TRANSFORMATION OF 5-AZA-2′-[^{3}H]DEOXYCYTIDINE IN *ESCHERICHIA COLI*

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1. Introduction

5-Aza-2′-deoxycytidine has preferential affinity for the lymphatic system [1,2] and displays pronounced growth-inhibitory action against various forms of experimental neoplasias [3,4]. Although 20-times less active in *Escherichia coli* B than 5-azacytidine the deoxy-aza analogue exhibits still remarkable antibacterial effects which can be completely removed by natural pyrimidine precursors [5].

The 5-azapyrimidine deoxyribonucleoside is an efficient donor of the deoxyribosyl group promoting in *E. coli* the deoxyriboside-dependent incorporation of thymine [6]. However, the deamination to 5-aza-2′-deoxyuridine seems to be necessary for the utilization of the deoxyribose moiety of 5-aza-2′-deoxycytidine since its uptake was lost in bacterial mutants deficient in cytidine deaminase [7] where 5-aza-2′-deoxycytidine had practically no inhibitory effect. It was proposed that the analogue enters the cells of *E. coli* via deamination followed by the phosphorolytic cleavage of the glycosidic bond [7].

The deamination of 5-aza-2′-deoxycytidine takes place also in mice and results in the formation of 5-azauracil which has been isolated from the urine of drug-treated animals [2]. Recently the phosphorylation of 5-aza-2′-deoxycytidine to higher 5′-phosphates in parallel with the incorporation of the fraudulent deoxyribonucleotide into DNA was described in different systems of eukaryotic cells [4,8]. The aim of the present study was to follow the metabolic conversion and incorporation of 5-aza-2′-deoxycytidine in *E. coli* using tritium-labelled drug of high specific radioactivity.

2. Materials and methods

Cultivation of *E. coli* B was performed at 37°C by the stationary method in a synthetic medium containing glucose [9]. Inoculation was carried out with a 15 h *E. coli* culture added in an amount of 2% of the total volume. 5-Azauracil and 5-aza-2′-deoxycytidine were prepared in the Department of Organic Synthesis of this Institute. Tetrahydrouridine was a gift from Dr G. L. Neil, The Upjohn Company, Kalamazoo.

The incorporation of 5-aza-2′-[^{3}H]deoxycytidine (19.5 Ci/mmol) which was prepared by Dr B. Černý from the Isotope Laboratory, Prague-Krč, was followed as described in the legend to fig.1. 5-Aza-[^{3}H]uracil was prepared from 5-aza-2′-[^{3}H]deoxycytidine using the cell-free extract of *E. coli* as is shown in fig.3. Description of individual experiments is presented in the legends to figures. The radioactivity on chromatograms was detected using automatic Frieseke-Hoepfner scanner and the radioactivity of samples was measured using liquid scintillation system Isocap/300 (Nuclear Chicago Division).

3. Results

5-Aza-2′-deoxycytidine inhibits the growth of *E. coli* B in a mineral medium supplemented with glucose at the concentration of 1 μg/millilitre by 50% [5]. 5-Azauracil displays similar inhibitory effect while 5-azacytosine is about 1000-times less active. Consequently, we followed incorporation of the labelled 5-aza-2′-deoxycytidine and 5-azauracil into

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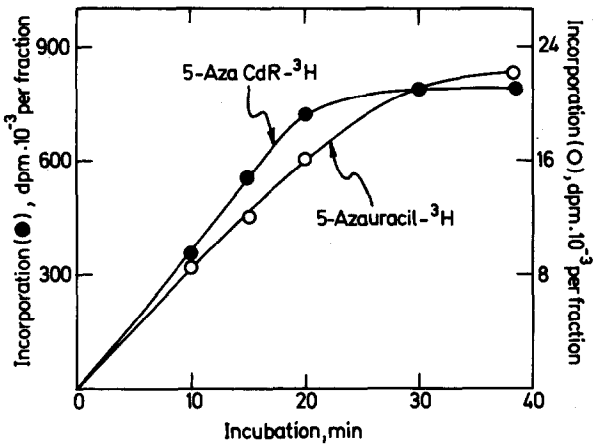

Fig.1. Incorporation of 5-aza-2′-[^{3}H]deoxycytidine and 5-aza-[^{3}H]uracil in *E. coli*. Washed bacterial cells collected in the logarithmic phase of culture growth were suspended in a mineral medium supplemented with glucose and incubated in total vol. 3 ml in the presence of 0.15 μmol 5-aza-2′-[^{3}H]deoxycytidine (800 μCi) or 5-aza-[^{3}H]uracil (100 μCi). At different time intervals (min) 0.5 ml portions of the incubation mixture were analyzed [2] for the uptake of the label into the acid-insoluble fraction of nucleic acids (dpm/fraction).

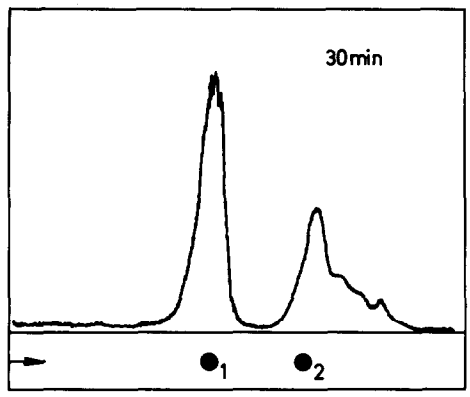

Fig.2. Radioactive compounds in the acid-soluble pool of *E. coli* cultivated in the presence of 5-aza-2′-[^{3}H]deoxycytidine. The cells incubated as in fig.1 were extracted under cooling with equal vol. 0.4 M $HClO_4$. The extract was neutralized by 2 M KOH, centrifuged and supernatant separated chromatographically on Whatman No. 3 paper using solvent system composed of isobutyric acid/ammonium hydroxide/water (44:1:22). (1) 5-Azauracil; (2), 5-aza-2′-deoxycytidine.

the fraction of nucleic acids in *E. coli*. The data presented in fig.1 show that the two azapyrimidines are incorporated into nucleic acids but to a different degree. 5-Aza-2′-[^{3}H]deoxycytidine is utilized about 10-times more effectively than 5-aza-[^{3}H]uracil. Using 25-times higher concentration of 5-aza-[2,4-^{14}C] uracil 0.4–0.7% of the drug was present in the acid-soluble pool of *E. coli* cultivated 16 h with the analogue in the form of ribosylated compounds [10].

Analysis of the acid soluble pool of *E. coli* incubated 30 min in the presence of 5-aza-2′-[^{3}H]deoxycytidine revealed a significant level of 5-aza-[^{3}H]uracil, unchanged deoxy analogue and respective degradation products with the highest R_F-values (fig.2). It seems that 5-aza-[^{3}H]uracil formed from 5-aza-2′-[^{3}H]deoxycytidine during the uptake of the fraudulent deoxynucleoside into bacterial cells is incorporated into nucleic acids more efficiently than 5-aza-[^{3}H]uracil added to baterial suspension. However, part of the radioactivity of tritium-labelled 5-aza-2′-deoxycytidine might be incorporated into nucleic acids also by other mechanisms which are mentioned below.

The transformation of 5-aza-2′-deoxycytidine

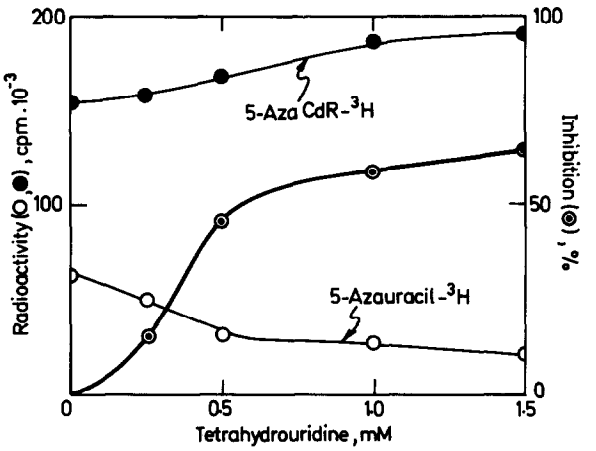

Fig.3. Inhibitory effect of tetrahydrouridine on metabolic conversion of 5-aza-2′-[^{3}H]deoxycytidine in the cell-free extract of *E. coli*. Bacteria harvested in the logarithmic phase of culture growth were suspended in 40 mM Tris–HCl buffer (pH 7.5), desintegrated (MSE ultrasonic desintegrator, 1 min, 2°C) and the fraction centrifuged (10 000 × *g*, 30 min, 2°C). Aliquots of the supernatant enzyme fractions were incubated 15 min at 37°C in total vol. 0.3 ml in the presence of 1 mM $MgCl_2$ and 0.05 mM 5-aza-2′-[^{3}H]deoxycytidine with increasing concentrations of tetrahydrouridine (mM). The separation of unreacted 5-aza-2′-deoxycytidine and of the newly formed 5-azauracil was carried out chromatographically on Whatman No. 1 paper as in fig.2. The corresponding spots were cut out and their radioactivity was measured (cpm).

resulting in the formation of 5-azauracil takes place also in the cell-free extract of *E. coli* (fig.3). Tetrahydrouridine affecting the deamination of cytosine arabinoside [11] inhibits also the deamination of 5-aza-2′-deoxycytidine as revealed by the lower level of the newly formed 5-azauracil. However, the intermediate component, 5-aza-2′-deoxyuridine, was not detected in the incubation mixture.

4. Discussion

5-Aza-2′-deoxycytidine is deaminated and undergoes nucleosidic cleavage resulting in the formation of 5-azauracil that was detected in vivo in the acid soluble pool of the drug-treated cells as well as in vitro using the cell-free extract of *E.coli* (figs 2 and 3). While 5-azauracil and 5-aza-2′-deoxycytidine have practically the same inhibitory efficiency in *E. coli* [2] both compounds are taken up into cellular nucleic acids to a different degree. It is supposed that the lower uptake of 5-aza[^{3}H]uracil (fig.1) reflects the incorporation of the drug into RNA since it reacts with 5-phosphoribosyl-1-pyrophosphate giving rise to 5-azauridine 5′-monophosphate [9].

On the other hand, the uptake of the label from 5-aza-2′-[^{3}H]deoxycytidine is likely to go both into RNA (through the formation of 5-azauracil) and DNA. 5-Aza-2′-deoxycytidine as an efficient donor of the deoxyribose supports the incorporation of thymine into DNA [6,7]. Moreover, the analogue itself can be incorporated into DNA. DNA with the labelled drug was recently isolated from the liver of leukemic mice injected with 5-aza-2′-[^{3}H]deoxycytidine [4]. However, since in *E. coli* the rate of deamination of the analogue deoxynucleoside is much higher than in eukaryotic systems only limited uptake of intact 5-aza-2′-deoxycytidine into bacterial DNA can be expected.

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