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Gratuitous Repression of *avtA* in *Escherichia coli* and *Salmonella typhimurium*

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avtA, which encodes transaminase C (alanine-valine transaminase), is repressed by excess-L-alanine or L-leucine, and also by limitation for any of a number of amino acids in *Escherichia coli* and *Salmonella typhimurium*. Amino acid limitation causes repression by promoting the accumulation of L-alanine or L-leucine or both. *avtA* is also repressed by L-α-aminobutyric acid and other nonprotein amino acids which are structurally similar to L-alanine. We hypothesize that L-alanine and L-α-aminobutyric acid, whose syntheses are catalyzed by transaminase C, are the true corepressors of *avtA*. Repression by structural analogs of the true corepressors is termed gratuitous repression.

In *Escherichia coli* and *Salmonella typhimurium*, the alanine-valine transaminase, transaminase C (TrC), catalyzes the transamination of L-valine, L-alanine, and L-α-aminobutyric acid (Fig. 1) (11). The gene encoding this enzyme, *avtA*, has some unusual regulatory properties. *avtA* is repressed: (i) by L-alanine (2, 5, 14); (ii) by L-leucine, whose synthesis is not catalyzed by TrC (2, 9); and (iii) upon limitation for any single amino acid (2, 14).

We show here that *avtA* is also repressed by nonprotein L-amino acids which are structurally similar to L-alanine and that *avtA* is not repressed when cells are limited for both L-alanine and L-leucine. We hypothesize that L-alanine and L-α-aminobutyric acid are the physiologically relevant corepressors of *avtA* and that the other L-amino acids repress because they are structural analogs of these amino acids, we term such nonphysiological corepressors gratuitous corepressors.

MATERIALS AND METHODS

Bacterial strains and phage. Table 1 lists the strains of *E. coli* K-12 and *S. typhimurium* LT2 used. Cultures were grown at 37°C, except for Mu dl(Ap *lac*) (3) lysogens which were grown at 30°C. Transductions were performed with P1 Cmcl-100 (*E. coli*) or P22 (HT, *int*) (*S. typhimurium*) as described previously (2, 14).

Chemicals. Antibiotics, substrates, Coomassie blue, bovine serum albumin, cofactors, and most amino acids were purchased from Sigma Chemical Co., St. Louis, Mo. D-Leucine and DL-α-aminoheptanoic acid were purchased from Aldrich Chemical Co., Milwaukee, Wis.

Media. Lennox (L) complex medium and Vogel and Bonner glucose-medium E salts were used as described previously (1). The carbon source was glucose at 0.5 or 0.05% (limiting glucose). For growth experiments, medium E was supplemented as required with L-alanine (0.23 mM), L-isoleucine (0.15 mM), L-valine (0.17 mM), L-leucine (0.15 mM), L-cysteine (0.17 mM), L-methionine (0.13 mM), L-histidine (0.14 mM), L-phenylalanine (0.24 mM), L-proline (0.26 mM), thymine (0.079 mM), or thiamine (0.006 mM). Growth-limiting amino acid concentrations were 0.1 the supplemental concentrations. For *avtA* repression experiments, the amino acid was added at 1.0 mM to medium E. Ampicillin (25 μg/ml), tetracycline (25 μg/ml), chloramphenicol (25 μg/ml), or kanamycin (30 μg/ml) was added to complex media where indicated. For P22 transductions with *galE S. typhimurium* strains, maltose (1 g/liter) was added to L broth.

Growth conditions and enzyme assays. Cells were grown overnight with aeration in glucose-limited medium E containing the required supplements plus 1.0 mM L-alanine to repress *avtA*. The following morning, glucose was added to 0.5%, and cultures were grown for 1 h, harvested by centrifugation, and washed in medium E salts. For growth limitation studies, cells were resuspended at an optical density of ca. 0.15 at 600 nm in medium E containing the required amino acids in supplemental or growth-limiting amounts, as indicated. Exponentially growing cultures were harvested after two doublings. Amino acid-limited cultures were harvested as growth started to plateau (2 to 3 h). For repression studies, cultures were resuspended at an optical density of ca. 0.10 at 600 nm in medium E containing the required supplements plus 1.0 mM of the indicated amino acid and grown for two doublings before harvesting.

Cultures were harvested by centrifugation and washed twice in cold medium E salts. *avtA* expression was measured by assaying TrC activity in *avtA*$^+$ strains and β-galactosidase activity in *avtA*::Mu dl(Ap *lac*) strains. TrC was assayed, using crude extracts, by measuring pyruvate production in an α-ketoisovalerate-dependent conversion of alanine to pyruvate (9, 14). TrC activity is expressed as nanomoles of pyruvate produced per minute per milligram of protein. The assays of β-galactosidase activity (14) and transaminase B activity (1) were described previously.

RESULTS

Effect of amino acid limitation on *avtA* expression. Limitation for any one of nine amino acids caused reduced TrC activity in one or both genera (Table 2). To determine whether this effect is pre- or posttranslational, we used *E. coli* CBK703 (14), in which phage Mu dl(Ap *lac*) (3) is inserted into *avtA*, creating an operon fusion of the *avtA* control region and *lac* structural genes. As a result, in CBK703, β-galactosidase activity is subject to repression by L-leucine and L-alanine (14). Upon amino acid limitation, the β-galactosidase activity in several amino acid auxotrophic derivatives of CBK703 is reduced (Table 2). This reduction parallels the reduction in TrC activity found in the corresponding *avtA*$^+$ strains, indicating that amino acid limitation affects *avtA* transcription, not a translational or posttransla-

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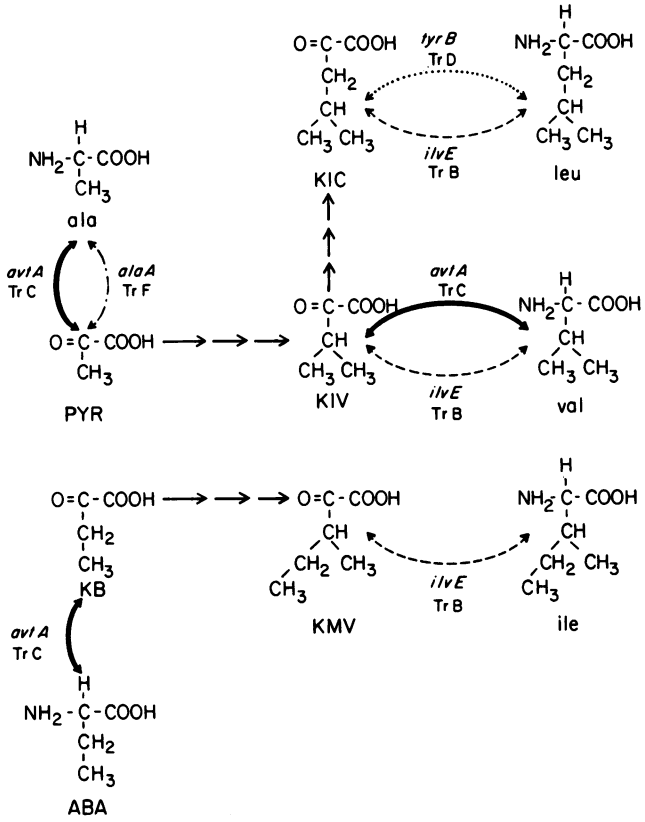

FIG. 1. Reactions catalyzed by TrC and the other general transaminases in the branched chain amino acid and L-alanine pathways. Symbols: ala, L-alanine; pyr, pyruvate; KIV, α-ketoisovaleric acid; KIC, α-ketoisocaproic acid; leu, leucine; val, valine; ABA, α-aminobutyric acid; KB, α-ketobutyric acid; KMV, α-keto-β-methylvaleric acid; ile, isoleucine. The transaminases are: TrB (*ilvE*), branched chain; TrC (*avtA*), alanine-valine; TrD (*tyrB*), tyrosine repressible; TrF (*alaA*), alanine-glutamate.

tional step. In addition, limitation for L-valine, one of the products of TrC, also causes repression, not derepression, of *avtA*. The reduction in TrC activity observed upon limitation of any single amino acid is not a general feature of operon regulation. Expression of *ilvE*, which encodes transaminase B, was found not to vary in *E. coli* upon limitation of any of several nonbranched chain amino acids (data not shown).

Since limitation for any single amino acid might lead to high endogenous levels of L-alanine or L-leucine or both, it appeared that *avtA* repression could be due to the accumulation of repressing levels of one or both of these amino acids. If *avtA* is regulated by a simple repression mechanism, then the only type of amino acid limitation which should not lead to repression is that in which both the L-alanine and L-leucine intracellular pools are below the threshold necessary for repression. The absence of simple alanine auxotrophs had previously made it impossible to test this. However, we have recently isolated a *S. typhimurium* mutant which requires L-isoleucine plus either L-alanine or L-valine (2; W. A. Whalen and C. M. Berg, unpublished data). A *leu*::Tn*5* derivative of this strain, CBS540, requires L-leucine and L-isoleucine plus either L-valine or L-alanine for growth. The data show that repression of *avtA* occurred under every amino acid limitation condition except when the culture was simultaneously limited for both L-alanine and L-leucine (Table 3).

TABLE 1. Bacterial strains used

Strain	Genotype	Source or reference
E. coli		
CBK001	*thyA pheA*::Tn*5*	(12)
CBK012	*thyA leu*::Tn*5*	(12)
CBK017	*thyA argE*::Tn*5*	(12)
CBK040	*thyA metE*::Tn*5*	(12)
CBK103	*thyA cysG*::Tn*5*	(12)
CBK130	*thyA proAB*::Tn*5*	(12)
CBK140	*thyA lysA*::Tn*5*	(12)
CBK236	*thyA hisG*::Tn*5*	(12)
CBK252	*thyA ilvC711*::Tn*5*	(1)
CBK699	*thyA* Δ(*proB-lac*)	(14)
CBK701	*thyA* Δ(*proB-lac*) *ilvE720*::Tn*5* *avtA21*::Mu dl(Ap *lac*)	(14)
CBK703	*thyA* Δ(*proB-lac*) *avtA21*::Mu dl(Ap *lac*)	(14)
CBK709	*thyA* Δ(*proB-lac*) *leu*::Tn*5* *avtA21*::Mu dl(Ap *lac*)	(14)
CBK746	*thyA* Δ(*proB-lac*) *avtA21*::Mu dl(Ap *lac*) *pheA*::Tn*5*	Transduction of CBK703 to Kan^r, using P1 · CBK001
CBK747	*thyA* Δ(*proB-lac*) *avtA21*::Mu dl(Ap *lac*) *metE*::Tn*5*	Transduction of CBK703 to Kan^r, using P1 · CBK040
CBK749	*thyA* Δ(*proB-lac*) *avtA21*::Mu dl(Ap *lac*) *hisG*::Tn*5*	Transduction of CBK703 to Kan^r, using P1 · CBK236
W3110 (*thy*)	*thyA*	J. Cairns
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*	Shaw and Berg, unpublished data
CBS531	*galE1122 ilvC2104*::Tn*10*	(2)
CBS537	*galE1122 ilvE2101*::Tn*10* *leu*::Tn*5*	(2)
CBS540	*galE1122 ilvE2101*::Tn*10* *ala-196*::Mu dl (Ap *lac*) *leu*::Tn*5*[a]	Transduction of CBS502 (2) to Kan^r, using P22 · CBS537
CBS541	*galE1122 leu*::Tn*5*	Transduction of JL3404 to Kan^r, using P22 · CBS537
JL3404	*galE1122*	L. N. Csonka (4)

[a] The *ala-196* mutation confers an alternate L-alanine or L-valine requirement in an *ilvE* (Ile$^-$) background (Whalen and Berg, unpublished data). This strain has reduced alanine-glutamate transaminase, but it is not known whether the lesion is in a structural or regulatory gene.

TABLE 2. *avtA* expression under conditions of amino acid limitation

Amino acid in growth-limiting amt	*S. typhimurium*		*E. coli*			
	Strain	TrC sp act	Strain	TrC sp act	Strain	β-Galactosidase (U)
None	JL3404	22.2	W3110 (*thy*)	18.6	CBK703	107
L-Isoleucine[a]	CBS531	7.2	CBK252	3.0	CBK701	19.5
L-Valine[b]					CBK701[c]	20.3
L-Valine + L-leucine[b,d]	CBS531	7.6	CBK252	4.3		
L-Leucine	CBS541	5.2	CBK012	5.6	CBK709	21.0
L-Phenylalanine			CBK001	6.1	CBK746	35.6
L-Lysine			CBK140	4.6		
L-Histidine			CBK236	5.6	CBK749	49.5
L-Cysteine	CBS101	10.6	CBK103	4.1		
L-Methionine	CBS106	6.3	CBK040	6.7	CBK747	56.7
L-Proline			CBK130	3.6	CBK703	30.0

[a] L-Valine was provided.
[b] L-Isoleucine was provided.
[c] *ilvE avtA* mutants require L-isoleucine and L-valine and can synthesize L-leucine.
[d] *ilvC* mutants cannot synthesize L-leucine in the absence of L-valine.

Amino acids which repress *avtA* are structurally similar. Repression of *avtA* in both *E. coli* and *S. typhimurium* by L-leucine (2, 14; Table 4) is surprising since TrC does not participate directly in L-leucine synthesis (although it can in strains blocked in the synthesis of α-ketoisovalerate from pyruvate [Fig. 1]. Since L-alanine and L-leucine are the only protein amino acids which repress *avtA* (14), it seemed possible that L-alanine, which is synthesized by TrC, is the physiologically relevant corepressor of *avtA* and that the L-leucine-mediated repression is simply a consequence of its structural similarity to L-alanine. The lack of repression by other protein amino acids (14), including glycine (which has no β-carbon) and L-valine and L-isoleucine (which are branched at the β-carbon), suggests characteristics of compounds which repress *avtA*: they must be amino acids with an alkyl group side chain which is unbranched at the β-carbon. To test this hypothesis, several structurally similar D-amino acids and nonprotein L-amino acids were tested for their effects on *avtA* expression. D-leucine did not repress *avtA* in either genus, and D-alanine did not repress *avtA* in *E. coli*. In *S. typhimurium*, however, D-alanine resulted in a small reduction in *avtA* expression (Table 4). To test whether this effect is due to repression by D-alanine or racemization and repression by L-alanine, we tested an *S. typhimurium* mutant lacking the major alanine racemase (*dadB*) (13). There was no repression of *avtA* by D-alanine in this strain (data not shown). Thus, neither D-alanine nor D-leucine exerts any direct effect upon *avtA* expression in either genus. L-α-aminobutyric acid, L-norvaline, L-norleucine, and DL-α-aminoheptanoic acid, which are L-amino acids with alkyl groups unbranched at the β-carbon, repress *avtA* in both genera (Table 4).

DISCUSSION

The general transaminases are unusual among biosynthetic enzymes in that each catalyzes terminal reactions in the biosynthesis of more than one amino acid (7). One of these, transaminase C (TrC), catalyzes the synthesis of L-alanine, L-valine, and L-α-aminobutyric acid (Fig. 1). *avtA*, which encodes TrC, is repressed (i) upon limitation for any single amino acid (Table 2); (ii) by excess L-alanine and L-α-aminobutyric acid, the amino acids whose synthesis is catalyzed by TrC; and (iii) by L-leucine, L-norvaline, L-norleucine, and L-α-aminoheptanoic acid, amino acids whose synthesis is not catalyzed by TrC (Table 4). These unusual regulatory properties of *avtA* are considered below.

During limitation of any single amino acid, protein degradation and the synthesis of other amino acids continues, resulting in the intracellular accumulation of free amino acids (including L-alanine and L-leucine). Since high concentrations of either L-alanine or L-leucine repress *avtA*, it seemed that the repression observed upon amino acid limitation might be due to the accumulation of one or both of these amino acids. To test this, a mutant requiring both L-alanine and L-leucine was constructed. Although *avtA* was repressed when the culture was limited for either amino acid, *avtA* was not repressed when the culture was simultaneously limited for both (Table 3). Repression upon amino acid limitation is, therefore, not a unique aspect of *avtA* regulation but is a simple consequence of repression by either of two amino acids; only when a culture is limited for both L-alanine and L-leucine is repression not found (Table 3).

Repression by nonprotein amino acids and also by L-leucine, whose synthesis is not catalyzed by TrC (Table 4), was more puzzling. We start with the assumption that repression by L-alanine evolved because the in vivo role of TrC is in L-alanine biosynthesis. But the role of TrC in the synthesis of L-α-aminobutyric acid, a nonessential (and

TABLE 3. *avtA* expression when *S. typhimurium* CBS540 is limited for amino acids[a]

Amino acid concn (×)[b]				TrC sp act[c]
L-Leu	L-Val	L-Ala	L-Ile	
1	1	1	1	16.3 ± 2.1[d]
0.1	1	1	1	7.6 ± 2.8
1	0.1	0.1	1	7.6 ± 1.5
0.1	0.1	0.1	1	15.0 ± 3.6

[a] CBS540 requires L-isoleucine and L-leucine plus L-valine or L-alanine (this alternate requirement for L-valine or L-alanine precludes limiting for either amino acid alone). *avtA* is not repressed when *S. typhimurium* is simultaneously limited for both L-alanine and L-leucine.
[b] The amino acid concentrations in the growth medium were supplemental (1×) or growth-limiting (0.1×) (see the text for exact concentrations). Leu, Leucine; Val, valine; Ala, alanine; Ile, isoleucine.
[c] Numbers represent the mean ± the standard error of six experiments.
[d] These concentrations of L-leucine (1×) and L-alanine (1×) are only marginally repressing.

TABLE 4. *avtA* expression after growth in the presence of added amino acids

Addition to minimal medium[a]	Enzyme activity		
	TrC sp act for *S. typhimurium*[b]	*E. coli*	
		TrC sp act[c]	β-Galactosidase (U)[d]
None	20.7	19.8	115
Glycine	21.3	20.5	107
L-Valine	19.0	20.6[e]	109[e]
L-Isoleucine	22.0	18.6	109
D-Alanine	17.0	18.9	117
D-Leucine	21.1	20.9	116
L-Alanine	6.8	7.2	60.5
L-Leucine	6.5	6.4	45.8
L-α-Aminobutyric acid[f]	7.0	9.8	45.8
L-Norvaline[g]	6.3	3.5	43.4
L-Norleucine[g]	10.1	12.4	87.6
DL-α-Aminoheptanoic acid	8.8	NT	NT

[a] Amino acids were added at 1 mM, except for DL-α-aminoheptanoic acid which was added at 2 mM.

[b] JL3404 *galE* was used.

[c] CBK699 Δ(*proB-lac*) *thyA* was used.

[d] CBK703 Δ(*proB-lac*) *thyA avtA*::Mu d1(Ap *lac*) was used. The effect of all 20 protein amino acids on β-galactosidase activity has been reported previously: only L-alanine and L-leucine repressed *avtA* (14).

[e] L-Isoleucine (0.15 mM) added to avoid isoleucine starvation (8).

[f] L-Valine (1 mM) and L-isoleucine (0.15 mM) added to avoid valine and isoleucine starvation (6).

[g] L-Methionine (1 mM) added to avoid methionine starvation (10).

[h] NT, Not tested.

inhibitory) L-valine analog (6), and the repression of *avtA* by amino acids other than L-alanine must also be explained. In TrC-catalyzed L-alanine synthesis, L-valine donates its amino group to pyruvic acid, with the release of α-ketoisovaleric acid (Fig. 1). We suggest that TrC catalyzes L-α-aminobutyric acid biosynthesis because TrC cannot discriminate against the L-α-aminobutyric acid precursor, L-α-ketobutyric acid, although it does discriminate against the L-isoleucine and L-leucine precursors, α-keto-β-methylvaleric acid and α-ketoisocaproic acid, respectively (perhaps because they are larger than α-ketoisovaleric acid) (see Fig. 1 for structures).

Since L-α-aminobutyric acid is synthesized by TrC, the accumulation of this toxic amino acid, which occurs when α-ketobutyric acid pools are elevated (8), must be prevented. It is probable, therefore, that the repression of *avtA* by L-α-aminobutyric acid, as well as by L-alanine, has evolved to limit the accumulation of L-α-aminobutyric acid under physiological conditions.

We hypothesize that repression by L-leucine, L-norvaline, L-norleucine, and L-α-aminoheptanoic acid is due to the structural similarity between these amino acids and L-alanine and L-α-aminobutyric acid. We refer to the amino acids which repress *avtA* but are not synthesized by TrC as gratuitous corepressors.

It is puzzling that *avtA* is repressible by L-leucine, which, although present in the cell, is not a TrC-related metabolite. This lack of specificity found in *avtA* repression could reflect the fact that TrC is a duplicate enzyme: other enzymes can catalyze L-alanine and L-valine biosynthesis (L-α-aminobutyric acid is nonessential). Therefore, gratuitous repression can be tolerated better by *avtA* than by a gene encoding an indispensible enzyme.

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