ISOLEUCINE AND VALINE METABOLISM IN ESCHERICHIA COLI

V. ANTAGONISM BETWEEN ISOLEUCINE AND VALINE

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The influence of the relative concentrations of amino acids in culture media on the growth of microorganisms was first brought to the attention of microbiologists by Gladstone (1939). He observed that isoleucine, valine, or leucine added singly to a synthetic medium inhibited the growth of the anthrax bacillus whereas a mixture of the three stimulated growth. Although Gladstone was unable at that time to explain the nature of the imbalance, he did suggest two possibilities. The more obvious possibility was that because of their similarity in structure, an excess of one amino acid (for example, leucine) might block the incorporation of another (for example, valine) into bacterial protoplasm. The second possibility suggested was that the amino acid added in excess might prevent the synthesis of the second.

To the knowledge of the authors, there have been no reports in the literature which serve as well documented examples of either of Gladstone's possibilities. The first possibility requires that the antagonism between the two amino acids be competitive. Unfortunately, most of the examples of competitive antagonism between pairs of structurally similar amino acids involve amino acids which are required for growth and, hence, must be supplied in the medium. In one such case, carefully analyzed by Prescott et al. (1953), it was concluded that the inhibition of L-serine utilization by L-alanine in the medium was occurring at the cell surface and not at some intracellular site where it is presumed the synthesis of protoplasm occurs. One might question, therefore, whether similar interpretations might eventually be applied to most other examples of antagonisms between amino acid pairs.

On the other hand, if an inhibitory amino acid prevented the intracellular synthesis of a structurally similar amino acid (Gladstone's second possibility), one would expect the antagonism between the two amino acids to be noncompetitive. An ideal system for testing whether one or the other of Gladstone's interpretations is applicable would be the reversal by L-isoleucine of the inhibitory effect of L-valine on the growth of Escherichia coli strain K-12 (Bonner, 1946). Since the strain can synthesize both valine and isoleucine, the possibility that valine is acting at the cell surface (by preventing penetration of isoleucine) can be eliminated. In this paper, experiments are described which seem best interpreted by Gladstone's second possibility, namely, that valine inhibits the synthesis of isoleucine in E. coli strain K-12 rather than the utilization of isoleucine. These results are in contrast to those obtained using an isoleucine and valine requiring mutant of E. coli in which the antagonism seems to occur at the cell surface quite analogous to the L-serine-L-alanine antagonism described by Prescott et al. (1953).

MATERIALS AND METHODS

The organisms employed in these experiments were the K-12 strain of E. coli and two mutants derived from it, strain 20A19 requiring both L-isoleucine and L-valine or the corresponding α-keto acids (Umbarger and Mueller, 1951) and strain JHM544 requiring either L-isoleucine or its 4-carbon precursors such as α-aminobutyric acid (Umbarger, 1953). An L-valine requiring mutant, strain M-48–62, derived from the W strain of E. coli was kindly supplied by Dr. B. D. Davis. The basal medium was that of Davis and Mingioli (1950) except that sodium citrate was omitted. The amino acids employed were obtained from Nutritional Biochemicals Corporation.

Except where specified, the growth experiments were performed using 10 ml of the basal medium in 125 ml Erlenmeyer flasks incubated at 37 C on a New Brunswick rotary shaking machine. Growth was measured turbidimetrically.

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RESULTS AND DISCUSSION

Isoleucine-valine antagonism in strain 20A19.

E. coli strain 20A19, as a result of a single mutation, is unable to convert the \( \alpha,\beta \)-dibhydroxy acid precursors of L-isoleucine and of L-valine to \( \alpha \)-keto-\( \beta \)-methylvalerate and \( \alpha \)-ketoisovalerate, respectively (step 2 in figure 1; Myers and Adelberg, 1954). Therefore, for the growth of this strain it is necessary to supplement the medium with both L-isoleucine and L-valine. Either or both of the amino acids may be replaced by the corresponding \( \alpha \)-keto acids. In experiments with this strain, it has always been observed that a certain balance between L-isoleucine and L-valine must be maintained in the medium for optimal growth. In figure 2, the growth response of mutant 20A19 is shown to increasing concentrations of L-isoleucine in the presence of several concentrations of L-valine. It can be seen that the optimal concentration of L-isoleucine was dependent upon the concentration of L-valine in the medium. L-Isoleucine, when present in relatively high concentrations, was inhibitory to the utilization of L-valine by the mutant.

When the experimental design was reversed, and the growth response to increasing concentrations of L-valine was determined, it was observed that, unlike its wild type parent (strain K-12), strain 20A19 was not sensitive to L-valine. As shown in figure 3, even with a concentration of 150 \( \mu \)g of L-valine per ml, the utilization of only 5 \( \mu \)g of L-isoleucine was not impaired. Again, however, the depressed utilization of lower concentrations of L-valine due to excessive amounts of L-isoleucine was demonstrated, as can be concluded from the low slope in that portion of each curve representing low concentration of L-valine.

Some indication of the specificity of this inhibitory effect of L-isoleucine is given in figure 4. The data in this graph are not completely comparable to that shown in figures 2 and 3 since the experiments were performed with tube cultures and the basal medium of Davis (1949) was employed. However, the principle is shown that isoleucine impaired valine utilization but not the utilization of \( \alpha \)-ketoisovalerate, the immediate precursor of valine. Conversely, the keto acid analogue of isoleucine, \( \alpha \)-keto-\( \beta \)-methylvaleric acid, depressed the utilization of \( \alpha \)-ketoisovalerate but not that of valine. These results strongly suggest that isoleucine in the medium prevents valine from entering the cell but does not interfere with the utilization of valine formed within the cell. This conclusion is in agreement with the fact that isoleucine is not inhibitory to the wild strain.

A further test of this hypothesis was modeled after the report of Prescott et al. (1953) regarding the role of peptides of L-serine in the nutrition of Lactobacillus delbrueckii. Accordingly, glycyl DL-valine was employed as a source of L-valine for strain 20A19 in the presence of a high concentration of L-isoleucine. The results of such an experiment are shown in figure 5. In plotting these data it was assumed that only the glycyl L-valine was utilized so that the curves are plotted in terms of L-valine. Regardless of how
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Figure 2. Effect of valine concentration on the growth response of Escherichia coli strain 20A19 to increasing concentration of isoleucine. O—O, 5 \mu g L-valine per ml; ●—●, 10 \mu g L-valine per ml; X—X, 20 \mu g L-valine per ml; Δ—Δ, 40 \mu g L-valine per ml. 125 ml Erlenmeyer flasks containing 10 ml of medium, supplemented as indicated. Inoculated 24 hours with shaking at 37 C.

Figure 3. Effect of isoleucine concentration on the growth response of Escherichia coli strain 20A19 to increasing concentrations of valine. O—O, 5 \mu g L-isoleucine per ml; ●—●, 10 \mu g L-isoleucine per ml; X—X, 20 \mu g L-isoleucine per ml; Δ—Δ, 40 \mu g L-isoleucine per ml. Conditions as in figure 2.

Figure 4. Specificity of isoleucine inhibition of valine utilization in Escherichia coli strain 20A19. Wasserman tubes containing 3 ml medium supplemented as indicated. Incubated 24 hours at 37 C.

little or how much of the peptide was utilized the important feature of the data is the shape of the two curves. The dip in the curve caused by L-isoleucine did not occur when the peptide was added.

Another difference in the growth response of strain 20A19 to the peptide and to free valine was noted. The extent of growth obtained in the presence of 5 \mu g of L-valine in the form of the peptide per ml was about twice as great as would have been anticipated from the results already shown using L-valine. The possibility was considered that the necessity of adding L-isoleucine to the medium to permit growth of strain 20A19...
also imposed a limitation on the efficiency of utilization of L-valine even when L-isoleucine was not relatively in excess. It was possible to test the relative effectiveness of the peptide and free L-valine in the absence of L-isoleucine by using strain M-48-62 as the test organism. This mutant has no L-isoleucine requirement and is probably blocked by the reaction (or one of the reactions) represented by step 3b in figure 1. Although the utilization of L-valine by this mutant is not depressed by L-isoleucine, the amount of L-valine needed for protein synthesis would probably be the same for both strains. As shown in figure 6, the growth response to the peptide and free L-valine were identical and at least at low concentrations quite similar to the growth response of strain 20A19 to the peptide. Because of the similarity in response to the peptide by the two mutants it seems reasonable to conclude that L-isoleucine even in fairly low concentrations depresses the uptake of L-valine by strain 20A19 from the medium.

Valine-isoleucine antagonism in strain K-12. In an earlier communication (Umbarger and Mueller, 1951) data were presented which suggested that the valine-isoleucine antagonism observed with the K-12 strain of E. coli was of the competitive type. In figure 7, a typical family of curves is shown from which some of the older data were taken. These experiments were performed in the manner described for those represented in figure 4. It can be seen that the inhibitor-antagonist ratios for 50 per cent inhibition are very nearly constant, characteristic of the behavior of a competitive antagonism. A competitive antagonism between valine and isoleucine would imply that valine blocks the utilization of isoleucine. Since the only known role of isoleucine is that of a protein constituent, the reaction inhibited would presumably be the incorporation of isoleucine into protein.

Such a role of valine as an inhibitor must be rejected because of the evidence presented above that mutant 20A19, derived from the K-12 strain, is not sensitive to valine. The insensitivity of strain 20A19, blocked in step 2 of figure 1, is not unique. The same is true of strain 11A6, which lacks the transaminase (Adelberg and Umbarger, 1953) which transfers the amino group of glutamic acid to the keto analogues of isoleucine and valine (step 1 in figure 1). However, L-valine sensitivity is found in strain JHM544, an isoleucine requiring mutant which can utilize several 4-carbon compounds as a substitute for L-isoleucine but does not have an L-valine requirement. Inhibition by L-valine of strain JHM544 occurs only when the isoleucine requirement is supplied in the form of its 4-carbon precursors. The results of an experiment comparing the effect of L-valine on the growth response of

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**Figure 5.** Utilization of valine and glycylvaline by Escherichia coli strain 20A19. Conditions as in figure 2.

**Figure 6.** Utilization of valine and glycylvaline in the absence of isoleucine. Conditions as in figure 2.
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strain JHM544 to α-aminobutyric acid and L-isoleucine are shown in table 1. Thus only when this strain was forced to convert the 4-carbon precursor of L-isoleucine to a 6-carbon chain, was its growth inhibited by L-valine. This would suggest that isoleucine lies after the blocked reaction rather than before it. If such were the case, the reversal of valine inhibition by isoleucine would be expected to be noncompetitive.

When the antagonism between valine and isoleucine was reexamined, using flask cultures, the basis of the apparent competitive antagonism was revealed. In figure 8, the results of such a series of experiments are shown. Several concentrations of L-valine were employed. For reference, the extent of growth of strain 20A19 to those same concentrations of L-valine (see figure 2) is also shown. It should be noted that the amount of growth was proportional to the concentration of L-isoleucine present up to a certain concentration depending on the amount

**TABLE 1**

<table>
<thead>
<tr>
<th>Effect of valine on Escherichia coli strain JHM544</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Growth Factor</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>None</td>
</tr>
<tr>
<td>L-Isoleucine, 10 µg/ml</td>
</tr>
<tr>
<td>L-Isoleucine, 20 µg/ml</td>
</tr>
<tr>
<td>L-Isoleucine, 30 µg/ml</td>
</tr>
<tr>
<td>DL-Aminobutyric acid, 10 µg/ml</td>
</tr>
<tr>
<td>DL-Aminobutyric acid, 20 µg/ml</td>
</tr>
<tr>
<td>DL-Aminobutyric acid, 30 µg/ml</td>
</tr>
</tbody>
</table>

Conditions as in figure 2.
of L-valine originally present. Beyond that point, the next increment in L-isoleucine resulted in a drastic increment in growth. Particularly important to note is the fact that the sharp inflexion in the curve obtained with a given concentration of L-valine was closely related to the amount of growth that same concentration of L-valine had permitted with strain 20A19 as the test organism. For example, in the presence of 10 μg of L-valine per ml, growth was proportional to the amount of L-isoleucine added up to 2 μg per ml. The actual final concentration of L-valine at the end of the incubation period would probably have been about 3 μg per ml—7 μg having been utilised during growth as judged by the utilization of L-valine by strain 20A19. The next flask in the series, containing 3 μg of L-isoleucine per ml, had sufficient growth to permit the utilization of the entire amount of L-valine after which the culture was no longer valine inhibited and could grow independently of L-isoleucine. Those flasks which had initially 40 μg of L-valine per ml did not achieve in the 24 hour incubation period a level of growth sufficient to have removed all the L-valine so that the sharp rise in the growth response curve was not observed. In other words, as long as there is valine in the medium, growth is dependent on L-isoleucine and is independent of the amount of L-valine present. This implies that the reversal of the inhibition is non-competitive and that L-valine exerts its inhibitory action by interfering with one of the steps in isoleucine synthesis.

An explanation of the basis of the sensitivity of strain K-12 to L-valine must account for the fact that the only three compounds known to reverse the valine inhibition are isoleucine, α-keto-β-methylvaleric acid, and α,β-dihydroxy-β-methylvaleric acid. Other known precursors to isoleucine such as ketobutyric acid are ineffective. There may be an exception to this in that Rowley (1953) has reported that his stock of strain K-12, also sensitive to valine, could utilize leucine or threonine to reverse the inhibition. With the stock used in this work the only effect of leucine on valine inhibition of growth in liquid media is to hinder to a very slight extent the reversing capacity of isoleucine.

Quite pertinent to an explanation of the mechanism of action of valine is the observation that valine in the growth medium blocks the synthesis of its own precursors and, therefore of itself (Adelberg and Umbarger, 1953). At the present time it is not possible to attribute the effect of valine to any known physicochemical interaction between the valine supplied in the medium and an enzyme system. However, results with mutants have narrowed the possible sites of the valine effect to one or two postulated steps.

Steps 1 and 2 in figure 1 are known not to be the enzymes inhibited by valine since the effect of valine on the synthesis of its own precursors can be demonstrated even in mutants in which these steps are missing. It seems reasonably certain that one of the requirements for a step to be inhibited is that its substrate should have a 5-carbon branched chain similar to that of valine. This eliminates the coupling reaction converting pyruvate to the initial 5-carbon compound (Adelberg, 1954). The identity of "C₁₅" has not been revealed. "C₁₅" in figure 1 represents the compound or compounds which are intermediate in the postulated pinacol-type rearrangement that essentially results in splitting the pyruvate moiety and the insertion of a 2-carbon fragment. It is probable that the formation of the dihydroxy precursor of valine from the initial product of condensing pyruvate with the 2-carbon fragment involves more than one enzyme (represented by step 3 in figure 1), but it does seem at present that one of these enzymes is the one at which L-valine inhibits its own synthesis. There is some evidence that the corresponding step in isoleucine synthesis is catalyzed by the same enzyme since one kind of mutant, E. coli strain 83 (Umbarger and Adelberg, 1951), requires both L-isoleucine and L-valine or, alternatively, the α-keto or α,β-
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Medium

- Glycylvaline
- Isoleucine
- Valine
- $\alpha$-Keto-$\beta$-Methyl-valerate
- $\alpha$-Keto-isovalerate

Cell

- Glycylvaline
- Valine
- Isoleucine
- $\alpha$-Keto-$\beta$-Methyl-valerate
- $\alpha$-Keto-isovalerate
- $\alpha$-Keto-butyrate

Proteins

Pyruvate

Dihydroxy C₅

$\alpha$-Keto-4-dehydroxy-
C₆
dihydroxy analogues of isoleucine and valine. This mutant is probably blocked at step 3 in figure 1. Thus if valine blocks its own synthesis at this step, it does not seem unreasonable that it would also block isoleucine synthesis at the same step.

A second type of mutant which can utilize the 6-carbon precursor of isoleucine but not the 4-carbon precursors, for example Bacillus subtilis strain 185X, has been reported (Umbarger and Adelberg, 1951). This mutant does not have a valine requirement and is probably blocked at step 3 in figure 1. The valineless mutant referred to above, E. coli strain M-48-62, is probably blocked at the corresponding reaction in valine synthesis, step 3b. Thus, it appears that L-valine and L-isoleucine are synthesized via different pathways until the formation of "C₄" and "C₆", thereafter single enzymes perform each of the final steps for both pathways. An exception occurs at the transamination step since there are two transaminases for L-valine and only one of these functions in L-isoleucine synthesis (Rudman and Meister, 1953).

This hypothesis for the inhibition of strain K-12 by L-valine has one obvious drawback in that it fails to account for the fact that not all organisms are inhibited by L-valine. It could well be that in most bacteria L-valine can be displaced from the enzyme catalyzing step 3.

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TABLE 2

Antagonisms between L-valine and L-isoleucine on Escherichia coli

<table>
<thead>
<tr>
<th>Strain of E. coli</th>
<th>Growth Requirement</th>
<th>Effect on L-valine</th>
<th>Effect on L-isoleucine</th>
<th>Antagonism</th>
</tr>
</thead>
<tbody>
<tr>
<td>K-12</td>
<td>None</td>
<td>Inhibits</td>
<td>Overcomes inhibition by L-valine</td>
<td>Noncompetitive</td>
</tr>
<tr>
<td>20A19</td>
<td>L-Valine and L-isoleucine</td>
<td>Overcomes inhibitory effect of high concentrations of L-isoleucine</td>
<td>In high concentrations inhibits</td>
<td>Competitive</td>
</tr>
<tr>
<td></td>
<td>$\alpha$-Ketoisovalerate and L-isoleucine Glycyl-DL-valine and L-isoleucine</td>
<td>—</td>
<td>No inhibition</td>
<td>—</td>
</tr>
<tr>
<td>JHM544</td>
<td>L-Isoleucine</td>
<td>No effect</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>DL-$\alpha$-Aminobutyric</td>
<td>Inhibits</td>
<td>—</td>
<td>Not determined</td>
</tr>
<tr>
<td>M-48-62</td>
<td>L-Valine</td>
<td>—</td>
<td>No inhibition</td>
<td>—</td>
</tr>
</tbody>
</table>

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Figure 9. Schematic summary of the interactions between isoleucine and valine.
by "C₄" but not by "C₃". Regardless of how wrong the proposed hypothesis might be, it would appear that the identity of both "C₄" and "C₃" would be of considerable importance in helping to understand the interactions between L-valine and L-isoleucine that are encountered so often in microorganisms.

Figure 9 and table 2 summarize the experiments and interpretations presented above. In mutants blocked after the condensing reaction in L-isoleucine synthesis, there is a simultaneous requirement for L-valine. The doubly deficient mutants require that L-isoleucine be supplied in not too great an excess relative to the amount of L-valine since L-isoleucine in the medium can prevent the uptake of L-valine. This inhibition can be by-passed by supplying either a peptide of L-valine or α-ketoisovaleric acid—the passage of either of which into the cell is not inhibited by L-isoleucine. The keto acid corresponding to L-isoleucine does, however, specifically depress the utilization of the keto analogue of L-valine. It is probable that this, too, is an inhibition occurring at the level of cell permeation.

Finally, in strain K-12 L-valine probably inhibits not the incorporation of L-isoleucine into protein but, rather, the formation of L-isoleucine. The site of L-valine inhibition probably occurs at an enzyme responsible not only for its own synthesis but also for that of L-isoleucine. What appeared to be a competitive release of L-valine inhibition by L-isoleucine is probably a non-competitive release complicated by the fact that in this case the inhibitor is gradually removed by cellular utilization. The inhibition of E. coli strain K-12 by L-valine seems at present to be an example of an amino acid preventing the synthesis of a structurally similar compound, L-isoleucine, an interaction anticipated 16 years ago by Gladstone.

SUMMARY

The growth behavior of an isoleucineless-valineless mutant of Escherichia coli has been examined. It was observed that when the concentration of L-isoleucine was high and the concentration of L-valine was relatively low, there was a depression of growth. Experimental evidence was presented which indicated that L-isoleucine in excess prevented the uptake of L-valine by the cells.

The inhibition of growth of E. coli strain K-12, by L-valine, previously considered to be competitively antagonized by L-isoleucine, has been reexamined. It was found that L-isoleucine was actually a noncompetitive antagonist of L-valine and hence a direct or indirect product of the inhibited reaction. While the inhibited reaction has not yet been revealed, considerable information regarding its identity could be deduced from observations on the wild strain and several isoleucineless mutants.

REFERENCES


