REGULATION OF ISOLEUCINE-VALINE BIOSYNTHESIS IN AN *ilvDAC* DELETION STRAIN OF *ESCHERICHIA COLI* K-12*

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Studies of isogenic strains of *Escherichia coli* K-12 with and without the *ilvDAC115* deletion described by Kiritani showed that a strain carrying this lesion does not have the *ilvA*, *ilvD* or *ilvC* structural genes but has normal multivalent regulation of the *ilvB* and *ilvE* structural genes. It was also shown that the regulatory locus (*ilvO*) for the *ilvADE* operon (defined by Ramakrishnan and Adelberg) affects the expression of the *ilvB* and *ilvC* structural genes and is located outside of the *ilvDAC115* deletion. These experiments demonstrate that there is multivalent control of at least two *ilv* biosynthetic enzymes in the absence of the *ilvA* gene product.

The gene-enzyme relationship in isoleucine and valine biosynthesis is shown in Fig. 1. Several kinds of evidence have suggested that the *ilvA* gene product can participate in the regulation of gene expression of the *ilvADE* operon and of the *ilvB* and *ilvC* structural genes (3,4,8,9,12,16,21,22). However, to date there has been no experimental test to determine whether the product of the *ilvA* structural gene plays an obligatory role in the regulation of the *ilv* gene cluster.

The availability of a mutation of *E. coli* K-12 deleted for the entire *ilvA* structural gene and at least portions of the *ilvC* and the *ilvD* structural genes (11,14) while retaining a functional operator region for the *ilvADE* operon has enabled us to examine the regulatory responses of the *ilvE* remnant of the *ilvADE* operon and the *ilvB* structural gene in the absence of the *ilvA* gene product, threonine deaminase.

EXPERIMENTAL

Organisms and basal medium. The *E. coli* K-12 strains used, their genotypes, growth rates, and derivations are given in Table 1. All strains were grown in the minimal medium of Davis and Mingioli (5) modified by omitting citrate and by increasing the glucose concentration to 0.5%.

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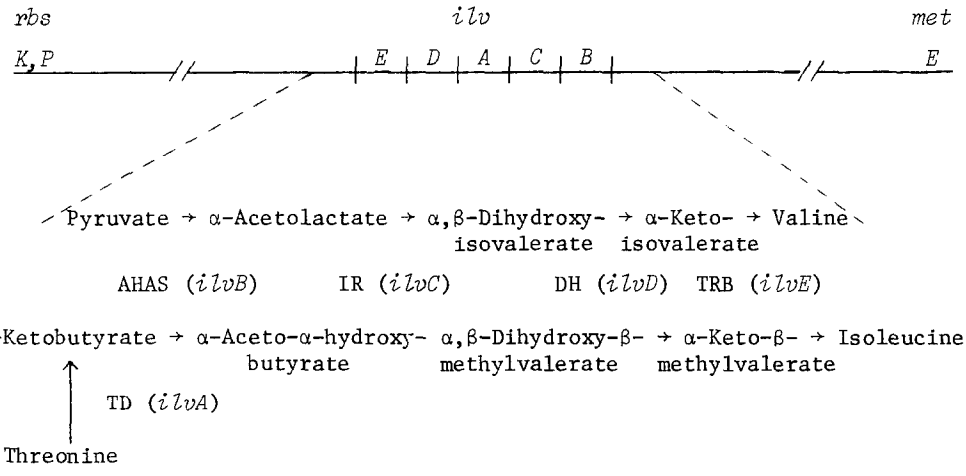

Figure 1. The enzymes encoded by the *ilv* gene cluster. Abbreviations of the enzymes are as follows: TD, threonine deaminase; AHAS, acetohydroxy acid synthase; IR, acetohydroxy acid isomeroreductase; DH, dihydroxy acid dehydrase; TRB, transaminase B. The order of the *ilv* structural genes is that reported by Ramakrishnan and Adelberg (17). For a review, see reference 20.

The effect of the *ilvDAC* deletion on multivalent repression of the *ilv* gene cluster. Table 2 shows the pattern of repression exhibited by the strains used in this study. Growth of the prototrophic strains in the presence of excess branched-chain amino acids resulted in a repression of all five isoleucine and valine biosynthetic enzymes. All the enzymes except the isomeroreductase can be derepressed by growing a leucine auxotroph (strain CU361) on limiting leucine. The isomeroreductase was not derepressed presumably because the excess valine in the culture prevented the endogenous production of the acetohydroxy acid, the inducer of isomeroreductase, as has been proposed by Pledger *et al.* (16). The effect of limiting either isoleucine or valine on the level of the isoleucine and valine biosynthetic enzymes could be demonstrated with a strain that also has an *ilvC* lesion (CU367). Again, leucine restriction resulted in derepression of the same four enzymes. Valine limitation produced a similar pattern. Isoleucine limitation, on the other hand, resulted in a derepression of only the three enzymes corresponding to the *ilvADE* gene cluster as has been previously observed (2,15).

The isogenic Ilv⁻ strain carrying the *ilvDAC* deletion (CU372) forms two

TABLE 1. Bacterial Strains.

Strain	Mating Type	Generation Time (hr^{-1})	Genotype	Source or Reference
CU1	F^+	0.66	wild type	H. E. Umbarger
CU1008	F^+		*ilvA454*	Spontaneous ile^- from CU1
CU339	F^+	0.66	*ilvO468*	P1kc/CU113 x CU1008
CU340	F^+	0.65	*ilvO469*	P1kc/CU114 x CU1008
CU341	F^+	0.66	*ilvO264*	P1kc/CU112 x CU1008
CU342	F^+	0.65	*ilvO266*	P1kc/CU115 x CU1008
CU16	F^-		*gal⁻ rbs-215 metE200*	J. Pledger
CU17	F^-		*gal⁻ rbs-215 ilvA467 metE200*	J. Pledger
CU112	F^-		*gal⁻ ilvO264 metE200*	P1kc/AB1005 x CU17
CU113	F^-		*gal⁻ rbs-215 ilvO468 metE200*	P1kc/CU2501 x CU17
CU114	F^-		*gal⁻ rbs-215 ilvO469 metE200*	P1kc/CU2506 x CU17
CU115	F^-		*gal⁻ rbs-215 ilvO266 metE200*	P1kc/AB1001 x CU17
CU12	F^-	0.65	*gal⁻ rbs-215*	J. Pledger
AB3590	F^-		*ilvDAC115 thi⁻ mtl⁻ malA⁻ str his⁻ trpC⁻ tsx⁻ lacZ⁻*	B. Bachmann
MI199	F^-		AB3590 lysogenic for $\phi 80\lambda$ CI857St68d*ilv*/$\phi 80\lambda$CI857St68	M. Iaccarino
CU1010	F^+		*ilvC462*	Spontaneous *ilv⁻* from CU1
CU344	F^-	0.65	*gal⁻ ilvDAC115*	P1kc/MI199 x CU12
CU356	F^-	0.66	*gal⁻ ilvDAC115 leu-455*	CU344 $\xrightarrow{EMS}$ *leu⁻*
CU357[a]	F^-	0.65	*gal⁻ ilvDAC115*	P1kc/CU12 x CU356
CU358	F^-	0.66	*gal⁻*	P1kc/CU12 x CU357
CU361	F^-	0.66	*gal⁻ rbs-215 leu-455*	P1kc/CU12 x CU356
CU367	F^-	0.65	*gal⁻ ilvC462 leu-455*	P1kc/CU1010 x CU361
CU372	F^-	0.66	*gal⁻ ilvDAC115 leu-455*	P1kc/AB3590 x CU361

[a]Reversion tests failed to give Ilv^+ derivatives in the presence of diethylsulfate (DES), ethyl methanesulfonate (EMS), N-methyl-N'-nitro-N-nitrosoguanidine (NG) or ICR-191E. Phage lysates grown on strain CU357 with seven *ilvC* mutants, six *ilvA* mutants and one *ilvD* mutant as recipients failed to yield Ilv^+ transductants although they did yield >200 Rbs^+ transductants with 13 *ilv⁻ rbs⁻* strains (7). *leu-455* is located in the *leu* operon.

ilv gene products, acetohydroxy acid synthase (*ilvB*) and transaminase B (*ilvE*). As shown in Table 3, the derepression and repression of the enzymes in strain CU372 is like that in strain CU367 which contains an intact *ilvA* gene. The experiments with strain CU357 (*ilvDAC115*) showed an identical pattern in the absence of the *leu* lesion.

The location of the *ilvO* locus. Since the *ilvE* gene responds identically to repressing and derepressing conditions (Table 2) in isogenic strains with or without the *ilvDAC* deletion, a genetic analysis of the location of *ilvO* was performed. It has been assumed from the experiments of Ramakrishnan and

TABLE 2. Effects of isoleucine, valine and leucine limitation on *ilv* gene expression in strains carrying the *ilvDAC115* deletion.

Strain	Genotype	Growth Conditions[a]	mμMoles/min/mg protein[b]					
			AHAS		IR	TD	DH	TRB
			-val	+val[c]				
CU358	isogenic prototroph	Excess	10	3	2	31	21	15
		Minimal	29	6	48	61	46	32
CU361	*leu-455*	Excess	10	3	2	28	18	16
		Lim leu	78	3	54	93	56	48
CU367	*ilvC462 leu-455*	Excess	9	3	0	30	20	17
		Lim leu	79	3	0	118	64	47
		Lim val	87	7	0	147	62	86
		Lim ile	3	1	0	329	129	70
CU372[d]	*ilvDAC115 leu-455*	Excess	9	3	0	0	0	16
		Lim leu	69	3	0	0	0	47
		Lim val	89	6	0	0	0	63
		Lim ile	4	2	0	0	0	69
CU357	*ilvDAC115*	Excess	9	4	0	0	0	15
		Lim val	87	6	0	0	0	52
		Lim ile	3	3	0	0	0	70

[a]Cultures in excess branched-chain amino acids initially contained 4×10^{-4} M isoleucine and leucine and 1×10^{-3} M valine and were grown at 37° to an OD_{660} of 0.55. For limitation experiments similar cultures were harvested at an OD_{660} of about 0.3, washed twice with prewarmed 0.05 M phosphate buffer, pH 7.5, and resuspended at the same density in medium supplemented with an excess of two amino acids and a limiting amount of the third. After 3 hours the OD_{660} was approximately 0.45. The limiting concentrations used were 2×10^{-5} for isoleucine and leucine and 8×10^{-5} M for valine.

[b]Extracts and enzyme assays for threonine deaminase (TD), dihydroxy acid dehydrase (DH), transaminase B (TRB) and acetohydroxy acid synthase (AHAS) were as previously described (20,6) except that 20 μg FAD per ml was used in the AHAS assay. The acetohydroxy acid isomeroreductase (IR) assay was a modification of the procedure of Arfin and Umbarger (1). The assays were performed at 37° instead of 30° and initiated with undiluted extract instead of NADPH. The activities were corrected for a magnesium-independent reductase activity as well as NADPH oxidase activities by using one cuvette in which $MgCl_2$ was replaced by 10^{-3} M tetrasodium EDTA. Proteins were determined by the method of Lowry *et al.* (15).

[c]10^{-3} M valine present in enzyme assay.

[d]CU356 has virtually the same physiological responses as strain CU372.

Adelberg (17) that the *ilvO* lesions that they described defined the operator controlling the *ilvADE* operon and that these lesions were located between the *ilvA* and *ilvC* structural genes. The *ilvO* lesions from the Adelberg stocks (*ilvO264*, *ilvO266*) as well as two from strains isolated by J. Jackson in this laboratory (*ilvO468*, *ilvO469*) have been examined. All gave rise to valine

TABLE 3. Effects of *ilvO* lesions on *ilv* gene expression.

Strain	Genotype	Growth Conditions	mμMoles/min/mg protein AHAS -val	AHAS +val[a]	IR	TD	DH	TRB
CU1	isogenic wild type	Excess[b]	12	2	2	31	20	17
		Minimal	40	8	56	69	48	38
CU239	*ilvO468*	Excess	25	6	1	369	97	40
		Minimal	72	12	31	626	121	94
CU340	*ilvO469*	Excess	28	5	1	468	80	44
		Minimal	83	11	22	747	97	96
CU341	*ilvO264*	Excess	6	2	1	361	49	39
		Minimal	20	6	22	686	61	62
CU342	*ilvO266*	Excess	20	3	1	333	72	36
		Minimal	29	7	21	581	90	59

[a] 10^{-3} M valine present in enzyme assay.

[b] Leucine, isoleucine and valine are in excess.

TABLE 4. Evidence that the *ilvO* lesions are outside of the *ilvDAC115* deletion.

Plkc Donor Strains	Ilv$^+$ transductants using CU357 (*ilvDAC115*) as recipient: Val^r	Val^s
CU112 (*ilvO468*)	891	3
CU113 (*ilvO469*)	1123	4
CU114 (*ilvO264*)	957	3
CU115 (*ilvO266*)	567	2

resistance and led to increases in the levels of *ilvADE* gene products (Table 3). They also resulted in an increase in the levels of *ilv* mRNA (R. Vonder Haar, personal communication). It should be noted, however, that although the *ilvADE* absolute enzyme levels were elevated, the degree of repressibility of only the dehydrase was affected. As would be expected from the presumed position of the *ilvO* locus, these four *ilvO* lesions were found to be closely linked (99%) to several *ilvA* and *ilvC* lesions. A convenient outside marker

(Fig. 1) for three point genetic crosses in the *ilv* region is *rbs* which is approximately 90 to 94% linked by P1 transduction to *ilvA* and *ilvC*. Transduction of Rbs^+ *ilvA* or *ilvC* strains to Ilv^+ prototrophy using *rbs-215 ilvO⁻ metE200* strains (Table 1) as donors yielded only 1-2% valine-sensitive transductants among greater than one thousand Rbs^- recombinants examined. None of the fewer Rbs^+ recombinants were valine sensitive. These results did not distinguish between *ilvO* being on the *rbs* distal side of either *ilvA* or *ilvC* (assuming rare quadruple crossover events) or on the *rbs* proximal side of *ilvC* and even of *ilvA*. Less equivocal, however, were the crosses between strains carrying any of the four *ilvO* lesions and strain CU357 (*ilvDAC115*). As shown in Table 4, several valine sensitive (Val^s) recombinants were found among the Ilv^+ transductants in each of the crosses. Thus, the *ilvO* lesions are outside the region covered by the *ilvDAC* deletion and are presumably on the *metE* side of the deleted region. Repeated examinations have failed to yield valine sensitive revertants among the *ilvO⁻* strains used as donors in these crosses.

The question arises regarding the nature of the locus defined by the *ilvO* lesions. It is difficult to envision that its earlier assumed role of a repression recognition site could be played if the *ilvC* gene were between it and the gene cluster it presumably controlled. It is interesting, in view of the apparent proximity of the *ilvO* locus to *ilvB* and *ilvC*, that the expression of both loci are modified in the isogenic *ilvO⁻* strains (Table 3). The relationship of this locus to the *ilv* biosynthetic pathway is now under investigation.

DISCUSSION

The hypothesis that some form of threonine deaminase is a central component in *ilv* regulation has been proposed by Hatfield and Burns (8) who observed that leucyl-tRNA binds to an immature form of *S. typhimurium* threonine deaminase. Similar studies with the *E. coli* enzymes have shown that isoleucyl-tRNA and valyl-tRNA also bind to immature threonine deaminase (4). Supporting evidence for the involvement of some form of threonine deaminase in *ilv* regulation was provided by Levinthal *et al.* (12) who showed that a single lesion in threonine

deaminase (*ilvA538*) resulted in sensitivity to leucine, reduced expression of the *ilv* gene cluster and decreased the levels of the branched-chain aminoacyl-tRNA synthetases. The derepression of the *ilvD* gene product (dihydroxy acid dehydrase) and of the *ilvB* gene product (acetohydroxy acid synthase) in repressing levels of the three branched-chain amino acids under conditions of pyridoxine restriction supported the idea that some pyridoxine containing component was required for the multivalent repression signal (21). That this component was indeed the *ilvA* product was indicated by similar results in a strain of *S. typhimurium* in which only the *ilvA* gene product could have been deprived of its coenzyme (4). The observations pointing to a role for the *ilvA* gene in regulation of *ilv* gene expression have led to the postulation of both positive control and negative control models.

It is possible to accommodate into a model for regulation of the *ilv* gene cluster the evidence that the *ilvO* region is not between the *ilvA* and *ilvC* genes as would be anticipated if the *ilvO* region were indeed the operator for the *ilvADE* operon as postulated by Ramakrishnan and Adelberg (17). It was shown above that *ilvO* mutations, while amplifying the expression of the *ilvADE* operon, also affect *ilvB* expression and restrict the expression of the *ilvC* gene (Table 3). Thus, the *ilvO* region may not be the *ilvADE* operator. Indeed it is possible that the *ilvA* and the *ilvD* structural genes may have a promoter-initiator-repression site adjacent to *ilvA* and that *ilvE* may have its own such site.

Another question is whether the regulation observed over the *ilvE* gene in the *ilvDAC* deletion strains is mediated via the *ilvO* region to which it is now more closely linked. Until *ilvO*$^-$ derivatives of the deletion strains can be isolated or prepared, it is not possible to answer this question.

That an apparently normal regulation of the *ilvE* and the *ilvB* genes occurs in the strains with the *ilvDAC115* deletion leads to the conclusion that regulation of these genes can occur in the absence of threonine deaminase. The possibility that the first enzyme of a pathway can exert effects on the

expression of other enzymes in the pathway without being an absolute requirement for the control mechanism of the pathway is not unprecedented. For example, Somerville and Yanofsky (18) found that some mutations in *E. coli* that led to the production of a more feedback resistant anthranilate synthetase (*trpE* gene product) also affected regulation of synthesis of the other tryptophan biosynthetic enzymes. However, Hiraga and Yanofsky (10) described an apparent normal repression mechanism for the *trp* operon in an *E. coli* K-12 strain in which most of the *trpE* gene was deleted.

The results presented in this paper establish that threonine deaminase, the *ilvA* gene product, is not essential for the multivalent regulation of the *ilv* gene cluster. However, these data do not rule out the possible involvement of threonine deaminase in the repression mechanism when it is present. Thus, there may be both an *ilvA*-independent control and an *ilvA*-dependent control for isoleucine and valine biosynthesis.

REFERENCES

1. Arfin, S. M., and Umbarger, H. E. (1969) J. Biol. Chem. 244, 1118.
2. Blatt, J. M., Pledger, W. J., and Umbarger, H. E. (1972) Biochem. Biophys. Res. Commun. 48, 444.
3. Bollon, A. P., and Magee, P. T. (1973) J. Bacteriol. 113, 1333.
4. Calhoun, D. H., and Hatfield, G. W. (1973) Proc. Natl. Acad. Sci. U.S.A. 70, 2757.
5. Davis, B. D., and Mingioli, E. S. (1950) J. Bacteriol. 60, 17.
6. Duggan, D. E., and Wechsler, J. A. (1973) Anal. Biochem. 51, 67.
7. Gross, J., and Englesberg, E. (1959) Virology 9, 324.
8. Hatfield, G. W., and Burns, R. O. (1970) Proc. Natl. Acad. Sci. U.S.A. 66, 1027.
9. Hatfield, G. W., and Umbarger, H. E. (1970) J. Biol. Chem. 245, 1736.
10. Hiraga, S., and Yanofsky, C. (1972) Nature New Biology 237, 47.
11. Kiritani, K., Matsuno, T., and Ikeda, Y. (1965) Genetics 51, 341.
12. Levinthal, M., Williams, L., Levinthal, M., and Umbarger, H. E. (1973) Nature New Biology 246, 65.
13. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265.
14. Marsh, N. J., and Duggan, D. E. (1972) J. Bacteriol. 109, 730.
15. O'Neill, J. P., and Freundlich, M. (1972) Biochem. Biophys. Res. Commun. 48, 437.
16. Pledger, W. J., and Umbarger, H. E. (1973) J. Bacteriol. 114, 195.
17. Ramakrishnan, T., and Adelberg, E. A. (1965) J. Bacteriol. 89, 654.
18. Somerville, R. L., and Yanofsky, C. (1965) J. Mol. Biol. 11, 747.
19. Szentirmai, A., Szentirmai, M., and Umbarger, H. E. (1968) J. Bacteriol. 95, 1672.
20. Umbarger, H. E. (1971) In *Metabolic Regulation*, Vol. 5 (H. J. Vogel, Ed.), pp. 447, Academic Press, New York.
21. Wasmuth, J., Umbarger, H. E., and Dempsey, W. B. (1973) Biochem. Biophys. Res. Commun. 51, 158.
22. Wasmuth, J., and Umbarger, H. E. (1974) J. Bacteriol. 117, 29.