Isoleucine and Valine Metabolism in
Escherichia coli

XVIII. Induction of Acetohydroxy Acid Isomeroreductase

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The regulation by substrate induction of the acetohydroxy acid isomeroreductase was studied in Escherichia coli. Induction was inhibited by chloramphenicol and rifampin. The addition of rifampin resulted in a decay of the capacity to form isomeroreductase. This was attributed to the breakdown of the isomeroreductase messenger which had a half-life of about 45 sec at 37°C. Induction of isomeroreductase was enhanced by including glucose in the medium. This effect was shown to be due in part to the lowering of the pH of the medium, which presumably made inducer entry more rapid.

The isoleucine and valine biosynthetic pathway (Fig. 1) consists of five enzymatic steps that are specified in Escherichia coli, and presumably in Salmonella typhimurium as well, by a cluster of five ilv genes designated A through E (18). Arfin et al. (1) showed that the expression of the ilvC gene which encodes the α-acetohydroxy acid isomeroreductase is not controlled directly by end-product repression by isoleucine, valine, and leucine, as are the other isoleucine- and valine-forming enzymes in E. coli and S. typhimurium (6, 7). Rather, the isomeroreductase is induced by its substrates, α-acetolactate and α-acetohydroxybutyrate, even when the levels of the other isoleucine- and valine-forming enzymes are repressed by the addition of all three branched-chain amino acids to the medium.

This finding complements the previous observations on the apparently anomalous behavior of this enzyme by Ramakrishnan and Adelberg (17). They observed, in E. coli K-12, that neither the ilvO gene, which is thought to be the repression recognition site for the ilvADE gene cluster, nor the ilvP gene, which is the corresponding site for the ilvB gene, governed the expression of the ilvC gene. It had therefore been inferred, but never demonstrated, that the ilvC gene would be under the control of a third regulatory element in the ilv cluster. Preliminary evidence for such a control element between ilvB and ilvC has been reported (B. Ratzkin, W. J. Pledger, and H. E. Umbarger, Fed. Proc. 30:1264, 1971).

This communication presents further evidence supporting the inducibility of isomeroreductase. In addition, studies on the mechanism of the induction process are reported.

MATERIALS AND METHODS

Organisms. The organisms used for experiments in this study are listed in Table 1. The organisms were kept on nutrient agar (Difco) slants at 4°C.

Media. The minimal medium of Davis and Mingioli (5) was used, except that citrate was omitted and the glucose was increased to 0.5%. Glycerol, when used instead of glucose, was also at a concentration of 0.5%. Leucine, isoleucine, and methionine were added at a concentration of 0.4 mM, and valine was added at a concentration of 1.0 mM. The rich medium, used in some experiments, contained 0.5% yeast extract, 0.5% NaCl, 1% tryptone, and, unless otherwise specified, 0.5% glucose. The buffered rich medium contained, in addition, the salts of the minimal medium.

Growth of organisms. For studies at constant pH and in some of the induction experiments, the culture vessel depicted in Fig. 2 was used. The pH was controlled with a New Brunswick Automatic pH Controller, model pH-151, with Leeds and Northrup (glass) measuring and calomel reference electrodes. The pH was adjusted with 1N NaOH. The vessel was a Kontes 1-liter, jacketed reaction flask with a top containing four standard tapered necks of 29/42. The whole culture vessel containing medium and the electrodes was autoclaved without the aluminum reaction flask clamp, which was placed on the flask after autoclaving and cooling. A rubber girdle, cut from an automotive inner tube, was used to seal the boiling necks.

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Fig. 1. Biosynthetic pathways to isoleucine and valine, and the genetic map of the ilv region which specifies the enzymes in E. coli. TD = threonine deaminase (EC 4.2.1.15 L-threonine hydro-lyase, deaminating; AHS = acetohydroxy acid synthetase; IR = isomeroreductase; DH = dihydroxy acid dehydrase; TRB = transaminase B (EC 2.6.1.6 L-leucine: 2-oxoglutarate amino transferase). Genes A through E specify the structure of the enzymes as indicated by the dash lines. Genes ilvP and ilvO are repression recognition elements controlling the expression of ilvB and ilvADE operon, respectively. Modified from Ramakrishnan and Adelberg (18).

### TABLE 1. List of strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>Relevant phenotype</th>
<th>Parent</th>
<th>Former designation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>K-12</td>
<td>Wild-type prototroph</td>
<td>W</td>
<td>M-48-62</td>
<td>21</td>
</tr>
<tr>
<td>W</td>
<td>Wild-type prototroph</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CU6</td>
<td>Low level acetohydroxy acid isomeroreductase; requires only valine</td>
<td>K-12</td>
<td>20A19</td>
<td>20</td>
</tr>
<tr>
<td>CU15</td>
<td>Lacks dihydroxy acid dehydrase; requires isoleucine and valine</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Top and bottom of the vessel during autoclaving. Maintenance of temperature was achieved by using a Forma Temp. Jr. water bath, model 2000-2, which pumped water of the desired temperature through the reaction flask jacket.

Compressed air was sparged through an immersion tube that had a 6-cm medium-porosity fritted disc. Additional mixing was provided by a magnetic stirrer. Air pressure was maintained at 6 to 8 psi by a Marco Pres-sure Set. In some experiments, a Cole-Parmier compact flowmeter, model 13, was added to the system. Air flow was kept between 1.5 and 2 liters per min.

In other experiments, cells were grown in Bellco triple-baffled Erlenmeyer flasks in a New Brunswick gyratory shaker or in a gyratory water bath shaker at 37°C.

Cultures in minimal medium containing 0.025% glucose were inoculated at night with an overnight nutrient broth culture (0.5 ml per liter). The next morning, the glucose concentration was increased to 0.5%.

Growth was determined by measuring turbidities in a Klett-Summerson photoelectric colorimeter with a no. 42 filter when minimal medium was employed and with a no. 59 filter when the rich medium was employed. Each Klett unit corresponded to $4.3 \times 10^4$ viable cells per ml with the no. 42 filter and $5.7 \times 10^4$ cells per ml with the no. 59 filter.

**Preparation of extracts.** All manipulations of extracts were performed at close to 0°C. Cells were harvested by centrifugation in a Sorvall RC-2 refrigerated centrifuge at 9,000 $x$ g for 10 min. The cells were washed once with 50 mM potassium phosphate buffer (pH 7.0) and resuspended in three volumes of disruption buffer, which was 50 mM potassium phosphate buffer, pH 7.0, containing 1 mM MgCl$_2$ and 3 mM $\beta$-mercaptoethanol. The suspension was disrupted by ultrasonic disintegration at 2 to 15 C with a 125-w Bronson Sonifier operating at full power. Cell pellets obtained from 25 ml of culture were resuspended in 1 or 2 ml of disruption buffer and disrupted with a microtip on the Sonifier. Debris and unbroken cells were removed by centrifugation at 27,000 $x$ g for 20 min.

**Assay of enzyme activities.** Isomeroreductase
activity was assayed at 37 C by the procedure described previously (2). The 0.5-ml reaction mixture contained 50 μmoles of phosphate buffer, pH 7.5, 1.5 μmoles of MgCl₂, at least 0.02 μmole of reduced nicotinamide adenine dinucleotide phosphate, 5 μmoles of dl-α-acetolactate or dl-α-aceto-α-hydroxybutyrate, and extract. Unless stated otherwise, α-aceto-α-hydroxybutyrate, against which the enzyme was more active, was employed.

Threonine deaminase activity was assayed in a 1-ml reaction mixture containing: 100 μmoles of phosphate buffer, pH 8.0, 100 μmoles of NH₄Cl, 0.1 μmole of pyridoxal-5'-phosphate, 40 μmoles of L-threonine, and extract. After 10 min, the reaction was terminated by the addition of 0.1 ml of 50% trichloroacetic acid. The α-ketobutyrate formed was determined by the method of Friedemann and Haugen (8).

One unit of enzyme activity is equal to that amount of enzyme that forms 1 μmole of product per min. Specific activity is expressed as units per milligram of protein. Protein was determined by the method of Lowry et al. (16).

**Reagents.** The substrates dl-α-acetolactate and dl-α-aceto-α-hydroxybutyrate were prepared by saponification of the ethyl acetox esters as described previously (22). The esters were obtained from Reef Laboratory (Santa Paula, Calif.). Pyridoxal-5'-phosphate and chloramphenicol were purchased from Sigma Chemical Co. Reduced triphosphopyridine nucleotide, sodium salt, was obtained from P-L Biochemicals. The amino acids (A grade) and rifampin (B grade) were obtained from Calbiochem. All other chemicals were reagent grade.

**RESULTS**

**Effects of α-methyllactate.** It had been shown earlier that α-methyllactate is a competitive inhibitor of purified isomeroreductase derived from *S. typhimurium* (2). Its effects on growing cells were therefore examined to see whether it would generate a starvation for isoleucine and valine by inhibiting the isomeroreductase, which, in turn, might lead to a derepression of the isoleucine and valine biosynthetic enzymes. When α-methyllactate was incorporated into a minimal medium that was inoculated with *E. coli* K-12 grown overnight in nutrient broth, a transient inhibition was observed. Whereas a control culture had only a 30-min lag, growth in the presence of α-methyllactate was delayed for almost 4 hr. Examination of the enzyme activities in cell extracts from the two cultures harvested during the late log phase revealed that the cells grown in the presence of methyllactate had more isomeroreductase activity than cells grown without the analogue. In contrast, the levels of threonine deaminase, acetohydroxy acid synthetase, and dihydroxy acid dehydrase were less in the methyllactate-grown cells (Table 2). In some experiments, however, these three activities remained nearly the same.

**Basis of the α-methyllactate effect.** The specific derepression of only the isomeroreductase might have been due either to a specific induction of the enzyme or to an enhanced maturation of enzyme through ligand binding. In either case, the effect might have been due to the α-methyllactate itself or to the acetohydroxy acids that had accumulated owing to the inhibition of isomeroreductase by α-methyl-

**Table 2. Effect of α-methyllactate on the activities of isoleucine and valine biosynthetic enzymes**

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Specific activity&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Minimal-grown culture</td>
</tr>
<tr>
<td>Threonine deaminase</td>
<td>0.065</td>
</tr>
<tr>
<td>Acetohydroxy acid synthetase</td>
<td>0.046</td>
</tr>
<tr>
<td>Acetohydroxy acid isomeroreductase</td>
<td>0.028</td>
</tr>
<tr>
<td>Dihydroxy acid dehydrase</td>
<td>0.012</td>
</tr>
</tbody>
</table>

<sup>a</sup> Extracts prepared from late-log-phase cultures of *E. coli* K-12.
lactate. The effect of adding methyllactate to the medium was, therefore, examined under "repressing"-conditions so that there would be only a low level of acetohydroxy acid synthetase present. Under these conditions, an accumulation of the acetohydroxy acids would be less likely to occur even if the basal level of the isomeroreductase were inhibited by methyllactate.

A culture of strain K-12 was grown in the presence of excess isoleucine, leucine, and valine so that the isoleucine- and valine-forming enzymes were at a repressed level. Furthermore, under these conditions, the valine would have prevented virtually all activity of any acetohydroxy acid synthetase that was present. At a cell density of 100 to 120 Klett units, 6 mM α-methyllactate was added; after 30 min, the cells were harvested, extracts were prepared, and the isomeroreductase was assayed. As Table 3 shows, the culture receiving methyllactate had 1.6 times as much isomeroreductase activity as did the culture receiving no addition. When the natural substrates, acetohydroxybutyrate and acetolactate, were added at a concentration of 6 mM, there were increases of 35- and 25-fold, respectively.

The results in Table 3 support the idea that the isomeroreductase is induced by its substrate. If the assumption is correct that there was no significant acetohydroxy acid production in the cultures, it would appear that α-methyllactate was itself a weak inducer rather than an inhibitor that allowed inducer accumulation.

Effect of chloramphenicol and rifampin upon the induction of acetohydroxy isomeroreductase. Evidence that the induction of the isomeroreductase was dependent upon protein synthesis was obtained by use of inhibitors of protein and ribonucleic acid synthesis. As shown in Fig. 3A, the appearance of isomeroreductase after the addition of acetohydroxybutyrate was prevented if rifampin, which blocks the initiation but not the completion of messages, was added to the culture 3 min before the inducer. If chloramphenicol was added immediately after inducer ("0 min"), a small burst of synthesis was observed, owing perhaps to initiation of the ilbC gene transcription for a brief period before the effect of rifampin had occurred. At later times after induction, the typical pattern was observed of decreasing rate of enzyme synthesis as the presumed message decayed and was not replaced. In contrast, with chloramphenicol the inhibition of increase in enzyme activity was immediate (Fig. 3B). These observations support the idea that the appearance of isomeroreductase was indeed the result of induced enzyme formation. Furthermore, maturation of newly formed polypeptide chains after synthesis is complete does not require a significant amount of time.

**Table 3. Induction of acetohydroxy acid isomeroreductase in repressed cells of strain CU15**

<table>
<thead>
<tr>
<th>Addition to culture</th>
<th>Isomeroreductase activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0.0032</td>
</tr>
<tr>
<td>α-Methyllactate, 6 mM</td>
<td>0.0053</td>
</tr>
<tr>
<td>α-Acetolactate, 6 mM</td>
<td>0.079</td>
</tr>
<tr>
<td>α-Acetohydroxybutyrate, 6 mM</td>
<td>0.111</td>
</tr>
</tbody>
</table>

*Activity in cells harvested 30 min after additions.

The half-life of the functional capacity for isomeroreductase synthesis. As noted in Fig. 3A, the addition of rifampin resulted in a gradual decrease in the rate of isomeroreductase appearance. This effect was probably due to inhibition of further initiations of isomeroreductase messages, although messages already initiated would have been completed. Thereafter, the potential to form enzyme decayed, owing, presumably, to the breakdown of the unstable message.

The rate of decay of enzyme-forming potential can be used, as it has been for other systems, to estimate the functional half-life of the isomeroreductase message. If one assumes a first-order decay of message, the data from experiments of the type shown in Fig. 3A can be treated by the method of Tyler and Magasanik (20) to estimate the half-life of the message. The slope of the linear portion of a plot of log \( (E_\text{max} - E_t)/(E_\text{max} - E_0) \) against time after rifampin addition, where \( E_{\text{max}} \) is the maximal amount of activity appearing after rifampin addition, \( E_t \) is the activity present at the time of addition, and \( E_0 \), the activity at \( t \) min after the addition, is proportional to the half-life. Theoretically, a similar cessation of message initiation can be achieved by removing the inducer by dilution. Figure 4 shows the results obtained by stopping message initiation either by adding 50 µg of rifampin to a rich medium culture induced with 6 mM acetoacidobutyrate or by a 25-fold dilution to remove inducer. The two sets of data appeared to resemble each other sufficiently to conclude that message decay in each case occurred at essentially the same rate. Both sets were therefore included in constructing the straight-line por-
tion of the curve in Fig. 4. It would appear that, 60 sec after rifampin addition or inducer removal, the decay was essentially first order and that the message half-life was about 45 sec. The lag before the onset of a first-order decay would be due not only to the time required for completion of the messages that were initiated last, but also to any additional time required for the protein to be completed and for intracellular inducer to be consumed or lost from the cell, or for rifampin to enter the cell and exert its effect. However, the immediate effect of chloramphenicol inhibition makes unlikely the possibility that the formation of mature, active isomeroreductase causes this lag.

Effect of pH upon induction. While performing experiments in rich media, we noticed that induction of the isomeroreductase in cultures growing in unbuffered, glucose-containing medium resulted in a faster rate of enzyme synthesis than that in cultures growing in a medium buffered with the salts of the minimal medium. Such experiments are represented in Fig. 5. A determination of pH during growth in both media revealed that a pH of 5.4 was reached at a Klett reading of about 250 in the unbuffered medium, whereas that pH was not reached until the Klett reading was about 550 in the buffered medium. The effect of pH was further demonstrated by comparing the rate of induction in rich medium maintained at constant pH values. These results are also shown in Fig. 5. At pH 5.5, the initial rate, which was constant for the first hour, was about 17 times the steady rate at pH 7.0, although it does appear that there was an initial rapid burst of isomeroreductase at the higher pH.

The correlation of pH with induction rate was further demonstrated by comparing induction in the rich medium with and without glucose. As shown in Fig. 6A, in the presence of glucose the pH dropped to 5.7 over the course of the experiment, and the specific activity rose to a maximum of 320 times the uninduced level. In the absence of glucose, the pH rose to 7.5 during the same period, and the maximal specific activity rose to 41 times the uninduced level. In Fig. 6B, a differential plot of the data is shown, and the inset shows a plot of the calculated rate of synthesis against pH. As can be seen, the differential rate in the two cultures was essentially the same until the pH had dropped to 6.5 (indicated by arrow). Thereafter, the differential rate steadily increased with decreasing pH. When the differential rate was plotted against the hydrogen ion concentration, a linear relationship between the differential rate and the hydrogen ion concentration above $3 \times 10^{-3}$ M (i.e., pH $< 6.5$) was revealed. Thus, the increase in the rate of induction that occurred when the pH dropped below 6.5 appears to have been directly proportional to hydrogen ion concentration and perhaps to the concentration of the undissociated form of the inducer. Perhaps, too, the constant induction rate that occurred while the pH was still above 6.5 may have been due to the anionic form of the inducer. The likely consequence of maintaining the inducer in the undissociated form would be
Fig. 4. Decay of the potential for isomeroreductase formation. The cells from a 500-ml culture of strain K-12 in buffered rich medium were resuspended in the same volume of fresh medium previously warmed to 37°C containing 6 mM acetoxybutyrate (O). After 10 min, 50 µg of rifampin per ml was added. Samples of 25 ml were transferred at the times indicated to 2.5 ml of 0.2 m sodium azide containing 2 mg of chloramphenicol per ml, and extracts were prepared. The cells from a second culture were resuspended in 20 ml of warm medium containing 6 mM acetoxybutyrate (●). After 10 min, induction was stopped by 480 ml of warm medium. Samples were removed as from the rifampin-treated culture. Abscissa is minutes after induction was interrupted by rifampin or dilution.

more rapid entry into the cell. Indeed, the internal pH of the cell would have changed little over the interval (7.0 to 5.7) through which the pH of the medium ranged (12).

Effects of carbon source. Although it did appear that the effect of glucose on induction occurred only at low pH, it was not clear that carbon source would have no effect. Accordingly, the effects of glucose and glycerol on induction in buffered rich medium were compared; the cultures were maintained at pH 7.0, so that the problem of pH variability could be eliminated. As shown in Fig. 7, less isomeroreductase was produced by cells of strain K-12 when they were grown in buffered rich medium with glycerol as a carbon source than when grown with glucose (graph A). The specific activity in the glucose culture continued to increase at a linear rate for at least the 120 min of the experiment. On the other hand, in the glycerol culture, the specific activity increased initially at about the same rate as did that in the glucose culture, but it appeared to be approaching a constant level at the time the experiment was terminated. These differences between the two cultures are also shown in the differential plots (Fig. 7B). The induced rate in the glycerol culture was constant, whereas there was a nearly exponential increase in the induced rate in the glucose culture. The pattern of induction shown in Fig. 7B is similar to that shown in Fig. 5B. In that experiment, however, a constant pH had not been maintained, and the increase in rate was attributed to decrease in pH. The question arose, therefore, whether the pH drop was only incidental to a carbon source effect.

Glycerol and glucose were also compared in minimal medium supplemented with excess branched-chain amino acids to reduce or prevent internal induction. The pH was again maintained at 7.0. As Fig. 8A shows, the in-
crease in specific activity for glucose was greater than that in glycerol for the first 20 min. Thereafter, the rates were approximately the same with the two carbon sources. Essentially the same relationship was shown in the differential plot (graph B), which corrects for any differences on the overall increase in cell mass between the two carbon sources. The addition of 3,5-cyclic adenosine monophosphate (AMP) to the glucose culture appeared to have prevented the initial burst of synthesis and reduced the rate of induction below that of either the glucose or the glycerol cultures.

A peculiar mutant with a noninducible isomeroreductase. In the first report from this laboratory on the isomeroreductase, a mutant derived from the W strain of E. coli, strain M48-62, was described which exhibited only a low level of that enzyme (22). The mutant (now designated strain CU6) was unique in that low isomeroreductase activity did not lead to either complete auxotrophy for isoleucine and valine or slow growth in the absence of the two amino acids but, rather, to an absolute requirement for valine. Since a satisfactory explanation for this peculiar requirement has not been proposed, an additional investigation seemed warranted now that more information about the regulation of the enzyme was available.

A culture of E. coli W and another of its derivative, strain CU6, in a glucose minimal medium supplemented with the three branched-chain amino acids were each divided into two portions. One portion of each culture was harvested immediately and an extract was prepared. The other portion was induced in the usual way with 6 mM acetoxybutyrate. After 30 min, the cells were harvested and extracts were prepared. The isomeroreductase activities at zero time and after 30 min of incubation for the two cultures are shown in Table 4. Whereas strain W exhibited an inducible isomeroreductase quite similar to that found in strain K-12 and its derivatives, strain CU6, with or without inducer, exhibited only a level of activity like the noninduced level in strain W.

The question arose whether the lesion in strain CU6 might have affected a controlling element rather than the structural gene for the isomeroreductase. Unfortunately, genetic studies with strain W mutants cannot be performed. However, it was thought that a decision might be made possible by taking advantage of the principle that a regulatory gene
mutation would probably not affect the catalytic properties of the protein whereas a structural gene lesion would.

The properties of the isomeroreductase in strain CU6 were therefore examined. These properties are also shown in Table 4. The enzyme present in cells of strain CU6 appeared to be identical with that of strain W with respect to heat stability, $K_m$ values, and ratio of activity against the two substrates. Moreover, these properties were the same in a crude extract as they were in partially purified preparations. Thus, the mutation in strain CU6 seems not to have affected the qualitative nature of the isomeroreductase. It appeared, rather, to have rendered the enzyme noninducible and the level "frozen" at the basal, non-induced level of the wild-type parent. It is interesting that this low level is sufficient to allow for isoleucine synthesis but under no conditions yet found can it provide for valine formation.

**DISCUSSION**

It was the initial aim in this study to employ methyllactate, an inhibitor of the acetohydroxy acid isomeroreductase, as a means of blocking the formation of valine or isoleucine (or both) and to achieve a resulting derepression. Analogous results had been obtained in other systems, for example, the derepression of the histidine biosynthetic enzymes by aminotriazole in *S. typhimurium* (10). That only the isomeroreductase became elevated brought our attention to the fact that this enzyme is an inducible enzyme and that, if there is a component of multivalent repression in its regulation, it must be very small indeed.

Whereas the normal pattern of control of the amount of isomeroreductase seems clear enough, the mechanism by which methyllactate "induced" the enzyme is unexplained. Two possibilities remain. Either methyllactate mimics the inducing effect of the two substrates or it inhibits the basal amount of isomeroreductase, causing an in situ accumulation of substrates that induce further isomero-reductase synthesis. The second possibility is less attractive, however, since induction is essentially as good when the acetohydroxy acid synthetase is strongly repressed as it is when the enzyme is highly derepressed. Nevertheless, the second possibility has not been eliminated.

Inducible enzymes in biosynthetic sequences have been described previously, but their occurrence does not appear to be frequent. One of the earliest examples was that reported by Lacroute (14) for the pyrimidine pathway in yeast. The pattern found was that the first enzyme (or enzyme complex) in the pathway is a repressible one and the product of that activity is the inducer of the enzymes catalyzing the next step in the sequence (15). The third and fifth enzymes in the pathway are also induced by the products of the second and third enzymes.

This mixed pattern of induction and repression is also to be found in other examples in which induction of a biosynthetic enzyme has been proposed. In two examples, the induction of tryptophan synthetase by indole glycerol phosphate in *Pseudomonas putida* (4) and the induction of the second and third enzymes in the leucine pathway of *Neurospora crassa* by α-isopropylmalate (9), the role of the proposed inducer cannot be examined directly since the "inducers" cannot be supplied from outside the cell. In contrast, O-acetyl serine, the inducer of the assimilatory sulfate reduction pathway essential for cysteine biosynthesis, can be supplied from outside the cell and the inducer role can be readily demonstrated (11, 13). Such is also the case with the acetohydroxy acid isomeroreductase.
ISOMEROREDUCTASE INDUCTION

**Table 4. Comparison of isomeroreductase in strain CU6 and its prototrophic parent, strain W**

<table>
<thead>
<tr>
<th>Determination</th>
<th>Strain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>W</td>
</tr>
<tr>
<td>Specific activity</td>
<td></td>
</tr>
<tr>
<td>Uninduced cells</td>
<td>0.012</td>
</tr>
<tr>
<td>Cells induced 30 min</td>
<td>0.050</td>
</tr>
<tr>
<td>$K_m$ values (mm)$^a$</td>
<td></td>
</tr>
<tr>
<td>Acetolactate as substrate</td>
<td>0.43</td>
</tr>
<tr>
<td>Acetohydroxybutyrate as substrate</td>
<td>0.37</td>
</tr>
<tr>
<td>Half-life of activity upon treatment at 45°C (min)$^b$</td>
<td>5.0</td>
</tr>
</tbody>
</table>

$^a$ Extracts subjected to steps 1 and 3 of purification (1).

$^b$ Extract subjected to first step of purification (1).

The demonstration of the induction of this enzyme accounts for several unexplained features of the control of the isoleucine- and valine-forming enzymes. It is now understandable that there was no effect on the isomeroreductase by either of the two regulatory mutations found by Ramakrishnan and Adelberg (17) to affect the multivalent repression separately of the ilvADE operon (the ilvO mutation) and of the ilvB gene (the ilvP mutation). The control of the expression of the ilvC gene by substrate induction implies that an entirely different kind of control element is involved. By analogy with other systems, one might anticipate that recognition of the acetohydroxy acids by the enzyme-forming system might involve at least two additional genetic elements. One, either a positive or negative control element, would be involved in the generation of the "induce" signal, and the other, an "induction recognition element," would pre-
sumably be contiguous with the ilvC gene and analogous to either the operator or the promoter element of the lac system (23). A preliminary report of a gene, ilvY, specifying a positive control element that in the presence of an acetohydroxy acid interacts with the ilvQ gene adjacent to the ilvC gene has been given (B. Ratzkin, W. J. Pledger, and H. E. Umbarger, Fed. Proc. 30:1264, 1971) and will be described in greater detail in the near future.

Another previously unexplained feature of the regulation of the isoleucine- and valine-forming enzymes was the fact that, in E. coli, regulatory mutations that are thought to have affected specifically the "isoleucine component" of multivalent repression affected expression of neither the ilvB nor ilvC genes but did cause derepression of the ilvA, D, and E genes (6, 19). In contrast, an analogous regulatory mutation affecting multivalent repression in S. typhimurium resulted in derepression of all five ilv gene products (2a). Mutations affecting the "leucine component" of multivalent repression in S. typhimurium lead to derepression of all five ilv gene products, whereas those in E. coli lead to derepression of all but the isomeroreductase itself (3; S. Dwyer, Ph.D. Thesis, Purdue Univ., Lafayette, Ind., 1970). In E. coli, only limiting valine was very effective in causing derepression of that enzyme, whereas, in S. typhimurium, it is derepressed by limitation of any branched-chain amino acid. This difference is attributed to the greater sensitivity of acetohydroxy acid synthetase to inhibition by valine in E. coli than in S. typhimurium. Thus, when the synthetase is elevated in S. typhimurium, even if valine is in excess, sufficient acetohydroxy acids are apparently formed to result in internal induction of the ilvC gene. In contrast, an elevated activity of the synthetase is accompanied by an elevated activity of the isomeroreductase in E. coli only if the synthetase is more valine-resistant than the wild-type organism. A detailed genetic and biochemical analysis of this difference will be reported in a future communication.

The observation that induction was better in the presence of glucose than in its absence appears to have been due in part to the effect of glucose in reducing the pH of the medium. Indeed, induction was better at pH 5.5 than at 7.0, and the differential rate of isomeroreductase formation increased with increase in acidity. It would be anticipated that the acetohydroxy acids penetrate the cell better in the undissociated form than as an anion, so that the effect of pH may simply have been to raise the effective inducer concentration.

The effect of glucose cannot be solely explained by its effect on pH, however. When the pH was maintained at 7.0, better induction was obtained in either rich or minimal medium containing glucose than in either medium containing glycerol. Interestingly, the addition of 3',5'-cyclic AMP to the minimal medium culture containing glucose caused a marked decrease in induction. This observation reveals an interesting symmetry between the effects of cyclic AMP on the induction of catabolic enzymes and on the induction of a biosynthetic enzyme. However, the correlation may merely be a consequence of the reduction in growth rate commonly observed when cyclic AMP is added to cultures growing on glucose.

Finally, the finding that one of the fairly rare and previously unexplained valine-requiring mutants of E. coli contains a noninducible isomeroreductase accounts for the low level of isomeroreductase that was found in that organism. What is not clear is why a basal level of the isomeroreductase is sufficient to permit isoleucine biosynthesis but not valine biosynthesis. Even when about two-thirds of the carbon flow from pyruvate to α-ketoisovalerate is spared by adding leucine and the carbon flow between threonine and isoleucine is virtually quenched by adding isoleucine, the isomeroreductase cannot be diverted to a valine-forming role. It appears that, for some reason, the first molecules of isomeroreductase to be formed, i.e., the basal, uninduced level, are completely committed to isoleucine biosynthesis.

To account for such a partition in function, the idea of a five-enzyme cluster ("isoleu-some") specifically committed to isoleucine biosynthesis and a four-enzyme cluster ("vali-some") specifically committed to valine biosynthesis is attractive. No solid, in vitro evidence for such enzyme aggregates has been encountered in E. coli, however.

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LITERATURE CITED


