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Multivalent Regulation of Isoleucine-Valine Transaminase in an *Escherichia coli* K-12 *ilvA* Deletion Strain

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In a strain of *Escherichia coli* K-12 lacking threonine deaminase, the enzyme converting α-ketoisovalerate and α-keto-β-methylvalerate to valine and isoleucine, respectively, was multivalently repressed by valine, isoleucine, and leucine. This activity was due to transaminase B, specified by the *ilvE* structural gene.

The regulation of three genes in the *ilv* cluster, *ilvE*, *ilvC*, and *ilvB* (specifying transaminase B, acetohydroxy acid isomeroreductase, and acetohydroxy acid synthase, respectively), in *Escherichia coli* K-12 was shown to be independent of the *ilvA* gene product, threonine deaminase, by analysis of a strain carrying a total deletion of the *ilvA* gene (5) and of a strain with Mu 1 phage inserted into the *ilvA* structural gene (4; J. M. Smith, M. Levinthal, and H. E. Umbarger, Abstr. Annu. Meet. Am. Soc. Microbiol. 1975, H23, p. 100). Recently, however, it has been questioned whether the assay devised by Duggan and Wechsler (2) and routinely used to determine transaminase B activity (*ilvE*) is a measure of transaminase B, the *ilvE* gene product, or some other transaminase in a strain carrying the *ilvDAC115* deletion (6). In this study we determined that the transaminase activity needed for isoleucine and valine formation under previously defined conditions, in a strain completely devoid of the structural gene for threonine deaminase (5), is attributable to transaminase B and not to some other transaminase.

A mutant strain unable to convert α-ketoisovalerate (αKIV) and α-keto-β-methylvalerate (αKMV) to valine and isoleucine, respectively, was isolated (from a strain carrying the *ilvDAC115* deletion) by penicillin counterselection after mutagenesis with diethylsulfate. The growth behavior of this strain on minimal medium supplemented with the three branched-chain amino acids or αKIV and αKMV revealed that this isolated strain ES30 is incapable of converting αKIV or αKMV to the respective amino acids, isoleucine and valine (Table 1). The loss of this transaminase activity, which results in an isoleucine, valine, and leucine requirement, is attributable to a lesion in the *ilvE* structural gene (3, 9; J. Guardiola, personal communication).

Genetic analysis of the isoleucine and valine transaminase-negative strain ES30 (*ilvDAC115 ilvE637*) showed that the lesion causing this response is linked to the ribose-minus mutation, *rbs-215*, as is the *ilvDAC115* deletion, and is very close to *ilvDAC115* as indicated by the very low frequency of separating *ilvDAC115* from *ilvE637* (Table 2). This would place the second lesion causing the transaminase-negative phenotype in the region of the *ilvE* structural gene.

Isolation of such a strain (ES30) would strongly suggest that the transamination of αKIV and αKMV in a normal genetic background is via the product encoded by the *ilvE* structural gene (transaminase B) in a strain carrying the *ilvDAC115* deletion.

The same physiological conditions used earlier (5) to show that the regulation of transaminase B was independent of threonine deaminase in strain CU357 (*ilvDAC115*) were used in this study to compare strains carrying this deletion alone or, in addition, a lesion that we now identify as an *ilvE* lesion. The increase in acetohydroxy acid synthetase and transaminase B during valine limitation in strains carrying the *ilvDAC115* deletion (CU357 and ES35), or acetohydroxy acid synthetase in strains carrying the two mutations *ilvDAC115* and *ilvE637* (ES30 and ES33), verifies the presence of a multivalent regulatory system (Table 3). The absence of a transaminase regulated multivalently by leucine, valine, and isoleucine in the *ilvDAC115 ilvE637* derivative strains (ES30 and ES33) and the presence of it in the *ilvDAC115* deletion strains (CU357 and ES35) substantiate the threonine deaminase independent multivalent regulation of the *ilvE* structural gene. To determine whether some other transaminase (1, 8) may be able to function during growth under these physiological conditions (5), limitation experiments were conducted as previously described (5) for valine or isoleucine, except for the addition of αKIV (1

TABLE 1. *Utilization of substrates for transaminase B*

Strain	Ile, Val, Leu[a]	αKIV, αKMV	Ile, Leu, αKIV	Ile, Val, αKIV	Ile, Val	Ile, Leu, Ala, αKIV
CU357 (*ilvDAC115*)	+	+	+	+	+	+
ES30 (*ilvDAC115 ilvE637*)	+	−	−	+	−	−

[a] Strains were scored on glucose minimal plates with the following concentrations of supplements when indicated: 1×10^{-3} M valine (Val), 4×10^{-4} M isoleucine (Ile) or leucine (Leu), and 1×10^{-3} M αKIV, αKMV, or alanine (Ala).

TABLE 2. *Linkage of rbs-215 with the transaminase-negative mutation*

Donor	Recipient	No. of *rbs*$^+$ transductants	No. requiring:		Linkage to *rbs-215* (%)
			Ile, Val, and Leu[a]	Ile and Val[a]	
CU357 (*ilvDAC115*)	CU12 (*rbs-215*)	831	0	673	81
ES30 (*ilvDAC115 ilvE637*)	CU12 (*rbs-215*)	1,072	824	2	77

[a] P1*kc* transductants were scored on glucose minimal plates with the following concentrations of supplements when indicated: 1×10^{-3} M valine (Val) and 4×10^{-4} M isoleucine (Ile) or leucine (Leu).

TABLE 3. *Branched-chain amino acid transaminase in a strain carrying an ilvDAC115, transaminase B mutation*

Strain	*ilv* genotype	Growth conditions[a]	nmol/min per mg of protein[b]				
			TRB[c]	DH	TD	IR	AHAS
ES30	*ilvDAC115* *ilvE637*	Excess	0	0	0	0[d]	14
		Lim Val	0	0	0	0	65
		Lim Ile	0	0	0	0	11
		Lim Leu	0	0	0	0	73
ES33	*ilvDAC115* *ilvE637*[e]	Excess	0	0	0	0	12
		Lim Val	0	0	0	0	61
		Lim Ile	0	0	0	0	14
		Lim Leu	0	0	0	0	69
CU357	*ilvDAC115*	Excess	17[f]	0	0	0	11
		Lim Val	61	0	0	0	69
		Lim Ile	73	0	0	0	12
ES35	*ilvDAC115*[e]	Excess	19	0	0	0	11
		Lim Val	65	0	0	0	68
		Lim Ila	69	0	0	0	15
CU12	Wild type	Excess	20	19	31	2	12
		Minimal	51	47	62	49	33

[a] All excess and limitation (Lim) experiments were conducted as described previously (5).

[b] Proteins were determined by the method of Lowry et al. (7).

[c] EC 2.6.1.42, Branched-chain amino acid:2-oxoglutarate aminotransferase (transaminase B [TRB]); EC 4.2.1.9, 2,3-dihydroxy acid hydrolase (DH); EC 4.2.1.16, deaminating threonine hydrolase (TD); EC 1.1.1.86, 3-alkyl-2,3-dihydroxy acid (isomeroreductase [IR]); EC 4.1.3.18, carboxylating acetolactate pyruvate lyase (acetohydroxy acid synthetase [AHAS]).

[d] Where there is no activity reported, values were <0.01. All strains were grown as before (5), using the assay methods described previously for the modified IR assay (5), and TD, AHAS, or DH assay (10).

[e] ES33 and ES35 are *rbs*$^+$ transductants of CU12 from ES30 requiring isoleucine (Ile), leucine (Leu), and valine (Val) or isoleucine (Ile) and valine (Val), respectively.

[f] The transaminase activity was determined by the assay method of Duggan and Wechsler (2).

$\times 10^{-3}$ M), αKIV (1×10^{-3} M) and alanine (1×10^{-3} M), or αKMV (1×10^{-3} M). Figure 1 shows that the growth limitations were virtually identical to those without added supplements. This further demonstrates that under the physiological conditions described (5) the enzyme encoded by the *ilvE* structural gene (transaminase B) is essential in strains CU357 and ES35 to form isoleucine or valine from αKMV and αKIV, respectively. Also shown is the necessity of αKIV for leucine biosynthesis, as has been described earlier (1, 2, 8, 9).

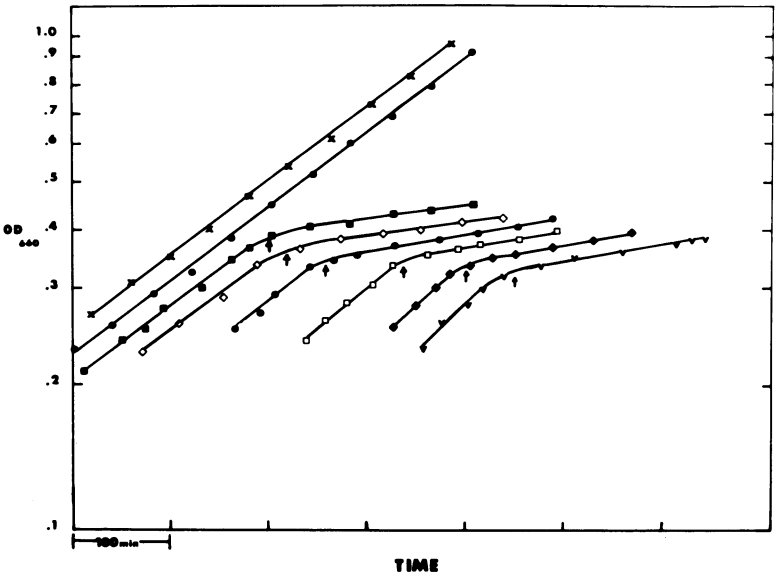

FIG. 1. *Effect of various limitation conditions on an E. coli strain (ES33) carrying ilvDAC115 and ilvE637 lesions. All cultures were initially grown in glucose minimal medium supplemented with isoleucine, leucine, and valine as described earlier (5), except that the growth temperature here was 25°C. For limiting conditions (indicated by arrows), all procedures were conducted as previously described (5), except the growth temperature here was 25°C and the supplements to separate glucose minimal medium were as follows: ×, control ES35, 0.4 mM L-isoleucine, 1 mM L-valine; ○, control ES33, 0.4 mM L-isoleucine, 0.4 mM L-leucine, 1 mM L-valine; ■, ES33, 0.4 mM L-isoleucine, 0.4 mM L-leucine; ◇, ES33, 0.4 mM L-isoleucine, 0.4 mM L-leucine, 1 mM αKIV, 1 mM alanine; ●, ES33, 0.4 mM L-leucine, 1 mM valine, 1 mM αKMV, 1 mM alanine; □, ES33, 0.4 mM leucine, 1 mM valine; ◆, ES33, 0.4 mM leucine, 0.4 mM isoleucine, 1 mM αKIV; ▽, ES33, 0.4 mM leucine, 1 mM valine, 1 mM αKMV. Identical limiting conditions were obtained with CU357 (ilvDAC115) and ES35 (ilvDAC115) when limited for isoleucine or valine. The limitation ceased and the control growth rates resumed when αKIV was added to a valine-limited culture or αKMV was added to an isoleucine-limited culture of CU357 or ES35.*

The above genetic and physiological evidence with various *E. coli* K-12 strains demonstrates that under the conditions previously specified (5) the transaminase activity that is multivalently regulated by the three branched-chain amino acids, in strains CU357 and ES35, is, in fact, specified by the *ilvE* gene. It also shows that the assay by Duggan and Wechsler measures the *ilvE* gene product in strains having a functional *ilvE* structural gene carrying the *ilvDAC115* deletion. Similar results have been obtained with ongoing research in this laboratory with various *E. coli* B/r *ilvA* deletion strains.

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