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Acetohydroxy Acid Synthase I Is Required for Isoleucine and Valine Biosynthesis by *Salmonella typhimurium* LT2 during Growth on Acetate or Long-Chain Fatty Acids†

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***Salmonella typhimurium* LT2 normally expresses two acetohydroxy acid synthases (AHAS I and AHAS II). The function of AHAS I in this organism was unclear, since AHAS I-deficient (*ilvBN*) mutants of LT2 grew well on glucose or succinate minimal media, whereas AHAS II-deficient (*ilvGM*) mutants required isoleucine for normal growth on glucose minimal media. We report that AHAS I-deficient mutants of *S. typhimurium* required isoleucine and valine for growth on acetate or oleate minimal media, whereas AHAS II-deficient mutants were able to grow on these media without isoleucine supplementation.**

Salmonella typhimurium LT2 and *Escherichia coli* K-12 generally regulate their biosynthetic pathways by the same mechanisms. A conspicuous and well-documented exception to this rule is found in isoleucine-valine biosynthesis, in which valine inhibits isoleucine biosynthesis in *E. coli* K-12 but not in *S. typhimurium* LT2 (4). This situation results from the presence of different sets of acetohydroxy acid synthase (AHAS) isozymes in the two organisms. AHAS catalyzes the first step in valine-leucine biosynthesis, the condensation of two pyruvate molecules to form acetolactate and CO_2, as well as the analogous reaction (the condensation of pyruvate and α-ketobutyrate) in isoleucine biosynthesis. *S. typhimurium* LT2 has two active AHAS isozymes, the valine-sensitive AHAS I encoded by the *ilvBN* operon and the valine-resistant AHAS II encoded by the *ilvGM* genes. Isoleucine synthesis in *E. coli* K-12 is valine sensitive because only the two valine-sensitive isozymes, AHAS I and AHAS II (encoded by the *ilvHI* operon), are expressed. Although wild-type strains of *S. typhimurium* LT2 and *E. coli* K-12 each fail to express an AHAS isozyme found in the other organism, mutants of both organisms which express the cryptic AHAS isozyme can be isolated (11, 13).

The reason for the presence of different sets of isozymes in *S. typhimurium* LT2 and *E. coli* K-12 is unclear, but it seems possible that seemingly homologous isozymes could have different functions in the two organisms. We have recently reported that *E. coli* strains lacking AHAS I fail to grow either on acetate or on a long-chain fatty acid (oleate) as the sole carbon source unless supplemented with isoleucine and valine (2). However, such strains grow normally without amino acid supplementation when other carbon sources, such as glucose, glycerol, or succinate, are provided (2). AHAS I expression is, therefore, essential for the adequate synthesis of valine and isoleucine in *E. coli* K-12 cultures growing on acetate or oleate as the sole carbon source. In this paper, we report that *S. typhimurium* LT2, like *E. coli* K-12, requires the expression of AHAS I for growth on these carbon sources.

The strains used in this work were all derivatives of *S. typhimurium* LT2. The media and AHAS assays were described previously (2). It should be noted that all three AHAS isozymes have recently been shown to be composed of two subunits encoded by two neighboring genes in an operon (10, 12, 16). The mutational lesions in the strains used are thought to reside in the genes encoding the large (catalytic) subunits, since the small subunits seem to play a regulatory role (4).

Strain MS1286 was isolated from a collection of Mu *d*I1734 (1) insertion mutants. The collected strains were unable to grow on acetate or oleate as the sole carbon source, whereas growth on succinate was normal (R. Wilson and S. Maloy, J. Bacteriol., in press). We screened the collection (22 strains) for strains that grew on acetate or oleate only when supplemented with isoleucine and valine. Strain MS1286 and one other strain had this phenotype and grew normally on succinate or glucose without amino acid supplementation (Table 1). In common with the results obtained with *E. coli* (2), supplementation with both α-ketoisovaleric acid and α-keto-β-methylvaleric acid (precursors of valine and isoleucine, respectively) also allowed growth on acetate or oleate (data not shown).

The close resemblance of the growth characteristics of strain MS1286 to those of the *E. coli* K-12 *ilvBN* strains (2) suggested that strain MS1286 lacked AHAS I. Indeed, the total AHAS activity was not only lower in extracts of this strain but was also resistant to valine inhibition and thus was identified as AHAS II (7, 9, 11) (Table 2). Moreover, the large increase in total AHAS activity seen in the wild-type strain grown on acetate plus limiting isoleucine and valine was not observed in extracts of strain MS1286 grown on the same medium. Most of the activity in the wild-type extract was inhibited by valine (Table 2), whereas the low AHAS activity remaining in strain MS1286 was resistant to valine. Thus, strain MS1286 lacked valine-sensitive AHAS 1. The mutational lesion in strain MS1286 was shown to be the result of the Mu *d*I1734 insertion, since all kanamycin-resistant (i.e., Mu *d*I1734 carrying) recombinants formed by transduction with phage P22 were unable to grow on acetate (Table 3). Moreover, the Mu *d*I1734 insertion was closely

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TABLE 1. Growth rates of *S. typhimurium* wild-type and mutant strains on various media

Strain	Genotype	Generation time (h) at 37°C on[a]: Glu	Suc	Ace	Ace + ILV	Ole	Ole + ILV
LT2	*ilv*$^+$	1.3	2.1	2.1	2.1	2.6	
MS1286	*ilvBN*::Mu *d*I1734	1.3	2.4	NG[b]	2.6	NG	3.2
TT56[c]	*ilvGM593*::Tn*10*	6.0	2.1	2.2	2.4	2.7	2.1

[a] The carbon source was glucose (Glu), sodium succinate (Suc), or sodium acetate (Ace) at a 0.4% final concentration. Oleate (Ole) was added to 0.1% as the potassium salt in 1% Brij 58 detergent. Isoleucine and valine (ILV) were added to 40 μg/ml each. The minimal medium was that of Neidhardt et al. (6).

[b] NG, No detectable growth. When cells were grown in medium plus limiting isoleucine and valine (20 μg/ml each), centrifuged, and suspended in the same medium minus isoleucine and valine, cell numbers doubled less than once.

[c] Strain TT56 was obtained from J. Roth and is described by Shaw et al. (11).

linked to a Tn*10* transposon inserted close to the *ilvBN* loci (Table 3) and cotransduced with the Tn*10* insertion at a frequency similar to that observed (K. Rudd, personal communication) between the Tn*10* insertion and *ilvBN* point mutations.

An *ilvGM*$^+$ strain carrying the *ilvBN101* lesion studied by Primerano and Burns (9) also grew on acetate only when supplemented with isoleucine and valine (data not shown), even though the *ilvBN101* lesion was originally identified by a different phenotype, a valine requirement in an *ilvGM* background.

We also investigated the effects of carbon source on the role of AHAS II. Several laboratories (8, 9, 11) have reported that AHAS II is an essential isozyme for isoleucine biosynthesis in *S. typhimurium* LT2 strains grown on glucose as the sole carbon source. We confirmed that strain TT56, an *ilvGM*::Tn*10* strain (11), required isoleucine for growth on glucose but found that the strain grew well in the absence of amino acids if acetate, oleate, or succinate was the carbon source (Table 1). Thus, AHAS I was able to synthesize the required isoleucine precursor but catalyzed the reaction inefficiently, thus requiring high enzyme levels to produce adequate levels of α-acetohydroxybutyrate. A similar conclusion concerning the specificity of AHAS I has been made with strains which overproduce AHAS I because of the presence of *ilvBN* on a multicopy plasmid (15). Primerano and Burns (9) also noted that the *ilvGM*::Tn*10* insertion mutant, strain TT56, had a less stringent requirement for isoleucine than did a strain carrying the *ilvGM236* point mutation. This unusual finding was attributed to the toxicity of α-ketobutyrate accumulated by the *ilvGM236* strain because of a lack of transcriptional polarity. We found no differences in growth on acetate, oleate, or succinate for strains carrying either the point or Tn*10* of *ilvGM* lesions, indicating that high levels of AHAS I eliminated the toxic accumulation of α-ketobutyrate (data not shown).

Weinberg and Burns (15) reported a lack of correlation between levels of AHAS I activity and carbon sources when an *ilvGM* strain was grown on carbon sources assumed to differ in their ability to elicit catabolite repression. Our data show a strong effect of carbon source (Table 2). However, it should be noted that Weinberg and Burns (15) did not report the carbon sources they tested. Moreover, the strain used (strain DU503) seems atypical in that the addition of cyclic AMP to glucose-grown cultures of that strain caused a decrease in AHAS I levels, although the formation of the cyclic AMP-cyclic AMP receptor protein complex was shown to increase the synthesis of AHAS I (15).

TABLE 2. AHAS activity in wild-type AHAS I- and AHAS II-containing *S. typhimurium* strains grown on various media

Strain	Genotype	Growth conditions[a]	AHAS activity[b] −	AHAS activity[b] +[c]	% Valine sensitive
LT2	*ilv*$^+$	Glucose	2.5	2.0	20
		Succinate	6.0	1.8	70
		Acetate	28.0	3.2	89
		Acetate + ILV[d]	25.0	3.6	86
		Oleate	6.0	1.5	75
		Oleate + ILV	7.8	1.3	85
MS1286	*ilvBN*::Mu *d*I1734	Glucose	1.8	1.8	0
		Succinate	1.6	1.6	0
		Acetate + ILV	1.6	1.6	0
		Oleate + ILV	1.2	1.2	0
TT56	*ilvGM593*::Tn*10*	Succinate	5.5	1.1	80
		Acetate	4.5		
		Acetate + ILV	5.0	1.2	76
		Oleate	5.4		
		Oleate + ILV	5.0	1.2	76

[a] Cells were grown as described in Table 1, footnote *a*.

[b] Essentially identical results were found in at least two independent experiments. One unit of activity is defined as 1 μmol of acetolactate formed per h at 37°C and pH 8.0.

[c] Valine was added to the assay to 1 mM. AHAS II activity is 100% resistant to valine inhibition, whereas AHAS I activity is only 10 to 30% resistant (9, 11, 14, 15).

[d] ILV, Limiting isoleucine and valine (see Table 1, footnote *b*).

In *S. typhimurium* LT2, as in *E. coli* K-12, the reason that AHAS I is specifically required for isoleucine-valine biosynthesis during growth on acetate or oleate is not yet clear. However, it seems likely that certain metabolic conditions known to be present in cells grown on acetate (and presumably oleate) are involved. First, the cyclic AMP levels in acetate-grown cells are high, greatly stimulating the synthesis of AHAS I but not the synthesis of the other isozymes (13, 15). Second, the decreased intracellular pyruvate levels found in acetate-grown *E. coli* K-12 cells (5) may necessitate a higher total AHAS activity for sufficient acetohydroxy acid production. Third, the presence of a metabolite accumulated

TABLE 3. Transductional mapping of the AHAS I defect in strain MS1286

Donor[a]	Recipient	Selected marker	Unselected marker[b] Ace	Tet	Kan	% Frequency[c]
MS1286	TV071	Kan^r	−	S		59 (118)
			−	R		41 (82)
			+	S		<0.5 (0)
			+	R		<0.5 (0)
TV071	MS1286	Tet^r	−		S	<0.5 (0)
			−		R	27 (54)
			+		S	73 (146)
			+		R	<0.5 (0)

[a] Phage P22 was grown on strain MS1286 (*ilvBN*::Mu *d*I1734 [Kan^r]) and strain TV071 (*ilv*$^+$ *zia*::Tn*10* [Tet^r]), and transductions were done as described by Davis et al. (3). Strain TV071 was obtained from R. LaRossa.

[b] The phenotypes are as follows: Ace, growth on acetate as the sole carbon source; Tet and Kan, resistance (R) or sensitivity (S) to tetracycline and kanamycin, respectively.

[c] Numbers in parentheses represent the number of colonies with a given phenotype out of 200 colonies scored.

when acetate or oleate is used as the sole carbon source (e.g., glyoxylate [2, 7]) could inhibit AHAS activity, thus necessitating the high AHAS levels found in acetate- or oleate-grown cells. Consistent with the possibility of inhibition of AHAS activity in vivo is the finding that although oleate-grown cultures have AHAS activities almost identical to those of succinate-grown cultures (Table 2), *ilvBN* strains grow normally on succinate without amino acid supplementation, whereas growth on oleate requires isoleucine and valine. It seems, therefore, that in oleate-grown cells the level of AHAS activity functioning in vivo is lower than it is in succinate-grown cells. The differences in AHAS I levels seen in *S. typhimurium* cultures grown on acetate or oleate are not understood, since both carbon sources must be converted to acetyl coenzyme A before utilization as carbon and energy sources (7). However, in contrast to *E. coli* K-12 (2), *S. typhimurium* LT2 grows more slowly on oleate than on acetate (Table 1), possibly reflecting a difference in the metabolism of these compounds by the two organisms.

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