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Formation of Valyl- and Isoleucyl-sRNA Synthetases in a Valine-Requiring Mutant of *Escherichia coli*

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The effect of valine, isoleucine, and leucine on the growth of *Escherichia coli* M4862, a mutant requiring valine, was studied. The increase of the cell mass during the exponential growth was roughly proportional to the concentration of valine in the glucose-salt medium in the range 0–30 mg/l. Isoleucine or leucine alone or both together did not promote cell growth, but leucine added together with valine decreased the amount of valine required to effect the same rate of growth. Isoleucine inhibited growth in the presence of valine, but this effect was counteracted by leucine.

When *E. coli* M4862 was cultivated in the presence of suboptimal concentrations of valine (10 mg/l), the specific activity of isoleucyl-sRNA synthetase rose specifically about 20 % above the control level immediately before growth slowed down owing to depletion of valine. No increase occurred in the specific activities of valyl- and tyrosyl-sRNA synthetases.

Nass and Neidhardt [1] showed that the formation of phenylalanyl- or isoleucyl-sRNA synthetase is specifically derepressed in *E. coli* bradytrophs requiring an amino acid when phenylalanine or isoleucine, respectively, is the growth-limiting factor. The derepression was, however, very slow and did not occur at all if the amount of leucine or histidine was so small that it limited growth. We have studied the effect of branched chain amino acids on the growth of *E. coli* M4862, a mutant requiring valine. Some of the results are presented in this paper. We also present some preliminary results concerning the formation of valyl- and isoleucyl-sRNA synthetases [L-valine:sRNA ligase (AMP), E.C. 6.1.1.9 and L-isoleucine:sRNA ligase (AMP), E.C. 6.1.1.5] in the same mutant in a valine-deficient medium.

EXPERIMENTAL

Escherichia coli M4862, a mutant requiring valine, was used as test organism. It had been isolated by Dr. H. E. Umbarger and kindly donated to us by Dr. M. J. Pine (Roswell Park Memorial Institute, Buffalo, N.Y.). It was stored and cultivated in the same way

as *E. coli* U5/41 in our recent work,[2] except that various amounts of branched chain amino acids were added to the minimal medium. The handling of the samples and the assay of aminoacyl-sRNA synthetase activities are described in Ref. 2.

RESULTS and DISCUSSION

First we determined some growth requirements of *E. coli* M4862. Umbarger *et al.*[3] have shown that *E. coli* M4862 has an altered isomero-reductase, which cannot convert α-acetolactate into α,β-dioxo-isovalerate. This mutant is thus able to grow only in the presence of valine. This is confirmed by our results, which are presented in Fig. 1a. This figure shows also that the increase of the cell mass during the exponential growth phase is roughly proportional to the amount of valine added. Thus the retardation of the growth rate after the normal exponential growth, *i.e.* after a period of 3—3.5 h, is evidently due to depletion of valine. Also Umbarger and Brown [4] studied the effect of added valine on the growth of *E. coli* M4862. Our results agree with theirs, but because they measured only the final turbidity of the medium after 24 h growth, they did not observe when the growth rate decreased.

Figs. 1b and 1c show that leucine or isoleucine alone or both together cannot replace valine, and isoleucine seems to inhibit growth in the presence

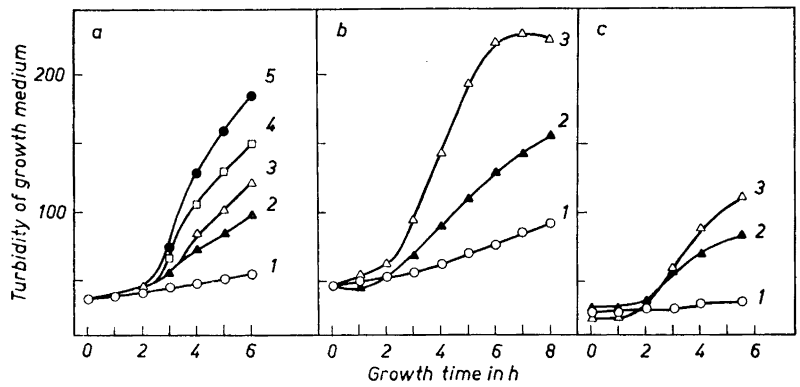

Fig. 1. Growth of *Escherichia coli* M4862 in a minimal medium supplemented with branched chain amino acids. The cells precultivated in GSHT medium were washed with cold sodium chloride solution and suspended in a small volume of the minimal medium, and a known volume of the suspension was pipetted to each of a series of vessels. Every vessel contained 0.5 l of minimal medium supplemented with branched chain amino acids as presented below. Cultivation took place in a thermostated water bath at 37° with mechanical stirring. Growth was followed by measuring the turbidity of the culture in a Klett colorimeter equipped with filter 62.

a. The effect of valine. Curve 1: 0 mg/l of valine.Curve 2: 10 mg/l of valine. Curve 3: 15 mg/l of valine. Curve 4: 20 mg/l of valine. Curve 5: 30 mg/l of valine.

b. The effect of leucine and isoleucine. Curve 1: 50 mg/l of leucine+50 mg/l of isoleucine. Curve 2: 50 mg/l of isoleucine+30 mg/l of valine. Curve 3: 50 mg/l of leucine+10 mg/l of valine.

c. The effect of leucine. Curve 1: 50 mg/l of leucine. Curve 2: 10 mg/l of valine. Curve 3: 10 mg/l of valine+10 mg/l of leucine.

of valine. Leucine added along with valine, however, enhances growth and can partly replace valine (curves 3 in Figs. 1b and 1c). This is evidently due to the fact that the keto analog of valine, α-ketoisovaleric acid, is a precursor of leucine,[5] and thus added leucine spares valine. Leucine cannot replace valine completely (Fig. 1c, curve 1), because leucine is not converted to valine in *E. coli*.[6]

The growth-inhibiting effect of isoleucine is evidently due to partial prevention of leucine biosynthesis, because isoleucine had no inhibitory effect, when all three branched chain amino acids were added to the medium.

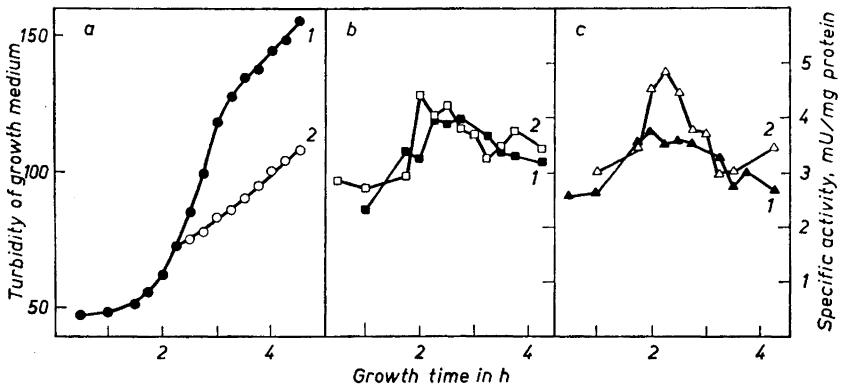

Fig. 2. The effect of valine depletion on the activities of valyl- and isoleucyl-sRNA synthetases in *E. coli* M4862. Cells precultivated in a GSHT medium were washed twice with cold 0.14 M sodium chloride solution and suspended in a small volume of minimal medium, and equal volumes of the suspension were pipetted into two cultivation vessels, which contained 0.5 l of minimal medium supplemented with 5 or 15 mg of valine. The cultivation vessels were immersed in a thermostated water bath (37°) and the cultures were stirred mechanically. Samples were withdrawn at regular intervals from both cultures, cooled rapidly and assayed as described earlier.[2] Growth was recorded by measuring turbidities in a Klett colorimeter equipped with filter 62.

a. Growth curves. Curve 1: 30 mg/l of valine. Curve 2: 10 mg/l of valine.

b. The specific activity ($\times 10^{-1}$) of valyl-sRNA synthetase. Curve 1: 30 mg/l of valine. Curve 2: 10 mg/l of valine.

c. The specific activity ($\times 10^{-1}$) of isoleucyl-sRNA synthetase. Curve 1: 30 mg/l of valine. Curve 2: 10 mg/l of valine.

In Figs. 2a, 2b, and 2c are plotted results of experiments where the variation of the activities of valyl- and isoleucyl-sRNA synthetases in two growth media containing 10 mg/l and 30 mg/l of valine was studied. These figures show that a valine deficiency had practically no effect on the specific activity of valyl-sRNA synthetase, but the specific activity of isoleucyl-sRNA synthetase increased above the level in the control culture immediately before the rate of growth decreased owing to depletion of valine. This increase was only transient, for the activity of this enzyme fell to the level in the control culture after the retardation of growth.

As a check we estimated also the activity of tyrosyl-sRNA synthetase in the two media. The activity curves of this enzyme were very similar in the two media.

We are not yet able to explain these unexpected results. It seems unlikely that they are due to any accidental technical error, because four independent experiments gave essentially the same result. Thus the increase in the activity of isoleucyl-sRNA synthetase must depend on some kind of activation of the enzyme or a specific increase in the rate of its synthesis.

It has been shown that valine competitively inhibits the isoleucyl-sRNA synthetase of *E. coli*.[7] Thus it may be possible that the increase in the activity of isoleucyl-sRNA synthetase is due to diminishing inhibition as the valine is exhausted. The inhibition in the presence of isoleucine requires, however, so high a concentration of valine that this should lead to a rapid endogenous reaction. Because the rate of ATP-PP exchange without added amino acid was only 5—10 % of the reaction rate in the presence of valine, the concentration of valine in the reaction mixture was too low to inhibit isoleucyl-sRNA synthetase.

Some investigators have presented results, suggesting that free sRNA may activate aminoacyl-sRNA synthetases,[8–11] but contradictory results have been obtained, too.[10,12–14] The rise in the activity of isoleucyl-sRNA synthetase may hence be due to a decreased degree of charging of sRNA specific for isoleucine. It can be easily calculated, however, that even though the charging of sRNA may be much slower than the ATP-PP exchange,[15] the activity of isoleucyl-sRNA synthetase in the reaction mixture is so high that all isoleucine-specific sRNA will be charged almost immediately after the reaction is initiated. Thus the effect of possible changes in the degree of charging of sRNA *in vivo* cannot be seen in our determinations.

Nass and Neidhardt,[1] using mutants of *E. coli*, found that the activity of phenylalanyl- or isoleucyl-sRNA synthetase increased specifically when phenylalanine or isoleucine was the growth-limiting factor. In the experiments described in this paper the activity of valyl-sRNA synthetase did not rise specifically, even though the amount of valine evidently was growth-limiting. Nass and Neidhardt [1] did not either find any specific change in the activities of leucyl- or histidyl-sRNA ligases when the amount of the respective amino acid was growth-limiting. It seems that amino acid repression occurs only in the case of some aminoacyl-sRNA synthetases or, more probably, that the limiting concentration for derepression is so low in the case of other aminoacyl-sRNA synthetases that protein synthesis is arrested before derepression occurs.

We do not yet know, if also all the isoleucine was consumed by the cells in our experiments, but this seems unlikely, because isoleucine inhibited growth when added together with valine (Fig. 1b). Determinations of the concentrations of free amino acids in the cells may be expected to clarify the problem.

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