4,013)	Aro$^+$ (94)	2.3
TT6197 (*pncB150*::Tn*10*)	TV062 (*aspC409*::*IE*)	Tc^r (136)	AspC$^+$ (3)[a]	2.2
TT6197	TV063 (*aspC409*::*IE*)	Tc^r (100)	AspC$^+$ (46)	46
TT6197	TV064 (*aspC409*::*IE*)	Tc^r (100)	AspC$^+$ (89)	89
TT6197	TV065 (*aspC409*::*IE*)	Tc^r (100)	AspC$^+$ (67)	67
TT6197	TV066 (*aspC409*::*IE*)	Tc^r (440)	AspC$^+$ (5)	1.1
TT6197	SA2978 (*aroA124*)	Tc^r (1,284)	Aro$^+$ (0)	<0.1

[a] AspC$^+$, Growth in the presence of 180 μM tyrosine.

typhimurium chromosome (26). Cotransduction experiments with markers in this region (Table 5) revealed that the Tn*10* insertion was 2.3% linked to *aroA* (19.3 min). To show linkage to *pncB* (20 min), five independent tetracycline-sensitive derivatives of strain SMS409 were selected. Using these strains as recipients and phage P22 grown on a strain carrying a *pncB*::Tn*10* mutation as the donor, we observed frequencies of cotransduction of tetracycline resistance and aspartate prototrophy in the presence of tyrosine of 1 to 89% (Table 5). The variation in the cotranduction frequencies observed was most likely due to differences in the molecular nature of the events which resulted in the tetracycline sensitivity of the recipients. We observed less than 0.1% cotransduction of the *aroA* and *pncB* markers (Table 5); therefore, the gene order is *aroA*-Tn*10*-*pncB*. In *E. coli*, *aspC* is located between the *aroA* and *pncB* loci (1, 18). Therefore, the position of Tn*10* in strain SMS409 is consistent with insertion of Tn*10* into the *aspC* locus, a previously unidentified gene in *S. typhimurium*.

Phage P22 preparations grown on the 14 other strains bearing Tn*10*-linked SM-sensitive mutations were used to test for cotransduction of *aroA* and Tn*10*. No cotransduction was observed; therefore, the mutation in strain SMS409 was unique in this set of 15 SM-sensitive mutants, in agreement with its observed phenotypic distinction in terms of MICs and conditional auxotrophy.

Phenotypes of an *E. coli aspC* mutant. The previously described *E. coli aspC* mutations were in strains which also contained mutations in the genes for other transaminases. To construct a strain lacking only the aspartate aminotransferase, a Tn*10* marker near *aspC* was introduced into strain DG30 by transduction with phage P1 grown on strain CY307, selecting for tetracycline resistance. Phage P1 grown on the resulting strain, strain TV1000, was used to transduce strain MC4100 to tetracycline resistance. The transductants were screened for the *aspC* mutation by scoring for growth in the presence of tyrosine. A total of 8% of the transductants were unable to grow in the presence of tyrosine; this growth inhibition was prevented by aspartate.

The aspartate aminotransferase activity of extracts of one of these *aspC*$^-$ *E. coli* strains, strain TV1006, was greatly reduced (18%) compared with the activity of extracts of an isogenic *aspC*$^+$ strain, strain TV1002 (Table 3). The SM MIC for strain TV1006 was 1.4 mM, a concentration that did not inhibit strain TV1002. This level of SM inhibition of an *aspC* *E. coli* mutant was not as dramatic as the level of inhibition observed in *S. typhimurium* mutant SMS409. The difference in sensitivity may reflect ALS isozyme differences between the two species (13). The reduced aspartate aminotransferase activity, the SM sensitivity, and the tyrosine-dependent aspartate auxotrophy of a known *aspC* mutant of *E. coli* were additional evidence that the mutation in *S. typhimurium* SMS409 was at the *aspC* locus.

AKB accumulation by *S. typhimurium* upon SM treatment. The in vivo conversion of exogenously added ^{14}C-labeled L-threonine to AKB after treatment of cells with 100 μM SM was monitored. AKB accumulated in both *aspC*$^-$ strain SMS409 and *aspC*$^+$ strain SMR102 (Fig. 3). The somewhat greater accumulation of AKB in strain SMS409 cultures may have been due to depletion of the endogenous threonine pool, which resulted from lower levels of the precursor, aspartate. The difference between the levels of AKB accumulation in strain SMS409 and SMR102 cultures was not substantial compared with the greatly increased rate of AKB accumulation observed after SM treatment of other SMS mutants. In contrast to the 5×10^{-18} and 9×10^{-18} mol of AKB per cell accumulated by strains SMR102 and SMS409, strain SMS429 accumulated 50×10^{-18} mol of AKB per cell 20 min after SM treatment and continued to accumulate AKB for an additional 20 min. This suggests that production of AKB is not greatly altered by the insertion in *aspC*. Rather, strain SMS409 cells may be unable to overcome the

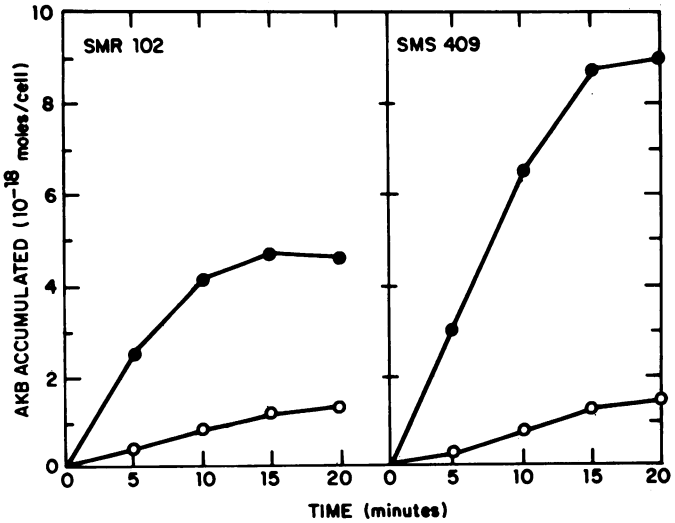

FIG. 3. Accumulation of AKB in SM-stressed cultures of strains SMR102 (*aspC*$^+$) and SMS409 (*aspC*$^-$). The accumulation of AKB from exogenously added [^{14}C]threonine was assayed as described in Materials and Methods. Symbols: ●, 100 μM SM added at zero time; ○, no addition.

toxicity associated with the normal accumulation of AKB caused by exposure of the cells to SM.

DISCUSSION

One mutant (strain SMS409) of a set of Tn*10*-linked SM-sensitive mutants had tyrosine-dependent aspartate auxotrophy and was hypersensitive to AKB. Strain SMS409 contained reduced levels of aspartate aminotransferase activity. The mutation in strain SMS409 mapped at 20 min on the *S. typhimurium* genetic map between *aroA* and *pncB*. A known *E. coli aspC mutation conferred similar phenotypes, and the aspC* gene of *E. coli* has a similar map position (1, 18). We conclude that the mutation in strain SMS409 is due to insertion of Tn*10*, which inactivates the *aspC* gene of *S. typhimurium*. Mutations in the *aspC* locus of *S. typhimurium* have not been described previously.

Loss of the *aspC* gene product (aspartate aminotransferase) in *E. coli* does not result in an auxotrophic requirement for aspartate because another transaminase, the product of the *tyrB* gene, can also convert OAA to aspartate (19) (Fig. 2). However, *tyrB* is repressed by tyrosine (19); thus, an *E. coli* strain with a mutation in the *aspC* gene required aspartate in the presence of tyrosine. Similarly, *S. typhimurium* SMS409 had a tyrosine-dependent requirement for aspartate due to inactivation of *aspC*.

Inactivation of *aspC* in *S. typhimurium* also resulted in sensitivity to SM and increased sensitivity to AKB. SM-stressed *S. typhimurium* SMS409 (*aspC*$^-$) and SMR102 (*aspC*$^+$) accumulated AKB (Fig. 3), an intermediate just prior to the metabolic block imposed by SM (Fig. 1). Danchin et al. have shown that the concentrations of several glycolytic intermediates drop after treatment of *E. coli* cells with AKB and have proposed that the input into the tricarboxylic acid cycle should also decrease, lowering the intracellular concentration of OAA (8). Since in *E. coli* the V_{max} of the *tyrB* product is much less than the V_{max} of the *aspC* product in aspartate formation (19), a lowered concentration of OAA may not be sufficient for the *tyrB* product alone to supply the cells with aspartate for growth. Thus, we propose that in an SM-stressed *S. typhimurium aspC* mutant, AKB accumulates and results in a lower level of OAA such that the cells are starved for aspartate. The observation that either aspartate or isoleucine reverses the SM inhibition of strain SMS409 is in agreement with this hypothesis. Aspartate reverses the inhibition by supplying the induced auxotrophic requirement; isoleucine reverses the inhibition by feedback inhibition of threonine deaminase such that synthesis of AKB is reduced. Alternatively, the aspartate requirement could result if the AKB which accumulates after SM addition were a competitive inhibitor of tyrosine aminotransferase in the aspartate-forming reaction. Initial experiments to test this hypothesis were hindered by a high background rate in a coupled assay. However, these possibilities may be distinguished by an analysis of pseudorevertants.

Only strain SMS409 from a collection of 15 SM-sensitive mutants contained an *aspC* mutation. Further genetic analysis of the SMS mutants showed that insertions into at least four other genes also gave rise to this phenotype (Van Dyk and LaRossa, unpublished data). Therefore, inactivation of many genes resulted in sensitivity to SM. The many ways that *S. typhimurium* can be mutated to SM sensitivity suggest the key place in metabolism affected by this inhibitor. The effect of AKB accumulation on metabolic pathways other than the pathway for branched-chain amino acid biosynthesis was demonstrated in the *aspC* mutant of *S. typhimurium*. Thus, the toxicity of SM in the *aspC* mutant was due to accumulation of AKB, as well as to depletion of branched-chain amino acids and pantothenate. This accumulation of AKB may also be an important factor in the herbicidal action of SM.

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