

The Effects of Pyruvate and Related Compounds on the Induced Formation of Tryptophanase in *Escherichia coli*

RAIMO RAUNIO

Department of Biochemistry, University of Turku, Turku, Finland

The formation of tryptophanase in *Escherichia coli* and the influence of various compounds, mainly sugars and keto acids, on this formation have been studied.

It was found that tryptophanase is formed in increasing amounts during the acceleration and exponential phases of cell growth and at the same time indole accumulates in the growth medium containing 1.0 % Bacto-tryptone as nutrient source.

Indole production is weak when glucose, galactose, mannose, fructose, ribose, arabinose, xylose, acetate, cysteine, fumarate or malate is present at a level of 0.5 % in the growth medium. Glycine, butanol, 2-oxoisovalerate, and 2-oxoisocaproate promote the production of indole. Glucose, pyruvate, and L-serine were found to repress the formation of tryptophanase.

Butanol, 2-oxoisovalerate, propionate, butyrate, 2-oxoisocaproate, and L-valine accelerate the formation of tryptophanase during bacterial growth.

L-Tryptophan and 2-oxoisocaproate effect a reversal of the repression of tryptophanase production by pyruvate.

Recent work has revealed that the so-called glucose effect, the repression of the synthesis of catabolic enzymes by degradative products of glucose, is specific.¹ For example, the formation of tryptophanase is more effectively repressed by pyruvate than by glucose, but the opposite is true in the case of β -galactosidase. The formation of catabolic enzymes is repressed also by the metabolites of mandelate.²

The prevailing views on the regulation of enzyme formation are largely based on the work of Jacob and Monod.^{3,4} According to these views a genetic repressor interacts either with a metabolic repressor or with a metabolic inducer; the genetic repressor is a macromolecule, in all probability a protein.

The aim of this study was to determine whether compounds structurally closely related to pyruvic acid, primarily keto acids, also influence tryptophanase production by *Escherichia coli*. Also the influence of other compounds of equal carbon chain length on this enzyme production was studied. It has previously

been shown that phenylalanine and tyrosine lower the concentration of tryptophan in cells and probably also reduce the inductive effect of tryptophan on the synthesis of tryptophanase.⁶

EXPERIMENTAL

The organism and its cultivation. The test organism was a wild type strain of *Escherichia coli* that had been grown on a medium containing Bacto-tryptone, glucose, sodium citrate and yeast extract. The composition of the medium and the culture method have been described previously.⁷ In the studies of the factors influencing the formation of tryptophanase *E. coli* bacteria were grown in a medium of pH 7.0 containing 1.0 % Difco Bacto-tryptone in distilled water. The formation of tryptophanase was rapid in this medium. Other compounds were added to the unsterilized medium at the beginning of bacterial growth to give a concentration of 0.5 %. *E. coli* was cultured in 10-ml volumes of the medium under aerobic conditions in 20 × 180 mm test tubes. The tubes were shaken in a Kunkel shaker in a 37° incubator during the growth period. 5 ml samples were taken at intervals and centrifuged in a Servall superspeed RC-2 centrifuge at 7000 *g* at 0–5° for 10 min.

Estimation of growth. The rate of growth was estimated by measuring the turbidities of the samples with a Klett-Summerson colorimeter employing filter 62 (590–660 *mμ*). The dry weights were computed from the turbidity values assuming, as found previously, that one Klett unit represents 2 *μg* of dry matter in 1 ml of bacterial suspension.

Estimation of tryptophanase activity. The centrifuged cells were washed twice with 0.15 M sodium chloride solution, cooled to –40°C, thawed at 37°C and cooled again to –40°C. The cells were suspended in 0.5 ml of distilled water, and 0.5 ml of a 0.1 M phosphate buffer of pH 7.8 containing 5 *μ*moles of L-tryptophan, and 10 *μ*moles of pyridoxal-5-phosphate was pipetted onto the suspension. The reaction was stopped by adding 5.0 ml of Ehrlich reagent to each tube after periods ranging from 5 to 15 min, *i. e.* in the early stage of the reaction. Each tube was then centrifuged at 7000 *g* for 10 min and the absorption of the supernatant was measured with the Klett-Summerson colorimeter employing filter 54 (520–580 *mμ*).

Estimation of indole. 2 ml of the cell-free medium was transferred to a test tube and 5.0 ml of the Ehrlich reagent was added, after which the absorption was measured using filter 54.

Unit of enzyme activity. The tryptophanase activity was determined as recommended by the Enzyme Commission by evaluating the number of *μ*moles of indole liberated per minute at 25°C and calculating the concentration of tryptophanase per ml of cell suspension.

Chemicals. The employed chemicals were commercial products from the following manufacturers: sodium pyruvate from C. F. Boehringer, GmbH, Mannheim, Germany, *α*-ketobutyric acid, *α*-ketoisovaleric acid, *α*-ketoisocaproic acid, L-serine, and L-valine, all purissimum grade from Fluka AG, Buchs, Switzerland, isovaleric acid, propionic acid, glucose, and butyric acid from E. Merck AG., Darmstadt, Germany and butanol from May and Baker Ltd., Dagenham, England.

RESULTS

In studies of enzyme formation in which batch cultures are employed, it is appropriate to estimate the enzyme activity during the different growth phases. The variations of the levels of tryptophanase and indole during the growth of *E. coli* in the 1.0 % Bacto-tryptone medium are shown in Fig. 1. It will be seen that the enzyme is not formed during the lag phase, but its formation begins when active cell division sets in. The tryptophanase level increases from nil to a maximum value of 10.0 mU/ml in the retardation phase and then begins to decrease when cell growth ceases (curve 1). Indole,

Table 1. The influence of various compounds on the production of indole by *Escherichia coli*.

Each compound was added to 10 ml of 1.0 % Bacto-tryptone growth medium to give a concentration of 0.5 % at the beginning of growth. The tubes were inoculated with drop inoculum and incubated without shaking at 37°C for about 19 h. The indole produced was assayed as described in the text and expressed as μ moles per milligram of dry weight of bacteria. The data in this table are percentages of the value for the growth medium which contained no added compound (100).

| Compound added | Relative indole production |
|----------------------|----------------------------|
| None | 100 |
| D-Glucose | 2 |
| D-Galactose | 12 |
| Na-pyruvate | 0 |
| D-Mannose | 21 |
| D-Fructose | 29 |
| D-Xylose | 94 |
| L-Arabinose | 88 |
| D-Ribose | 59 |
| Sucrose | 91 |
| Lactose | 6 |
| L-Alanine | 114 |
| L-Serine | 4 |
| L-Cysteine | 0 |
| Na-acetate | 52 |
| Na-fumarate | 77 |
| Na-malate | 86 |
| Butanol | 261 |
| 2-Oxoisocaproic acid | 191 |
| Glycine | 170 |

the end-product of tryptophanase action, is excreted into the growth medium and its final concentration when the growth ends is 68 μ g/ml. The curve plotting the indole content variation (curve 2) is similar in form to the curve plotting tryptophanase activity although it rises somewhat less rapidly than the latter curve in the early stages of bacterial growth.

Curves 2 and 3 in Fig. 1 show that the formation of tryptophanase can be studied by following the production of indole. The preliminary data in Table 1 show the influence of sugars, metabolites of the Krebs' cycle, amino acids, and compounds structurally related to pyruvic acid on the production of indole in *E. coli* cultures. The data reveal that sugars which are metabolised to compounds on the glucose-pyruvate axis inhibit the indole production. Similarly, amino acids (L-serine and L-cysteine) whose degradation end-product is pyruvic acid inhibited indole production, whereas L-alanine had no effect and glycine promoted indole production. The Krebs' cycle intermediates malate and fumarate slightly suppressed the indole formation. Particularly noteworthy are the results obtained with butanol, 2-oxoisocaproate, and glycine which all promoted indole production. The influence of keto acids and compounds containing three to five carbon atoms on the formation of tryptophanase was studied in greater detail. The results of the experiments with glucose, propionic acid, butyric acid, and butanol are plotted in Fig. 2. It will be seen that whereas glucose clearly repressed the formation of tryptophanase, propionic

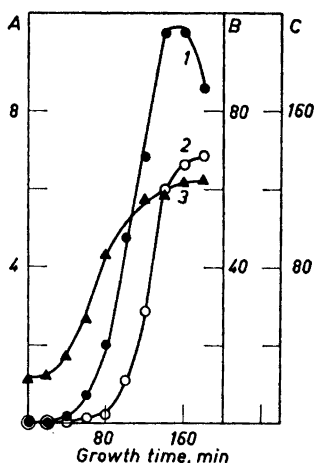


Fig. 1. Variation of tryptophanase and indole levels in batch cultures of *Escherichia coli*. 1, tryptophanase concentration, mU/ml of growth medium (scale A); 2, indole concentration, $\mu\text{g/ml}$ of growth medium (scale B) and 3, growth curve as Klett-readings (scale C).

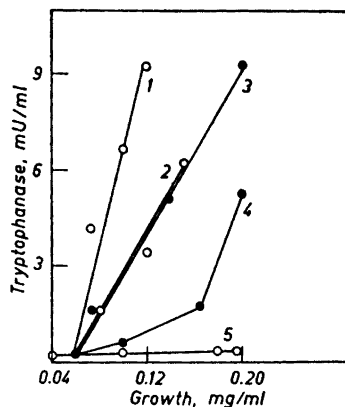


Fig. 2. Effect of butanol (1), propionate (2), butyrate (3), and D-glucose (5) on the induced formation of tryptophanase in *Escherichia coli* cultures containing 1.0% Bacto-tryptone. Each compound was added before inoculation and samples were taken during the acceleration and exponential phases. The cell concentration when growth began was 0.4 mg dry weight/ml. Curve 4 refers to the culture containing no added compound.

acid, butyric acid, and butanol "induced" the formation. Because the samples were taken for analysis after identical periods of time, it may be concluded that the last three compounds repressed the growth at the level of 0.5%.

The influence of L-serine, sodium pyruvate, L-valine, sodium 2-oxoisocaproate, and sodium 2-oxoisovalerate on the formation of the enzyme is shown in Fig. 3. L-Serine and sodium pyruvate almost completely repressed tryptophanase production. Particularly marked was the increase in enzyme activity induced by sodium 2-oxoisovalerate and sodium 2-oxoisocaproate.

Figs. 2 and 3 show that sodium pyruvate represses the production of the enzyme, whereas various C_3 – C_5 compounds promote the production. The results of experiments in which an additional inducer, L-tryptophan or 2-oxoisocaproate, was added to the Bacto-tryptone medium to determine whether they are able to counteract the repression of sodium pyruvate are presented in Fig. 4 and show that both compounds at the level of 0.1% were able to counteract the repression of sodium pyruvate whose initial concentration was varied from 0.05 to 0.4%. It is further seen that sodium 2-oxoisocaproate was more effective in this respect than L-tryptophan. Both these compounds promote the production of tryptophanase in media that contained no sodium pyruvate. The culture samples were taken in the middle of the exponential phase.

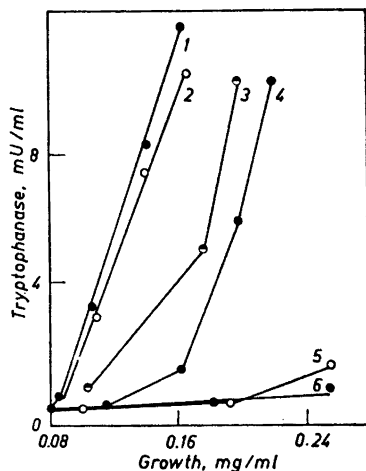


Fig. 3. Effect of 2-oxoisovalerate (1), 2-oxoisocaproate (2), L-valine (3), L-serine (5), and pyruvate (6) on the induced formation of tryptophanase. Growth conditions as in Fig. 2. (4), no added compounds.

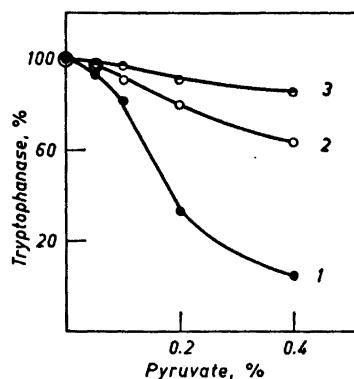


Fig. 4. Reversion of pyruvate repression of tryptophanase formation by tryptophan and 2-oxoisovalerate. 1% L-tryptophan (curve 2) and 1% 2-oxoisovalerate (curve 3) were added to separate cultures containing 0–0.4% sodium pyruvate at the beginning of cell growth and the samples were withdrawn in the middle of the exponential phase for the determination of tryptophanase activity. Curve 1 refers to cultures containing sodium pyruvate, but no other added compound.

DISCUSSION

Curve 1 in Fig. 1 shows that the enzyme is not formed in the lag phase in amounts that would permit its analysis by the method employed. The reason may have been the high pyruvate concentration in the early phases of growth, for this compound is formed at a high rate during the lag and acceleration phases and at a very high rate in the late exponential phase in cultures of *E. coli* in a medium containing glucose as carbon source.⁸ Fig. 1 shows further that the production of indole, which parallels the variation of the tryptophanase activity, can be employed, as was done in the experiments in Table 1, as a measure of the induction of tryptophanase. Evans *et al.*⁹ have found arabinose, lactose, glucose, fructose, mannitol, and gluconate to inhibit the production of indole in *E. coli* cultures. Earlier results as well as those presented in Table 1 show that compounds, as the mentioned sugars, L-serine (Table 1 and Fig. 3) and L-cysteine, which are converted to pyruvate in bacterial cultures, repress tryptophanase production. Also L-tryptophan is decomposed to indole and pyruvate by tryptophanase, but according to curve 2 in Fig. 1 the maximal level of indole, 68 $\mu\text{g}/\text{ml}$, in the cell suspension corresponds to a level of 52 μg of pyruvate per millilitre or about 0.005%. This concentration is too low to

repress tryptophanase production (curve 1 in Fig. 4). It should be noted further that L-alanine did not repress and glycine, on the contrary, promoted indole production. With the aid of isotopes, it has previously been shown that glycine is not converted to L-serine during active cell growth in *E. coli* cultures containing glucose. This would explain the effect of glycine. The results of the experiments with serine and alanine are in accord with previously reported results.¹²

It is probable that all *E. coli* cultures contain varying amounts of pyruvic acid which is the dominating keto acid in such cultures.⁸ It was for this reason that a 1.0 % Bacto-tryptone solution in which tryptophanase production is rapid was chosen as the medium in the experiments described in this paper. Keto acid chromatograms (not presented in this paper) revealed that ketoleucines, ketovaline, and pyruvate are present in the bacterial culture during the exponential phase. The data plotted in Figs. 2 and 3 are in agreement with results in that glucose and sodium pyruvate at the level of 0.5 % repress the formation of tryptophanase but 2-oxoisovalerate, butanol, L-valine, butyrate, and propionate "induce" the formation of tryptophanase. The keto acid chromatograms revealed further that butanol and pyruvate do not lead to the accumulation of keto acids in the growth medium, and hence the formation of keto acids is not necessary for the induction of tryptophanase. It has, however, been found that addition of L-valine and L-serine leads to the accumulation of the corresponding keto acids in the media.⁸ This would explain the induction of tryptophanase by L-valine (2-oxoisovalerate) and repression by L-serine (pyruvate). With regard to the induction of enzyme formation by the compounds mentioned in Figs. 2 and 3, the hypothesis may be proposed that these compounds prevent complex formation by the metabolic repressor, pyruvate, and the genetic repressor. The results presented in Figs. 2 and 3 show that the competitive repressor-inhibitor that competes with the pyruvate repressor does not have to be a definite compound because C₃–C₅ compounds are able to induce the formation of tryptophanase. It has been found previously that phenylalanine and tyrosine inhibit the induction of tryptophanase by tryptophan, probably because they compete with the latter for the inducer.^{5,6,10} A similar repression of anabolic enzyme production has been observed with compounds that are similar to the repressor in molecular structure.¹⁵

The curves 2 and 3 in Fig. 4 show that both the inducer and sodium 2-oxoisovalerate (competitive repressor-inhibitor) at the level of 1.0 % counteract the repression by pyruvate. This reversion was, in addition, more complete when the keto acid than when tryptophan was present. It has previously been shown that an inducer is unable to reverse the repression of β -galactosidase production, but tryptophan has been found to induce tryptophanase production when glucose is present.^{6,14} The results in Fig. 4 suggest that tryptophan and pyruvate, the true inducer and repressor, compete for the genetic repressor.

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