THE METABOLISM OF VALINE AND ISOLEUCINE IN ESCHERICHIA COLI XVII THE ROLE OF INDUCTION IN THE DEREPRESSION OF ACETOHYDROXY ACID ISOMEROREDUCTASE*

Stuart M. Arfin+, Barry Ratzkin‡, and H. E. Umbarger

Department of Biological Sciences
Purdue University
Lafayette, Indiana 47907

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Summary: Under conditions that repress the formation of four of the five enzymes required for isoleucine and valine biosynthesis in Escherichia coli and Salmonella typhimurium (growth in the presence of excess isoleucine, valine and leucine), the fifth enzyme, acetohydroxy acid isomeroreductase, is induced by its substrates, acetohydroxybutyrate and acetolactate. Substrate induction appears to be the major if not the only means for regulating the formation of the isomeroreductase in these organisms. The same process seems to occur in Aerobacter aerogenes.

An interesting feature of the isoleucine and valine forming enzymes in Escherichia coli, and most probably Salmonella typhimurium as well, is that two operator regions control four of the five genes of the ilv gene cluster (1). The fifth gene, ilvC, lying between the ilvB and ilvADE genes, responds similarly to, but not coordinately with, the ilvB gene (1). The protein product of the ilvC gene, the isomeroreductase, is formed in much larger amounts than is that of the adjacent gene ilvA, threonine deaminase (2, 3). In this paper, we present evidence that the control of isomeroreductase level is mediated by substrate induction.

Methods The organisms were Escherichia coli strain K-12, Salmonella typhimurium strain LP-2, Aerobacter aerogenes strain 1033, and several mutants derived from them. The medium was that of Davis and Mingioli (4) modified by omitting the citrate and increasing the glucose to 0.5%.

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+ Present Address: Department of Biochemistry, University of Pittsburgh, School of Medicine
‡ NIH Predoctoral Trainee, Grant GM-00779

Amino acids were obtained from Calbiochem. Acetolactate and acetohydroxybutyrate were obtained from Reef Laboratory, Lafayette, Indiana. The other chemicals were reagent grade.

Induction of acetohydroxy acid isomeroreductase was performed in one liter cultures growing in minimal medium supplemented with excess ("repressing" levels) L-isoleucine (0.4 mM), L-leucine (0.4mM) and L-valine (1mM) (5). Cultures were grown overnight with shaking at 37°C with 0.01% glucose. In the morning, glucose was increased to 0.5% and incubation continued. Exponential growth was rapidly obtained.

Extracts were prepared and assayed as described previously except that a microtip was used on the Branson sonifier because of the small volumes (1-2ml) of cell suspensions employed (6). Protein was determined by the method of Lowry et al.(7).

Results In order to determine whether acetohydroxy acid isomeroreductase might be induced by its substrate, acetohydroxybutyrate was added to repressed cultures of several strains. As Table I shows, there was significant change in specific activity only for the isomeroreductase in each of the strains examined. In these experiments, the activity of the fifth enzyme, transaminase B, was not measured. In one experiment, in which an analog of the substrate, α-methyllactate, was employed as an inducer for a culture of the wild type strain K-12 in minimal medium, transaminase B activity was also measured. The isomeroreductase increased by a factor of three while the other four enzymes remained essentially constant. It is interesting that in stationary phase cells of A. aerogenes, the isomeroreductase was four fold higher than it was in midlog phase cells, while the pH 8.0 or biosynthetic acetohydroxy acid synthetase was at a lower activity than in midlog. Thus, the high activity of the pH 6.0 or fermentative acetolactate forming enzyme, which is valine insensitive (8), probably provided inducer.

The specificity of the inducer in E. coli strain CU-15 is shown in

Table I. Effect of Acetohydroxybutyrate Supplementation of the Isoleucine and Valine Forming Enzymes in Several Bacterial Strains

Strain	Specific Activity (μmoles/mg prot/hr)							
	Threonine Deaminase		Acetohydroxy Acid Synthetase		Dihydroxy Acid Dehydrase		Isomero-reductase	
	0'	30'	0'	30'	0'	30'	0'	30'
E. coli								
K-12 (*ilv*$^+$)	1.51	1.90	0.85	0.69	0.55	0.63	0.19	4.13
CU-2 (*ilvE*)	1.42	1.75	0.75	0.71	0.66	0.86	0.34	4.02
CU-15 (*ilvD*)	0.40	0.38	0.67	0.55	Absent		0.28	2.53
S. typhimurium								
LT-2 (*ilv*$^+$)	11.2	11.3	4.25	4.24	0.72	0.72	0.53	5.17
MD-16 (*ilvE*)	6.35	5.83	1.37	1.77	1.00	1.04	0.18	1.72
A. aerogenes								
1033 (*ilv*$^+$)								
mid log	7.50	9.43	2.39* 0.27‡	2.45* 0.54‡	2.59	2.45	0.35	4.27
stationary	--	--	0.43* 4.93‡	--	--	--	1.40	--

* Assayed at pH 8.0
‡ Assayed at pH 6.0

To exponentially grown cultures with excess branched-chain amino acids, 6mM acetohydroxybutyrate was added and incubation continued 30 minutes. Cells were harvested before and after the experimental period and enzyme activities determined in extracts. Other conditions as described in Methods.

Table II. It can be seen that the natural substrates, acetolactate and acetohydroxybutyrate, were the most effective in inducing isomeroreductase activity. Of the analogs tested, α-hydroxy-α-methylbutyrate was the most effective.

Some preliminary studies have been made on the kinetics of induction of the isomeroreductase using strain CU-15. In one experiment, the log phase

TABLE II Effect of Substrates and Analogs on the Level Of the Isomeroreductase in Repressed Cells

Supplement	Specific Activity μMoles/hr/mg prot
None	0.193
α-Acetohydroxybutyrate	6.661
α-Acetolactate	4.717
α-Methyllactate	0.322
Acetoin	0.258
3-Hydroxy-3-methyl butanone	0.289
α-Hydroxy-α-methyl butyrate	1.812
α-Hydroxyisovalerate	0.315

Supplements were added at 6mM concentration. Cells were harvested and extracts prepared 30 minutes later. Other conditions as in Table I.

cells were deprived of valine for 20 minutes. Figure 1 shows that the addition of valine allowed an immediate burst of isomeroreductase synthesis comparable to the burst of synthesis of ornithine transcarbamylase observed by Faanes and Rogers (9) upon addition of arginine to arginine-starved cells. If acetohydroxybutyrate was added simultaneously, the burst was prolonged. A second experiment, also represented in Fig. 1, was performed like the experiments shown in Tables I and II except that samples were removed during the induction period. The two experiments differ in that, in the absence of the "derepression" signals generated during the valine starvation, a lag of about 2 minutes was observed before the increase in enzyme activity. Furthermore, the rate of increase was less.

Discussion The evidence reported here strongly supports the view that the acetohydroxy acid isomeroreductase is induced directly or indirectly by its substrates. The finding that acetohydroxy acid isomeroreductase is substrate-induced is quite in accord with the fact that this enzyme is not controlled by either of the two operator genes demonstrated in the ilv gene

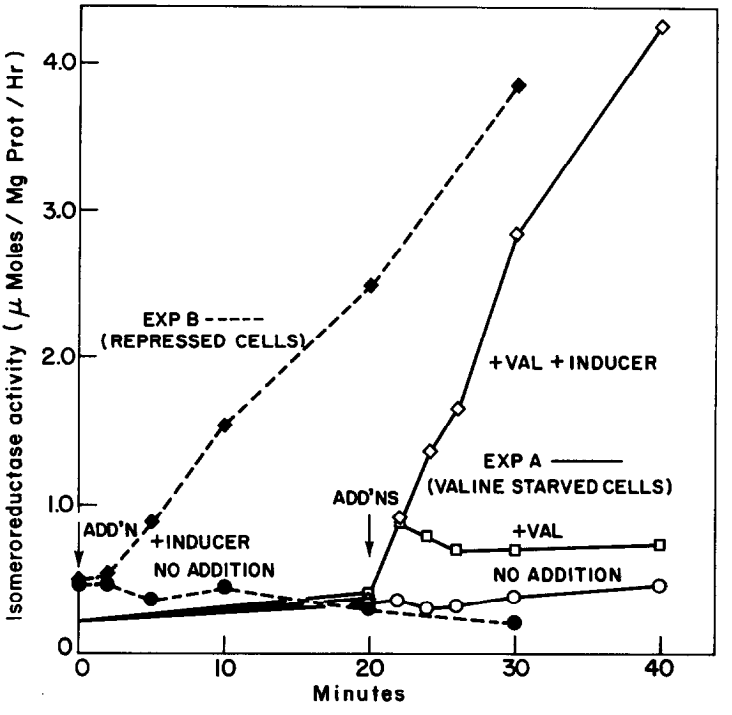

Figure 1. Induction of isomeroreductase activity in E. coli strain CU-15.

Experiment A. A one liter culture of E. coli strain CU-15 in the repressing medium was harvested by centrifugation at room temperature and resuspended in four 250 ml quantities of medium with leucine and isoleucine but no valine. All were incubated with shaking at 37° C; after 20 minutes, valine was added to flask 1 (□ — □), valine plus 6 mM acetohydroxybutyrate to flask 2 (◇ — ◇), and nothing to flask 3 (○ — ○). Samples of 25 ml were removed and chilled by pouring over frozen, crushed 0.05 M potassium phosphate buffer, pH 7.0. Other conditions as in Methods.

Experiment B. A repressed culture of E. coli strain CU-15 was induced as described in Table I. Samples removed at intervals as in Experiment A. ◆ — ◆ 6mM acetohydroxybutyrate added at arrow. ● — ● No additions.

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cluster (1). Thus, the previously observed multivalent repression of the isomeroreductase is mediated not by the repressor-operator mechanism that presumably regulates the formation of the other isoleucine and valine forming enzymes (10), but by the extent to which the second enzyme in the pathway is allowed to function and by the level of that enzyme in the cell.

To a limited extent, analogs of the substrates are able to induce the

enzyme. However, it is not clear whether they mimic the inducer effect of the substrates or inhibit the enzyme thus allowing the substrates to accumulate. An effective competitive inhibitor of the enzyme, α-methyllactate, was an effective growth inhibitor but a poor inducer. Another substrate analog, α-hydroxy-α-methylbutyrate, was a less effective inhibitor of both enzyme activity and growth, but it was a better inducer.

Compatible with the results described here are observations on derepressed mutants. Thus, isomeroreductase activity is partially derepressed in S. typhimurium strain CV-19 in which at least one component necessary for multivalent repression is missing (10). In this strain, valine is not an effective inhibitor of acetohydroxy acid synthetase. Therefore, the large amounts of the substrates formed presumably induce the isomeroreductase as a secondary consequence of the derepression of the acetohydroxy acid synthetase. In contrast, a derivative of strain K-12 that is deficient in multivalent repression exhibits derepressed levels of only those four enzymes shown by Ramakrishnan and Adelberg (1) to be controlled by the two ilv operator genes ($ilvO_A$ and $ilvO_B$). Presumably, endproduct inhibition prevents even the 80-fold derepressed level of acetohydroxy acid synthetase in this strain from producing a significant amount of "inducer." Interestingly, Mr. W. J. Pledger has found that in a derivative of that strain containing a valine-insensitive acetohydroxy acid synthetase, the isomeroreductase is derepressed.

While it is clear that a "derepression" signal is not required for the induction of isomeroreductase, we are not certain that such a signal does not contribute to the magnitude of the induction. For example, in Fig. 1, the rate of enzyme appearance following valine addition to starved cells was greater than it was in unstarved cells, and this rate was prolonged at least for a few minutes in the presence of inducers. However, the significance of the difference in the two cases is open to question since a day to day variation in the inducibility of cultures of the same strain has been observed but not explained.

Finally, it should be mentioned that there is no evidence favoring either a transcriptional control (i.e. substrate or its derivative interacting with some regulatory element) or a translational control (perhaps by substrate binding to "nascent" enzyme).

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