Uracil Catabolism by *Escherichia coli* K12S

Š. Šimaga and E. Kos

Laboratory for Cellular Biochemistry,
Institute "Rudjer Bošković", Zagreb

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Experiments designed to elucidate the mechanism of uracil degradation by *E. coli* K12S showed that in contrast to uracil, dihydrouracil — the postulated intermediate of a reductive mechanism — did not stimulate the growth of bacteria as additional source of nitrogen, nor it was catabolized to ureido carbon dioxide. However, the chromatographic analysis of dihydrouracil metabolic products, revealed the presence of an enzyme converting dihydrouracil to β-ureidopropionic acid. Results of growth and biochemical studies indicated that barbituric acid — the postulated intermediate of an oxidative pathway — is not involved in uracil degradation.

Introduction

Catabolism of pyrimidine bases in microorganisms has been recently reviewed by Vogels and Van Der Drift [1]. On the basis of growth and biochemical studies two mechanisms of pyrimidine degradation have been found to be operative in bacteria: 1. Reductive pathway, by which uracil is degraded via dihydrouracil and β-ureidopropionic acid to β-alanine, and by analogy thymine via dihydrothymine and β-ureidoisobutyric acid, to β-aminoisobutyric acid; 2. Oxidative pathway, in which the initial step is an oxidative attack in position 6 of the pyrimidine nucleus, yielding barbituric acid for uracil or 5-methylbarbituric acid for thymine. Barbituric acid is further transformed to urea and malonic acid, while the fate of 5-methylbarbituric acid is still unknown.

As far as pyrimidine catabolism in *E. coli* is concerned, it has been demonstrated that bacteria convert the carbon 2 of uracil and thymine to carbon dioxide, and that the catabolic process is repressed in the presence of ammonium ions, but escapes the repression under ammonia starvation [2].

The aim of present studies was to get insight in the mechanism of uracil degradation *i. e.* whether either of the above mentioned pathways is operative in these bacteria.

Requests for reprints should be sent to Dr. Š. Šimaga, Laboratory for Cellular Biochemistry, Institute "Rudjer Bošković", Bijenička c. 54, 41001 Zagreb, Yugoslavia.

Materials and Methods

We used bacteria *Escherichia coli* K12S, a stock organism maintained in our laboratory. The basal growth medium was Davis and Mingioli medium [3]. This medium without ammonium salts, but supplemented with 5 mM sodium glutamate as principal source of nitrogen, was designated G-medium. Bacteria were grown at 37 °C with aeration. Growth rate was followed by reading the absorbance at 650 nm.

Bacterial extracts were prepared as follows: Bacteria grown in appropriate medium until the late exponential phase were filtered on membrane filters (pore size 0.45 μm), washed and resuspended in 0.1 M Tris-HCl buffer pH 8.0 at 100 mg wet wt/ml. The suspensions were sonicated for 3×1 min in a MSE ultrasonic disintegrator. The mixtures were centrifuged at 0 °C and $10\,000 \times g$ for 10 min. The supernatant fluid was used in the experiments.

We used the following radioactive chemicals: [2-^{14}C]uracil (52 mCi/mmol, BioResearch, Orangeburg, New York, USA); [2-^{14}C]dihydrouracil (0.82 mCi/mmol, Isocommerz GmbH, Dresden, G.D.R.); [2-^{14}C]barbituric acid (2 mCi/mmol, I.R.E., Mol, Belgium).

Degradation of [2-^{14}C]pyrimidines to $^{14}CO_2$ was measured by pipetting 0.1 ml of the suspensions on the planchets, dried and counted on a D-47 gas flow counter (Nuclear Chicago Corporation, Des Plaines, Illinois, USA).

Separations of pyrimidines and their derivatives were performed by thin layer chromatography using glass plates coated with cellulose (Microcrystalline Cellulose, Merck, Germany). The radioactivity on the chromatograms was detected by autoradiography using X-ray films (Kodirex, Kodak, USA). Dihydrouracil, β-ureidopropionic acid and urea were detected according to Fink *et al.* [4]. Uracil and barbituric acid were detected in ultraviolet light.

Results and Discussion

First we tested the effect of uracil and the postulated intermediates of reductive or oxydative pathway on the growth of bacteria. As seen in Fig. 1 A, the addition of uracil to G-medium enhances the growth, while dihydrouracil and barbituric acid resp. have no effect. In addition β-ureidopropionic acid, β-alanine or urea (not shown) do not stimulate the growth.

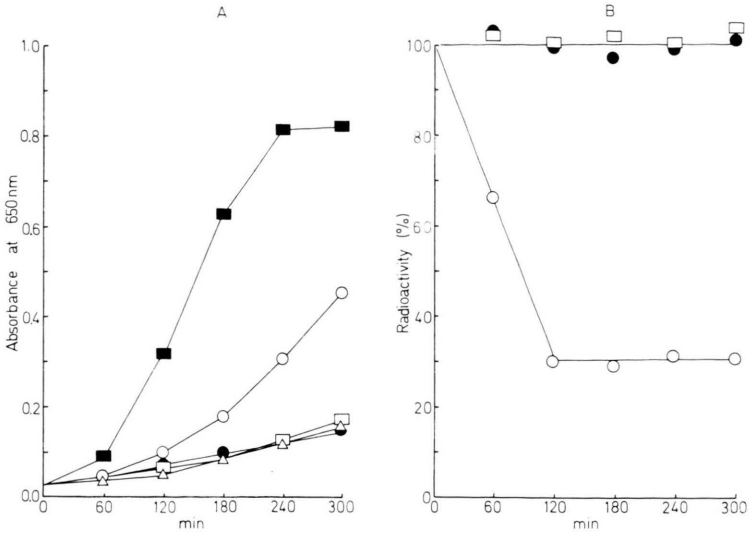

Fig. 1. A. Growth of bacteria *E. coli* K12S. Log phase cultures grown in basal medium were collected on membrane filters, washed with the same medium lacking glucose and $(NH_4)_2SO_4$ and regrown in the following media: ■ basal growth medium; △ G-medium alone, or with 1 mM of ○ uracil, ● dihydrouracil, and □ barbituric acid. B. The degradation of pyrimidines to ureido CO_2. Bacteria were incubated in G-medium in the presence of 0.1 mM [2-^{14}C]pyrimidines (each being of spec. activity 0.82 mCi/mmol): ○ uracil, ● dihydrouracil, □barbituric acid. The remained radioactivity determined as described in Materials and Methods was expressed as percentage of radioactivity initially present.

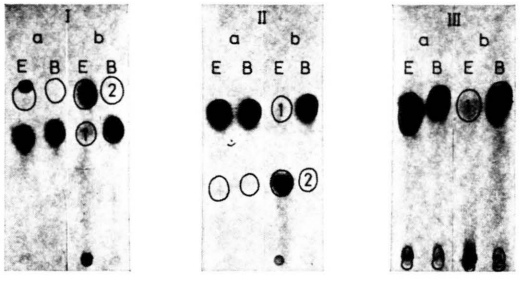

Fig. 2. Transformation of [2-^{14}C]dihydrouracil by cell free extracts of *E. coli* K12S. Bacterical extracts were prepared as described in Materials and Methods, from the cultures grown in G-medium and tested for their ability to degrade [2-^{14}C]uracil to $^{14}CO_2$; only the active extracts were used. Assays were made in 1.6 ml of bacterial extract-(E), to which 1 mM [2-^{14}C]dihydrouracil (0.82 mCi/mmol) was added. In controls -(B), 0.1 M Tris-HCl buffer pH 8.0 instead of bacterial extract was used. At 0 (a) and 180 min (b) of incubation aliquots of 270 μl were precipitated by 30 μl of 50% trichloracetic acid-TCA and after standing 30 min in ice-water, the precipitates were removed and TCA-soluble fractions used for chromatography and autoradiography. Aliquots of 10 μl were mixed with 2 μg of dihydrouracil (1) and β-ureidopropionic acid (2) resp. as standards, and separated by the following solvent systems: I. *tert*-butyl alcohol, methylethylketone, formic acid, water (4:3:1.5:1.5, v/v), II. *tert*-butyl alcohol, methylethylketone, water, ammoniumhydroxide (4:3:2:1, v/v) according to Fink *et al.* [5], III. pyridine, 17% ammonia (9:1) prepared in our laboratory.

In G-medium bacteria degrade uracil to the ureido carbon dioxide, which is not the case concerning dihydrouracil and barbituric acid (Fig. 1 B). When bacterial extracts prepared from the bacteria grown in G-medium were tested, we obtained the same results.

Chromatographic analysis of [2-^{14}C]dihydrouracil metabolic products revealed the conversion of dihydrouracil to β-ureidopropionic acid (Fig. 2). The chromatographic analysis of [2-^{14}C]barbituric acid (not shown) demonstrated that barbituric acid remained intact.

Our attempt to identify directly intermediate(s) of uracil degradation on chromatograms remained so far unsuccessful, because ^{14}C lost from the spot corresponding to uracil escaped as $^{14}CO_2$ and could not be detected in either of possible intermediates used as standards.

Discussing our results we may point out the following:

The metabolic inertia of bacteria *E. coli* in respect of barbituric acid supports the assumption that uracil is not catabolized by oxidative pathway. As far as reductive pathway is concerned, the ability of bacteria to catabolise dihydrouracil to β-ureidopropionic acid cannot be taken as evidence that dihydrouracil or β-ureidopropionic acid are involved in the main pathway of uracil degradation. This is supported by the findings, that in contrast to uracil, dihydrouracil is not catabolized to ureido carbon dioxide and that neither dihydrouracil nor β-ureidopropionic acid are utilized as additional source of nitrogen.

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