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Role of Alanine-Valine Transaminase in *Salmonella typhimurium* and Analysis of an *avtA*::Tn*5* Mutant

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In *Salmonella typhimurium*, as in *Escherichia coli*, mutations in *avtA*, the gene encoding the alanine-valine transaminase (transaminase C), are silent unless they are combined with mutations involved in isoleucine-valine biosynthesis. *avtA* is repressed by leucine or alanine but not by valine. Transaminase C is found at reduced levels upon starvation for any one of several amino acids. We hypothesize that this is due to repression of *avtA* by the elevated alanine and leucine pools found in amino acid-starved cells.

In *Escherichia coli*, four general transaminases with overlapping specificities, encoded by unlinked genes, have been described (9, 10, 14). In *Salmonella typhimurium*, only one of these, the branched-chain amino acid transaminase (transaminase B) has been described, and mutations have been isolated only in the gene (*ilvE*) encoding this enzyme (1, 15). We show here that *S. typhimurium* also possesses the alanine-valine transaminase (transaminase C). We describe some properties of transaminase C and of Tn*5*-induced mutations in *avtA*, the gene encoding this enzyme. The scheme used to isolate *avtA* mutants was to select isoleucine- and valine-requiring derivatives of an *ilvE* (Ile$^-$) strain which could not grow on isoleucine plus α-ketoisovalerate and which cross-fed an *ilvD* mutant (see Fig. 1).

MATERIALS AND METHODS

Bacterial strains and phage. Table 1 lists the strains of *S. typhimurium* LT2 and *E. coli* K-12 used. Cultures were grown at 30°C except where noted. The phage used were P22 (HT, *int*) (16) and P1Cmc1.100 (13), herein called P22 and P1, respectively.

Chemicals and media. Antibiotics, substrates, Coomassie blue, bovine serum albumin, and cofactors were purchased from Sigma Chemical Co., St. Louis, Mo.

Lennox (L) complex medium and Vogel and Bonner glucose-medium E salts were employed as described previously (2). The carbon source was glucose at 0.5 or 0.05% (limiting glucose). Medium E was supplemented as required with a millimolar concentration of the following: α-ketoisovalerate, 0.14; L-alanine, 0.23; L-isoleucine, 0.15; L-valine, 0.17; L-leucine, 0.15; L-cysteine, 0.17; or L-methionine, 0.13. Ampicillin (25 μg/ml), tetracycline (25 μg/ml), or kanamycin (30 μg/ml) was added to complex media where indicated. For transductant selection, sodium chloride was replaced by 5 μg of sodium citrate per ml. For P22 transduction of Gal$^-$ strains, L broth was supplemented with 1 g of D-galactose per liter (6).

Isolation and characterization of mutants. CBS501 (*ilvE*::Tn*10*) (Ile$^-$) was mutagenized by Mu *d*1 (3), using the procedure of Csonka et al. (4), or by Tn*5*, using the procedure of Berg and Simpson (manuscript in preparation). For Tn*5* mutagenesis, P1Cmc1 · 100 (13) carrying Tn*5* (Kan), herein called P1::Tn*5*, was used as the transposon vector. CBS501 was infected by P1::Tn*5* (grown on *E. coli*) at a multiplicity of infection of ~1. Since the restriction enzymes in *S. typhimurium* restrict most vector molecules which lack the *S. typhimurium* modification, P1::Tn*5* grown on *E. coli* serves as a suicide vector for Tn*5*.

Mutagenized cells were grown to saturation with aeration in L broth plus ampicillin (Mu *d*1 mutagenized) or kanamycin (Tn*5* mutagenized). Penicillin or D-cycloserine enrichment was used to kill nonauxotrophs (5). The permissive medium contained isoleucine, alanine, valine, and α-ketoisovalerate, whereas the starvation medium contained isoleucine and α-ketoisovalerate. After enrichment, the cells were plated on L agar plus the appropriate antibiotic and then replica plated to medium E plus isoleucine. Valine-requiring derivatives were characterized as described previously (18).

Enzyme assays. Cells were grown overnight in glucose-limited medium E containing the required supplements plus 1.12 mM alanine to repress *avtA*. Cells were harvested by centrifugation, washed in medium E salts, suspended at an optical density of 0.15 at 600 nm in medium E containing the required supplements plus the indicated amino acids, and grown for 2 h (at least two doublings). Cells were harvested by centrifugation and washed twice in cold medium E salts. Transaminase C was assayed by using alanine and α-ketoisovalerate as substrates, with activity measured by the production of pyruvate, as described by McGilvray and Umbarger (11) except that crude extracts were used (18). Specific activity is expressed as nanamoles of pyruvate produced per minute per milligram of protein.

RESULTS

Isolation of *avtA* mutants: By the isolation procedure described above, nine Tn*5*-induced

TABLE 1. Bacterial strains used

Strain	Genotype	Derivation or source
E. coli		
W3110(P1Cmc1.100[::Tn5])	*thyA*	D. A. Simpson and C. M. Berg (unpublished data)
S. typhimurium		
CBS101	*ilvG593*::Tn*10* *ilvB2771*::Tn*5* *ilvHI cys*::Tn*9*	K. J. Shaw and C. M. Berg (unpublished data)
CBS106	*ilvG593*::Tn*10* *ilvB2771*::Tn*5* *ilvHI met*::Tn*9*	K. J. Shaw and C. M. Berg (unpublished data)
CBS501	*ilvE2101*::Tn*10* *galE1122*	Transduction of JL3404 to Tet^r with P22 · TT79[a]
CBS514	*ilvE2101*::Tn*10* *avtA1*::Tn*5* *galE1122*	Mutagenesis of CBS501 with P1::Tn*5* · *E. coli*
CBS521	*avtA1*::Tn*5* *galE1122*	Transduction of CBS514 to Ile$^+$ with P22 · JL3404
CBS526	Δ*ilvGE* *avtA1*::Tn*5* *galE1122*	Tet^s derivative of CBS514[b]
CBS527	Δ*ilvGE* *galE1122*	Transduction of CBS526 to Val$^+$ with P22 · JL3404
CBS529	*ilvA2115*::Tn*10* *galE1122*	Transduction of JL3404 to Tet^r with P22 · TT93
CBS530	*ilvA2115*::Tn*10* *avtA1*::Tn*5* *galE1122*	Transduction of CBS521 to Tet^r with P22 · TT93
CBS531	*ilvC2104*::Tn*10* *galE1122*	Transduction of JL3404 to Tet^r, using P22 · TT82
CBS532	*ilvC2104*::Tn*10* *avtA1*::Tn*5* *galE1122*	Transduction of CBS521 to Tet^r, using P22 · TT82
CBS533	*ilvG593*::Tn*10* *galE1122*	Transduction of JL3404 to Tet^r, using P22 · TT56
CBS534	*ilvG593*::Tn*10* *avtA1*::Tn*5* *galE1122*	Transduction of CBS521 to Tet^r, using P22 · TT56
CBS535	*ilvE2157*::Tn*10* *galE1122*	Transduction of JL3404 to Tet^r, using P22 · TT1284
CBS536	*ilvE2157*::Tn*10* *avtA1*::Tn*5* *galE1122*	Transduction of CBS521 to Tet^r, using P22 · TT1284
CBS537	*ilvE2101*::Tn*10* *leu*::Tn*5*	Mutagenesis of CBS501 with P1::Tn*5* · *E. coli*
JL3404	*galE1122*	L. Csonka (4)
TT56	*ilvG593*::Tn*10*	J. Roth (1)
TT79	*ilvE2101*::Tn*10*	J. Roth (1)
TT82	*ilvC2104*::Tn*10*	J. Roth (1)
TT83	*ilvD2105*::Tn*10*	J. Roth (1)
TT93	*ilvA2115*::Tn*10*	J. Roth (1)
TT1284	*ilvE2157*::Tn*10*	J. Roth (1)

[a] Transduction was carried out as described by Schmeiger (16) except that galactose (1 g/liter) was added to L broth when the recipient was Gal$^-$ (6).

[b] Isolated by penicillin counterselection (8). Tetracycline (25 μg/ml) was added to mid-log-phase cells, and the culture was incubated in L broth with aeration for 30 min. Penicillin (2,000 U/ml) was added, and the incubation was continued for 90 min. Dilutions of the culture were plated on L agar, and survivors were tested for tetracycline sensitivity.

and one Mu *d*1-induced presumptive *avtA* derivatives of CBS501 (*ilvE*::Tn*10*) were obtained. These mutants required valine in addition to isoleucine, none could grow on medium supplemented with isoleucine plus α-ketoisovalerate in place of valine, and all cross-fed an *ilvD* tester strain (Fig. 1). Transaminase C activity was absent from extracts of three of the nine Tn*5*-induced mutants. One of these mutants, CBS514, was chosen for further study. The other mutants, which had normal transaminase C levels, appear to have a defect in an alanine-glutamate transaminase (Whalen and Berg, manuscript in preparation).

Additional growth requirements conferred upon some *ilv* strains by *avtA*. Because valine synthesis is catalyzed by either transaminase B or transaminase C and alanine synthesis is catalyzed by transaminase C plus at least two other enzymes (unpublished data), a mutation in *avtA* alone does not affect the ability of the strain to grow on minimal medium. It is when *avtA* is combined with *ilv* mutations that *avtA*-dependent phenotypes are observed (Table 2).

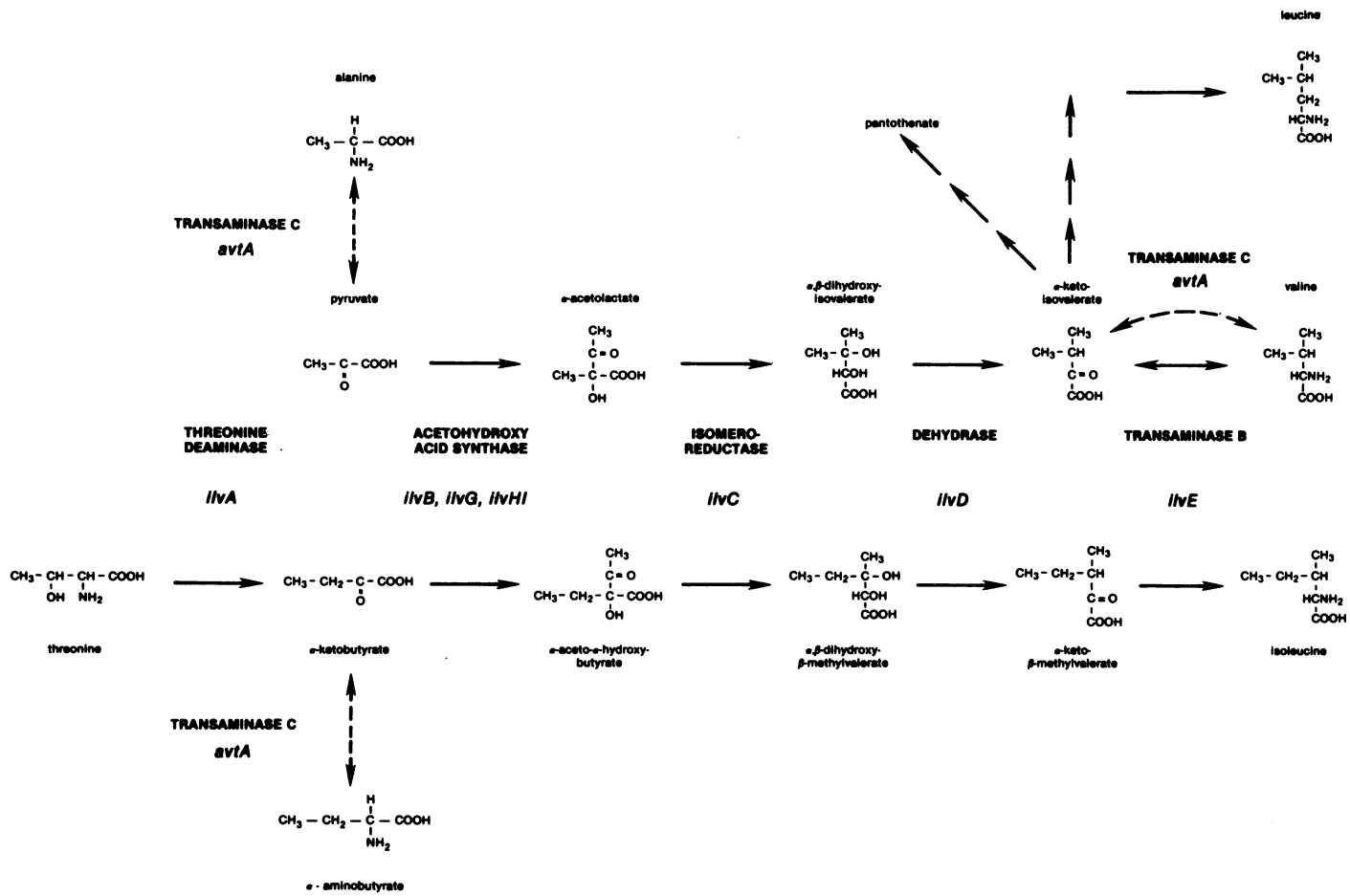

FIG. 1. Biosynthetic pathways for isoleucine, valine, leucine, pantothenate, and α-aminobutyrate and one of the alanine biosynthetic routes. The genes specifying these enzymes are given beneath the respective enzymes. Since *avtA* mutants do not require alanine for growth, alanine biosynthesis can proceed by at least one other route.

TABLE 2. Growth requirements of mutants

Strain	Relevant genotype	Supplement added[a]							
		None	ABA	αKB	Ile	Ile + Leu	Ile + KIV	Ile + Val	Ile + Val + Leu
CBS521	*avtA*	+	+	+	+	+	+	+	+
CBS529	*ilvA*	−	+	+	+	+	+	+	+
CBS530	*ilvA avtA*	−	±	+	+	+	+	+	+
CBS533	*ilvG*[b]	−	+	+	+	+	+	+	+
CBS534	*ilvG*[b] *avtA*	−	−	+	+	+	+	+	+
CBS501	*ilvE*[b]	−	−	−	+	+	+	+	+
CBS514	*ilvE*[b] *avtA*	−	−	−	−	−	−	+	+
CBS527	Δ*ilvGE*[b]	−	−	−	+	+	+	+	+
CBS526	Δ*ilvGE*[b] *avtA*	−	−	−	−	−	−	i, +	+
CBS535	*ilvE*[c]	−	−	−	−	±	+	+	+
CBS536	*ilvE*[c] *avtA*	−	−	−	−	−	−	±	+
CBS531	*ilvC*	−	−	−	−	−	+	+	+
CBS532	*ilvC avtA*	−	−	−	−	−	+	+	+

[a] Abbreviations: ABA, α-aminobutyrate; αKB, α-ketobutyrate; Ile, isoleucine; Leu, leucine; KIV, α-ketoisovalerate; Val, valine. Growth was determined by spreading ~10^6 cells on a minimal medium plate or partially supplemented minimal medium plate and spotting ~0.05 ml of the missing supplement: +, good growth; ±, poor growth; −, no growth; i, +, inhibited for ~15 mm around the valine spot with good growth beyond that.

[b] Constitutive expression of distal *ilv* genes (1).

[c] Very weak constitutive expression of distal *ilv* genes (1).

ilvA strains, unable to synthesize threonine deaminase, can grow on medium containing α-ketobutyrate (Fig. 1). *ilvG* strains, unable to synthesize acetohydroxy acid synthase (AHAS) II, one of the isozymes catalyzing the next step, are able to grow on medium supplemented with α-ketobutyrate, a substrate of AHAS, because AHAS I is able to condense α-ketobutyrate and pyruvate when α-ketobutyrate levels are elevated (17). Both strains can utilize α-aminobutyrate since it is efficiently converted into α-ketobutyrate by transaminase C.

The introduction of *avtA* into *ilvA* and *ilvG* strains interferes with their ability to utilize α-aminobutyrate. CBS530 (*ilvA avtA*) grows slowly on medium supplemented with α-aminobutyrate, in contrast to the comparable *E. coli* mutant which is unable to utilize α-aminobutyrate at all (18). These observations suggest that another transaminase can catalyze the conversion of α-aminobutyrate to α-ketobutyrate to a limited extent in *S. typhimurium* but not in *E. coli*. CBS534 (*ilvG avtA*) does not respond to α-aminobutyrate, indicating that the low level of α-ketobutyrate synthesized is insufficient to satisfy the isoleucine requirement in the absence of AHAS II (*ilvG*).

Two *ilvE*::Tn*10* mutations and a deletion derivative of one were compared. CBS501 (*ilvE2101*::Tn*10*) and CBS527, an *ilvGE* deletion derivative, have a relatively high constitutive level of expression of the operator-distal genes *ilvD* and *ilvA*, whereas CBS535 (*ilvE2157*::Tn*10*) has a barely detectable level of expression of *ilvD* and *ilvA* (1; unpublished data). All three strains have an absolute requirement for isoleucine since they lack transaminase B. CBS535 requires valine or leucine, in addition to isoleucine, because synthesis of α-ketoisovalerate, the precursor of valine, leucine, and pantothenate, is limited by low *ilvD* expression in this strain (1).

The presence of *avtA* in CBS501 and CBS527 (CBS514 and CBS526, respectively) confers a requirement for valine, in addition to isoleucine. Also, CBS526 is valine sensitive, probably because valine feedback inhibits AHAS I, the remaining major AHAS isozyme (12), thereby reducing the amount of leucine synthesized from pyruvate.

CBS535 (*ilvE2157*::Tn*10*) grows well on medium supplemented with valine plus isoleucine and poorly on medium supplemented with leucine plus isoleucine, because this strain expresses *ilvD* weakly and so produces only a limited amount of α-ketoisovalerate, the valine, leucine, and pantothenate precursor. Since valine is converted to α-ketoisovalerate by transaminase C, CBS535 grows well on medium supplemented with isoleucine plus valine. Since leucine inhibits leucine synthesis from α-ketoisovalerate, the addition of exogeneous leucine

serves to spare the limited endogeneous α-ketoisovalerate for valine and pantothenate synthesis, allowing CBS535 to grow, albeit poorly, on medium supplemented with isoleucine plus leucine (1). Introduction of *avtA* into this strain (CBS536) confers an absolute requirement for valine, in addition to isoleucine, and a partial requirement for leucine. We find that CBS536 (*ilvE2157*::Tn*10* *avtA*::Tn*5*) grows at about the same rate on medium supplemented with valine and isoleucine as CBS535 (*ilvE2157*::Tn*10*) grows on medium supplemented with leucine plus isoleucine, showing that the sparing interpretation is correct: sufficient α-ketoisovalerate is synthesized to completely satisfy the pantothenate requirement and to partially satisfy one, but not both, amino acid requirements.

Regulation of *avtA*. In most strains of *E. coli*, *avtA* is repressed by alanine or leucine (7) but not by any other amino acid (18), although there has been one report of a mutant in which valine represses *avtA* (11). We find that in *S. typhimurium*, *avtA* is also repressed by alanine or leucine (two- to threefold) but not by isoleucine. The addition of valine led to a small decrease in *avtA* activity (to about 90% of the unrepressed level), which was not found in *E. coli* (18). Since valine can be converted to leucine and can serve as an amino donor in alanine biosynthesis, the effect of valine is probably indirect: the addition of valine results in increased synthesis of alanine, leucine, or both amino acids and concomitant repression of *avtA*.

We have previously found that leucine, isoleucine, or proline limitation results in a severalfold reduction of *avtA* expression in *E. coli* (18). Similarly, limitation of *S. typhimurium* auxotrophs for leucine, valine, isoleucine, cysteine, or methionine resulted in a two- to fourfold reduction in transaminase C activity.

DISCUSSION

The alanine-valine transaminase, transaminase C, catalyzes the interconversion of alanine, valine, and α-aminobutyrate with their corresponding α-keto acids (14). Transaminase C is not required for the synthesis of alanine or valine since mutants devoid of transaminase C do not require either amino acid (Table 2). *avtA*, the gene encoding transaminase C, is repressed by alanine and not by valine. *avtA* is also repressed by leucine. Since transaminase C does not directly participate in leucine biosynthesis, the primary role of transaminase C, therefore, is probably in alanine biosynthesis.

This study shows that the expression and regulation of *avtA* in *S. typhimurium* are very similar to those of most strains of *E. coli* (7, 18). Only two differences have been detected. (i) An *S. typhimurium* *avtA ilvA* double mutant can synthesize α-ketobutyrate from α-aminobutyrate (a growth-limiting amount), whereas a comparable *E. coli* mutant cannot (18). The responsible enzyme has not been identified. (ii) The addition of valine to the medium results in a small, but reproducible, reduction in transaminase C activity in *S. typhimurium* (see above) but not in *E. coli* (18; unpublished data). This could be due to valine being a weak inhibitor of *avtA* expression in *S. typhimurium* but not in *E. coli* or to differences in leucine or alanine pool sizes in the two genera in the presence of exogenous valine. We favor this latter interpretation because an investigation of the effects of other amino acids, including nonprotein amino acids, upon *avtA* expression indicates that to have a repressor effect, a molecule must, unlike valine, be unbranched at the β-carbon (Whalen and Berg, submitted for publication). In addition, a gene is expected to be repressed by the end product and not by the precursor in a biosynthetic pathway.

Limitation for any of several amino acids causes significantly reduced transaminase C activity in *S. typhimurium* (see above) and *E. coli* (18). We hypothesize that this puzzling finding is not an effect of limitation for a specific amino acid but rather is the result of elevated alanine, leucine, or both pools in the starved cells (although we have not excluded the possibility of a stringent response). This is supported by our finding that in a multiple mutant requiring isoleucine, leucine, and either alanine or valine, transaminase C activity is not reduced when the strain is starved for leucine and alanine (and also valine) (Whalen and Berg, submitted).

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ADDENDUM IN PROOF

We have isolated a putative apoprepressor mutant in which *avtA* is not repressible by alanine, leucine, or amino acid starvation, in support of the hypothesis presented above.

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