Thymine and Uracil Catabolism in *Escherichia coli*

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SUMMARY

Growing *Escherichia coli* wild-type and pyrimidine-deficient strains break down exogenously added pyrimidine bases, thymine and uracil, whose 2-carbon atom appears as CO₂. By using mineral salts glucose media with different sources of nitrogen the degradation processes are shown to be inducible. We conclude that thymine and uracil catabolism are regulated by the amount of metabolically available nitrogen in the cell.

INTRODUCTION

Our knowledge of the degradation of thymine and uracil in bacteria stems from the studies on *Bacterium* sp. (Wang & Lampen, 1952), *Mycobacterium* sp., *Corynebacterium* sp. (Hayashi & Kornberg, 1952), *Nocardia corallina* (Lara, 1952) and *Clostridium uracilicum* (Campbell, 1957). All these organisms were isolated by enrichment culture for ability to use thymine and/or uracil as a sole carbon and nitrogen source.

Although *Escherichia coli* has been intensively studied, and the biosynthesis of pyrimidines and its control is well understood (O'Donovan & Neuhaud, 1970), the data concerning the catabolism of thymine and uracil and its regulation are still lacking.

We report here evidence for the breakdown of exogenous thymine and uracil in *Escherichia coli* and describe some factors governing its induction. *Escherichia coli* strains used were not able to grow when thymine and/or uracil were used as the sole carbon and nitrogen source, but were able to catabolize them in nitrogen-restricted glucose media.

METHODS

Bacterial strains and growth conditions. *Escherichia coli* K-12S requiring thymine and uracil obtained from D. Novak (Department of Biology, Institute 'Rudjer Bošković', Zagreb, Yugoslavia), its parent strain *E. coli* K-12S, obtained from Dr R. Latarjet (Institut du Radium, Paris), *E. coli* B and a uracil-requiring mutant *E. coli* BU⁻ (S. S. Cohen, Philadelphia, Pennsylvania, U.S.A.) were used.

The basal growth medium (Davis & Mingioli, 1950) contained (g/l of distilled water): (NH₄)₂SO₄, 1.0; K₂HPO₄, 10.5; KH₂PO₄, 4.5; trisodium citrate.H₂O, 0.5; MgSO₄.7H₂O, 0.1; glucose, 4.3; pH 7.0. This medium is designated as buffer M when made without glucose and (NH₄)₂SO₄, and as medium M when lacking only (NH₄)₂SO₄.

Auxotrophic bacteria were grown overnight in basal growth medium supplemented with 7.9×10^{-5} M-thymine and/or 2.7×10^{-4} M-uracil (Schwartz BioResearch, Orangeburg, New York, U.S.A.). Cultures were diluted to 1×10^8 bacteria/ml with the same medium which was now supplemented with 6.3×10^{-5} M-thymine and/or 1.8×10^{-4} M-uracil for deficient strains, and regrown to 5×10^8 bacteria/ml. Bacteria were collected on membrane

Fig. 1. Growth of *Escherichia coli* K-12ST-U⁻ in basal growth media supplemented with: ○, nothing; ●, 6.3×10^{-5} M-thymine and 1.8×10^{-4} M-uracil; ×, 6.3×10^{-5} M-thymine, 1.8×10^{-4} M-uracil and 5.0×10^{-3} M-glutamic acid. Growth in medium M containing 6.3×10^{-5} M-thymine and 1.8×10^{-4} M-uracil and additionally supplemented with: □, nothing; ■, 5.0×10^{-3} M or 5.0×10^{-2} M-glutamic acid; ▲, 5.0×10^{-3} M-glutamic acid and 6.3×10^{-4} M-thymine; △, 5.0×10^{-3} M-glutamic acid and 8.9×10^{-4} M-uracil.

filters, washed with buffer M, and inoculated into various modified basal growth media, in which the rate of growth and the fate of exogenously supplied thymine and uracil were followed during 5 h of incubation.

Bacteria were grown at 37 °C with aeration (390 ml of air/100 ml of medium/min).

Growth rate was followed by reading the extinction at 650 nm and by conventional viable count determinations using, for thymine-deficient strains, 50 µg thymine/plate.

Pyrimidine assimilation was followed by determining the incorporation of ^{14}C -labelled thymine and uracil into bacterial constituents insoluble in cold 5% trichloroacetic acid (TCA). $[2\text{-}^{14}\text{C}]\text{Thymine}$ or $[2\text{-}^{14}\text{C}]\text{uracil}$ (Schwartz BioResearch, Orangeburg, New York, U.S.A.) were added at 0.05 µCi/ml of bacterial culture, the final specific activity achieved being 797 µCi/mmol thymine and 292 µCi/mmol uracil. Bacteria from 1 ml of culture, taken at hourly intervals, were collected on membrane filters, washed with (2×1 ml) saline and treated with 1 ml of 5% TCA at 4 °C for 30 min. Soluble material was removed by suction, the sediment washed with 1 ml 5% TCA, transferred to planchet, dried and counted on a D-47 gas flow counter (Nuclear-Chicago Corporation, Des Plaines, Illinois, U.S.A.).

Pyrimidine degradation was followed by determining $^{14}\text{CO}_2$ evolved from $[2\text{-}^{14}\text{C}]\text{thymine}$

Fig. 2. Distribution of ^{14}C from $[2\text{-}^{14}\text{C}]\text{thymine}$ during growth of *Escherichia coli* K-12ST-U $^{-}$ in medium M supplemented with 6.3×10^{-5} M-thymine and 1.8×10^{-4} M-uracil with either 5.0×10^{-3} M-glutamic acid (a), or 7.6×10^{-3} M- $(\text{NH}_4)_2\text{SO}_4$ and 5.0×10^{-3} M-glutamic acid (b). ●, Growth medium without bacterial cells; Δ, cell constituents soluble in cold 5% TCA; ○, cell constituents insoluble in cold 5% TCA; ■, $^{14}\text{CO}_2$ evolved; ×, total ^{14}C .

or $[2\text{-}^{14}\text{C}]\text{uracil}$. A stream of CO_2 -free air (16 ml air/3 ml medium/min) was blown through the growing bacterial culture and then through 10 ml of 0.1 M-NaOH and 10 ml of 0.3 M- $\text{Ba}(\text{OH})_2$. All CO_2 was absorbed in the NaOH solution, from which 0.1 ml samples were pipetted on planchets, dried, and counted as an infinitely thin layer.

Within the accuracy of the method, the recovery of radioactivity in CO_2 was the same as the loss of ^{14}C from the culture determined by plating 0.1 ml samples of bacterial culture on planchets.

RESULTS

Growth of *Escherichia coli* wild-type and pyrimidine-deficient strains in different media showed that bacteria do not grow in media where thymine and uracil are the only carbon and nitrogen source, or the only carbon source.

The first indication of pyrimidine degradation in nitrogen-restricted media was with thymine-dependent *Escherichia coli* K-12ST-U $^{-}$ grown in medium M with glutamate as a source of nitrogen: in this medium bacteria after the initial growth underwent death reminiscent of thymineless death (Fig. 1) (Barner & Cohen, 1954), although thymine was present in a concentration which allows normal growth of bacteria in basal growth medium. However, higher concentrations of thymine in the medium completely prevented the decrease in viability, whereas higher concentrations of uracil merely postponed it.

To prove that thymine was degraded, the fate of $[2\text{-}^{14}\text{C}]\text{thymine}$ was followed during 5 h of growth (Fig. 2). In medium M with glutamate as principal nitrogen source, the breakdown

Fig. 3. Distribution of ^{14}C from $[2\text{-}^{14}\text{C}]\text{uracil}$ during growth of *Escherichia coli* K-12ST $^{-}\text{U}^{-}$ in medium M supplemented with 6.3×10^{-5} M-thymine and 1.8×10^{-4} M-uracil, with either 5.0×10^{-3} M-glutamic acid (a) or 7.6×10^{-3} M- $(\text{NH}_4)_2\text{SO}_4$ and 5.0×10^{-3} M-glutamic acid (b). ●, Growth medium without bacterial cells; △, cell constituents soluble in cold 5% TCA; ○, cell constituents insoluble in cold 5% TCA; ■, $^{14}\text{CO}_2$ evolved.

of exogenously supplied base, whose 2-carbon atom appears as CO_2 , took place concomitantly with the incorporation of thymine into bacterial mass (Fig. 2a). In a medium supplemented with glutamate and $(\text{NH}_4)_2\text{SO}_4$, thymine degradation did not occur, and the loss of radioactivity from the medium corresponded to its incorporation into macromolecules (Fig. 2b). The amount of $[^{14}\text{C}]\text{thymine}$ in the acid-soluble fraction of the bacterium was not significantly different in the two media.

Identical experiments with $[2\text{-}^{14}\text{C}]\text{uracil}$ (Fig. 3) showed that uracil was also catabolized by *Escherichia coli* K-12ST $^{-}\text{U}^{-}$ in a medium where glutamate was used as principal nitrogen source. At the same time there was no degradation when NH_4^+ was also added to the medium.

The rate of uracil breakdown was about 2.5 times greater than the rate of thymine breakdown but as the ratio of these two bases in the medium was 1:2.8, they are, in fact, degraded at approximately the same relative rate. The higher incorporation of uracil corresponded to a greater need of the bacterium for this pyrimidine base.

Using the evolution of $^{14}\text{CO}_2$ or the loss of radioactivity from the bacterial culture as a measure for thymine degradation, the catabolism of this base was further tested (i) in the absence of a nitrogen source, (ii) at two concentrations of $(\text{NH}_4)_2\text{SO}_4$, and (iii) in the presence of different amino acids as nitrogen donors (Fig. 4). Exogenously added thymine was not degraded with higher concentration of $(\text{NH}_4)_2\text{SO}_4$, and in media without glucose. It was, however, degraded in the absence of the nitrogen source, at low concentration of $(\text{NH}_4)_2\text{SO}_4$, and in media supplemented with different amino acids as a source of nitrogen.

To find out whether the catabolism of pyrimidines under the described conditions is a more general characteristic of *Escherichia coli* strains, the behaviour of *E. coli* K-12S, *E. coli* B

Fig. 4. Degradation of [2-¹⁴C]thymine by *Escherichia coli* K-12ST-U⁻. Degradation in: ○, buffer M: 6.3×10^{-5} M-thymine and 1.8×10^{-4} M-uracil alone (a); or with 7.6×10^{-3} M-(NH₄)₂SO₄ (b); 5.0×10^{-3} M-glutamic acid (c); 7.6×10^{-3} M-(NH₄)₂SO₄ and 5.0×10^{-3} M-glutamic acid (d). Degradation in medium M: ●, 6.3×10^{-5} M-thymine and 1.8×10^{-4} M-uracil alone (a); or with 1.9×10^{-3} M-(NH₄)₂SO₄ (b); 7.6×10^{-3} M-(NH₄)₂SO₄ (c); 5.0×10^{-3} M-glutamic acid (d); 5.0×10^{-3} M-aspartic acid (e); 5.0×10^{-3} M-alanine (f); 5.0×10^{-3} M-serine (g); 5.0×10^{-3} M-glycine (h); 5.0×10^{-3} M-methionine (i); 5.0×10^{-3} M-histidine (j).

and *E. coli* BU⁻ under identical conditions was followed. All strains examined initiated thymine and uracil degradation in media where amino acids or low concentrations of (NH₄)₂SO₄ were used as a nitrogen source. The only difference observed was the somewhat delayed start of the ¹⁴CO₂ evolution from the *E. coli* BU⁻.

The rapid onset of degradation of pyrimidine bases in medium M with glutamate probably indicates the induction of the appropriate degradative enzymes. This conclusion was supported by adding chloramphenicol, at 10 µg/ml, at zero time of incubation. This prevented degradation of pyrimidine, but when added 30 or 60 min later it only diminished the rate of breakdown. Also, (NH₄)₂SO₄, which does not allow the induction of pyrimidine degradation if present from the beginning of incubation, when added later, did not interfere with degradation processes which had been induced in its absence.

DISCUSSION

All the experiments reported in this paper are consistent with the conclusion that, in the examined *Escherichia coli* strains, the breakdown of exogenously provided pyrimidine bases, thymine and uracil, is an inducible process. The degradative enzymes are repressed when bacteria are grown in media optimally supplied with a carbon source (glucose) and a nitrogen source (NH₄⁺ salts or with other nitrogen donors).

The fact that degradation of pyrimidine bases can only be detected in mineral salts media with glucose, suboptimally supplied with a suitable nitrogen source (e.g. absence or low concentrations of ammonium salts) speaks in favour of a close relationship between pyrimidine degradation and nitrogen metabolism.

That the lack of the ¹⁴CO₂ evolution from [2-¹⁴C]thymine or uracil in the presence of NH₄⁺ is not a result of its inhibitory action on one of the degradation enzymes indicates that NH₄⁺

prevents thymineless death as well. The inability of NH_4^+ to stop pyrimidine catabolism when added to the medium after the degradation enzymes have been synthesized points to its role in the regulation of enzyme induction.

In media supplemented with different amino acids, thymine and uracil degradation was shown to depend on the amino acid used as a source of nitrogen. With glutamic and aspartic acids, the induction of pyrimidine degradation was fast and the evolution of CO_2 vigorous. With thymine-dependent strains the death of bacteria, which was reminiscent of thymineless death, occurred after the initial growth because of the rapid exhaustion of thymine from media. With all other amino acids tested, a lag in CO_2 evolution from thymine was observed, being shortest with glycine and longest with serine.

On the basis of these results we conclude that the induction of catabolic processes is somehow regulated by the amount of metabolically available nitrogen in the cell, which in turn may depend on the rate of uptake and metabolic transformations of various nitrogen donors present in growth media together with pyrimidine bases.

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REFERENCES

- BARNER, H. D. & COHEN, S. S. (1954). The induction of thymine synthesis by T2 infection of a thymine requiring mutant of *Escherichia coli*. *Journal of Bacteriology* **68**, 80–88.
- CAMPBELL, L. L. JUN. (1957). Reductive degradation of pyrimidines. I. The isolation and characterization of a uracil fermenting bacterium, *Clostridium uracilicum* nov.spec. *Journal of Bacteriology* **73**, 220–224.
- DAVIS, B. D. & MINGIOLI, E. S. (1950). Mutants of *Escherichia coli* requiring methionine or vitamin B₁₂. *Journal of Bacteriology* **60**, 17–28.
- HAYASHI, O. & KORNBERG, A. (1952). Metabolism of cytosine, thymine, uracil and barbituric acid by bacterial enzymes. *Journal of Biological Chemistry* **197**, 717–732.
- LARA, F. J. S. (1952). On the decomposition of pyrimidine by bacteria. I. Studies by means of the technique of simultaneous adaptation. *Journal of Bacteriology* **64**, 271–278.
- O'DONOVAN, G. A. & NEUHARD, J. (1970). Pyrimidine metabolism in microorganisms. *Bacteriological Reviews* **34**, 278–343.
- WANG, T. P. & LAMPEN, J. O. (1952). Metabolism of pyrimidines by a soil bacterium. *Journal of Biological Chemistry* **194**, 775–783.