THE ACETOHYDROXY ACID SYNTHASE III ISOENZYME OF ESCHERICHIA COLI K-12: REGULATION OF SYNTHESIS BY LEUCINE

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SUMMARY: Acetohydroxy acid synthase III (AHAS III) is one of the three isoenzymes which catalyze the condensation reaction for the biosynthesis of the branched chain amino acids in Escherichia coli K-12. The synthesis of this enzyme is repressed by leucine. As a consequence of this regulatory feature, strain PS1035, in which AHAS III is the only AHAS isoenzyme expressed, does not grow in minimal medium containing leucine. The other two branched chain amino acids, isoleucine and valine, do not have regulatory effects on AHAS III synthesis.

Acetohydroxy acid synthase III (AHAS III) is one of three isofunctional enzymes, each able to catalyze the synthesis of both acetohydroxy acid precursors of the branched chain amino acids in Escherichia coli K-12 (Fig. 1). This enzyme is composed of two different polypeptide subunits. One of these subunits, the product of the ilvI gene, bears the catalytic properties (3), while the other, the product of the ilvH gene, regulates the activity of the enzyme by making it sensitive to end product inhibition by valine (4). The ilvH and ilvI genes are closely linked and are both cotransducible at high frequency (about 85%) with the leu operon (4). These two genes are apparently coordinately expressed (M. De Felice, unpublished results), although there is no direct evidence putting them under the control of common regulatory elements.

We analyzed the effect of the branched chain amino acids on the regulation of AHAS III. The results reported here show that the synthesis of this enzyme is fully repressed by leucine. A preliminary account of this work has been reported (M. De Felice, T. Newman, C. Squires and M. Levinthal, Abstract K159, 77th Meeting of the American Society for Microbiology, May 1977).

MATERIALS AND METHODS

Bacterial strains. Table 1 lists the E. coli K-12 strains used.

Reagents, media and growth conditions.

All amino acids were the L-isomers, with the exception of α, ε-diaminopimelic acid (DAP), which was a mixture of the LL, DD and meso isomers. The minimal medium was that described by Vogel and Bonner (5), containing

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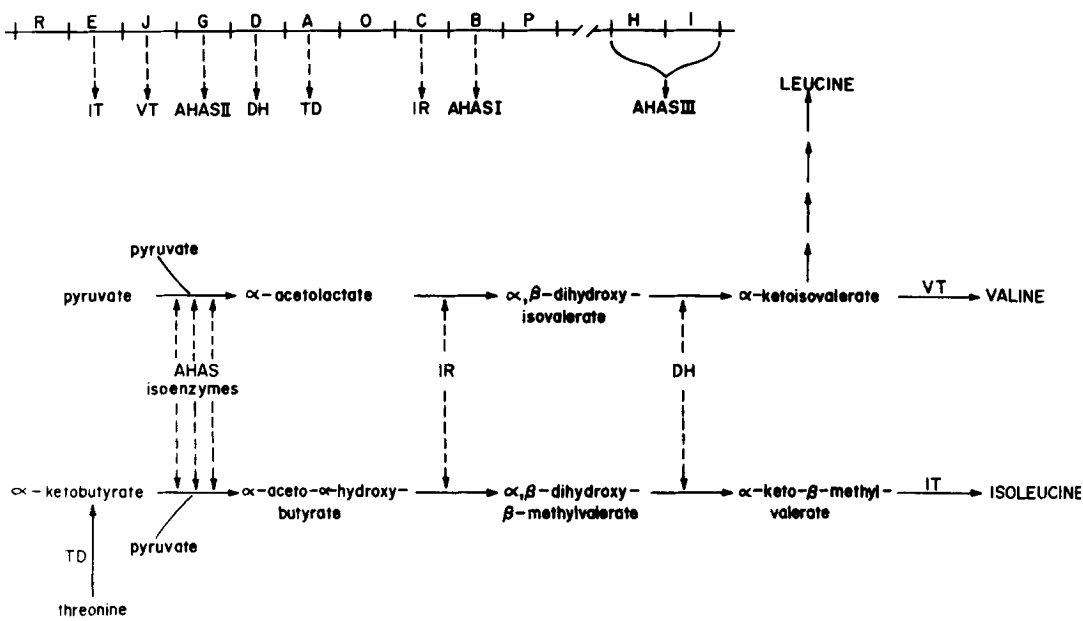


Figure 1. Biosynthetic pathway for isoleucine and valine and the corresponding ilv genes in E. coli K-12. The enzymes are abbreviated as follows: TD for threonine deaminase (EC 4.2.1.16), AHAS for acetohydroxy acid synthase (EC 4.1.3.18), IR for isomeroreductase (EC 1.1.1.86), DH for dehydrase (EC 4.2.1.9), VT for valine transaminase, and IT for isoleucine transaminase. Genetic designations are as given by Bachmann et al. (1) and Iaccarino et al. (2). All the ilv genes are located on the E. coli K-12 map (1) at 83 minutes, with the exception of ilvH and ilvI, which are located at 2 minutes.

glucose (0.4%) as a carbon source. Growth supplements were thiamine (10 µg/ml), glycine (50 µg/ml) and DAP (100 µg/ml). Since strains altered in the AHAS isoenzymes have a partial requirement for DAP and a temperature-sensitive phenotype (6), all experiments were performed at 32 C, in media containing DAP. Growth was measured in a Klett-Summerson colorimeter with a No. 42 filter.

Enzyme assays.

For the preparation of extracts, cells growing exponentially were centrifuged, washed with minimal medium, suspended in 0.1 M potassium phosphate (pH 8.1) and sonicated for 30" with a Biosonik III sonicator (Bronwill Scientific, Rochester, N.Y., U.S.A.) at 30% of its maximum intensity. Following centrifugation (30' at 25 000 x g) the threonine deaminase and AHAS activities in the supernatants were determined as described by Iaccarino and Berg (7) and Størmer and Umbarger (8), respectively. Specific activities are expressed as nmoles of product formed per minute per mg of protein.

RESULTS

The E. coli K-12 strains used in this work, MI158a, PS1035 and PS1036 (Table 1) are three isogenic derivatives of strain AT739. The only rele-

Table 1

Bacterial strains

Genetic symbols are those used by Bachmann et al. (1)

Strain	Genotype
AT739	HfrH thi-1 thr-19 car-53 (from A.T. Taylor)
MI158a	HfrH thi-1 (this laboratory)
PS1035	HfrH thi-1 bgl-20 glyA ilvB619 (this laboratory)
PS1036	HfrH thi-1 ara glyA ilvH612 ilvI614 (this laboratory)

Table 2

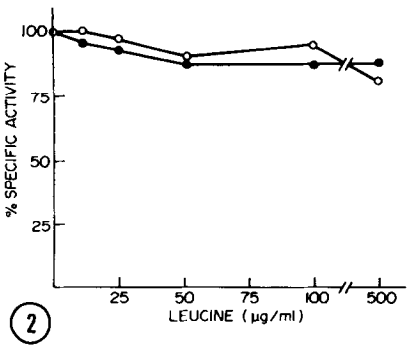
Doubling times of strains MI158a, PS1035 and PS1036

The growth medium was minimal medium containing 10 µg/ml thiamine, 50 µg/ml glycine and 100 µg/ml α, ε-diaminopimelic acid (MM). Where indicated, 40 µg/ml isoleucine (I), 40 µg/ml leucine (L) and 80 µg/ml valine (V) were also added. The doubling times are expressed in minutes.

Strain	MM	MM+L	MM+ILV
MI158a	85	91	87
PS1035	98	∿280	95
PS1036	88	93	94

vant difference among them is in the genes coding for the AHAS isoenzymes. Strain MI158a (ilvB$^+$ilvH$^+$ilvI$^+$) expresses both AHAS I and AHAS III; strain PS1035 (ilvB619) expresses only AHAS III; and strain PS1036 (ilvH612 ilvI-614) expresses only AHAS I. AHAS II (the product of the ilvG gene) is not present in any of these strains, because the ilvG gene is only expressed in E. coli K-12 mutants carrying the ilvO603 lesion (9, 10).

The doubling times of these three strains were determined in different growth media. As shown in Table 2, growth of strain PS1035 is inhibited by leucine, and this inhibition is reversed by isoleucine and valine (we report an approximate value for the doubling time of strain PS1035 in the culture containing leucine, because no exponential growth was observed



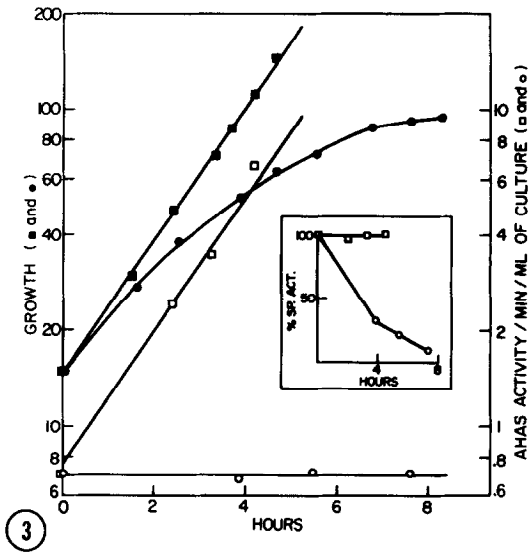


Figure 2. Effect of leucine on the AHAS activity in crude extracts of strains PS1035 (used as a source of AHAS III, •) and PS1036 (used as a source of AHAS I, o). 100% specific activity is equivalent to 38 for AHAS I and 15 for AHAS III.

Figure 3. Synthesis of AHAS III in strain PS1035 during growth in minimal medium (squares) and in minimal medium containing 40 µg/ml leucine (circles). Growth is expressed as Klett units. 100% specific activity is equivalent to 12.

in it). This result can be explained by assuming that either the activity or the expression of AHAS III is negatively affected by leucine.

Fig. 2 shows that in extracts, leucine has no specific inhibitory effect on the AHAS III activity as compared to the AHAS I activity. This observation suggests that the specific growth restriction of strain PS1035 by leucine is a regulatory response.

We performed the experiment reported in Fig. 3 to measure the rate of synthesis of AHAS III in the presence of leucine. An exponentially growing culture of strain PS1035 was diluted into minimal medium and minimal medium containing 40 µg/ml leucine. The AHAS activity was measured at the beginning of and during growth. Fig. 3 shows that, as expected, during growth in minimal medium, an exponential synthesis of AHAS III occurred, and therefore the specific activity remained constant. On the contrary, in the presence of leucine there was no measurable synthesis of AHAS III. In fact, the specific activity decreased as the pre-existing molecules were diluted into a larger population of cells. From the growth profiles

Table 3

Effect of α-ketobutyrate and leucine on the synthesis of threonine deaminase (TD) and AHAS III in strain PS1035

A culture of strain PS1035 was grown at 32 C in minimal medium to middle exponential phase and then divided into three parts. The first part was kept for enzyme assay, while the other two were grown for 90 more minutes with the indicated additions.

Additions to minimal medium	Specific activity	
	TD	AHAS III
---	101	14
α-ketobutyrate (20 μg/ml)	294	41
α-ketobutyrate (20 μg/ml) + leucine (50 μg/ml)	231	11

reported in Fig. 3, it can be noticed that in the presence of leucine, growth of strain PS1035 decreased gradually until it stopped. This phenomenon is most likely the consequence of the gradual decrease in AHAS III specific activity which, during the experiment, dropped about eight-fold until it reached a threshold level that is probably too low to support the synthesis of isoleucine and valine. The addition of isoleucine and valine to minimal medium has no effect on either growth or AHAS activity. In this case (not shown in the figure), cell concentration and AHAS units/ml increased at the same rate over a 10-fold interval.

From these data we conclude that expression of AHAS III is under a negative control in which leucine is the only branched chain amino acid obligatorily involved.

We have recently found (M. De Felice, C. Squires and M. Levinthal, submitted for publication) that growth of strain PS1035 is restricted in the presence of α-ketobutyrate, because this isoleucine intermediate inhibits the formation of α-acetolactate and, therefore, limits the synthesis of leucine and valine. Table 3 shows that, as a consequence of this phenomenon, strain PS1035, grown in the presence of 20 μg/ml α-ketobutyrate, has three-fold derepressed levels of threonine deaminase and AHAS III. However, when leucine (50 μg/ml) is also present, there is no effect on AHAS III, while threonine deaminase is still derepressed, demonstrating that in these conditions a limitation for valine is still occurring. These results confirm the regulatory role of leucine illustrated above.

DISCUSSION

The results reported in this paper provide evidence that leucine is the only branched chain amino acid involved in the regulation of the AHAS III isoenzyme of E. coli K-12. The negative regulatory role of this amino acid is powerful enough to block the synthesis of the enzyme. A physiological consequence of this pattern is that growth of strain PS1035, in which the other two isoenzymes are not expressed, is inhibited by leucine, because of a restriction in the synthesis of isoleucine and valine. This inhibition was not observed in strains MI158a (wild type) and PS1036 (ilvB$^+$ ilvH612 ilvI614), because an excess of only leucine does not affect the synthesis of AHAS I, the ilvB gene product. We report elsewhere that the expression of this gene is multivalently controlled by leucine and valine (M. De Felice, T. Newman and M. Levinthal, submitted for publication).

The present observation that leucine inhibits the growth of strain PS1035, has provided a direct method for the selection of regulatory mutants in which AHAS III is affected. Such mutants, presently under study, should permit us not only to analyze the mechanism of expression of the ilvH and ilvI genes, but also to investigate the possibility that the expression of these genes share common cis-acting and/or trans-acting genetic elements with the leucine operon.

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