THE EFFECTS OF 5-AZACYTIDINE AND 5-AZAURIDINE ON PROTEIN SYNTHESIS IN ESCHERICHIA COLI

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In cultures of E.coli deficient in cytidine deaminase 5-azacytidine is a weak inhibitor of total protein synthesis in spite of being extensively incorporated into RNA. Strong inhibition of protein synthesis in wild-type strains is due to 5-azauridine formed from 5-azacytidine by deamination. However, the blocking of replication of phage T4, shown previously to be due to primary inhibition of replication of phage DNA, is a function of 5-azacytidine itself.

5-Azacytidine, a synthetic analog of cytidine, is a strong antibacterial agent (Šorm et al., 1964). Inhibition of total protein synthesis (Doskočil et al., 1967), concurrent with extensive incorporation into rapidly labeled RNA (Pačes et al., 1968) has been shown to account for the bacteriostatic activity of the analog in E.coli. The blocking of replication of phage T4 has a different cause, being due to the interference of the analog with the replication of phage DNA, while the synthesis of phage-specific proteins is much less sensitive than in uninfected bacteria (Doskočil and Šorm, 1967).

E.coli contains a strong cytidine deaminase activity, so that 5-azacytidine is rapidly deaminated in the bacterial culture, being converted primarily to 5-azauridine (Čihák and Šorm, 1965). Until recently it has been impossible to resolve the biological effects of these two compounds. The availability

of mutants of E.coli deficient in cytidine deaminase (Munch-Petersen, 1968) has made possible to determine the effects of 5-azacytidine itself. These strains have recently been found useful for the elucidation of the mode of action of 5-azadeoxy-cytidine (Doskočil and Šorm, submitted).

Strain OK 408, deficient in cytidine deaminase, was isolated by Dr.O.Karström, Karolinska Institutet, Stockholm, and kindly given to us by Dr.Munch-Petersen, Copenhagen. This strain has no requirements for growth, but is also deficient in deoxyribomutase and purine nucleoside phosphorylase. The bacteria were grown in shaken flasks at 37°, using a glucose-mineral salts medium according to Spizizen (1958), as previously described. 5-Azacytidine was prepared in the Department of Organic Syntheses of this Institute, using the method previously described (Píškala and Šorm, 1964). /^{14}C/ 5-azacytidine has been synthesized by Dr.J.Morávek. 5-Azauridine cannot be prepared in pure form, since 5',6-anhydro-6-hydroxy-5,6-dihydro-5-azauridine is obtained using the general synthetic method. However, in a mildly acidic aqueous medium, this compound is in equilibrium with 5-azauridine. Spectroscopic evidence indicates that if the material is dispersed in water, about 15-20% of the material is present in the form of 5-azauridine. This solution has been used as a source of 5-azauridine in the present experiments. All inhibitory effects of this preparation are counteracted by uridine.

Total protein synthesis as measured by the incorporation of leucine is slightly inhibited in a deaminaseless strain in contrast with the wild-type strain, where a complete inhibition is observed (Fig.1). In order to exclude the possibility that the mild effect of 5-azacytidine in the deaminaseless mutant is

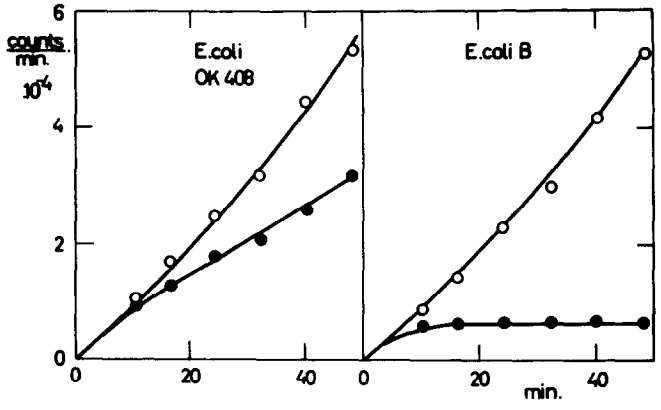

Fig.1. Incorporation of /^{14}C/leucine (10 /ug/ml, 0.2 /uC/ml) by liquid cultures of strain OK 408, deficient in cytidine deaminase, and by a wild-type strain E.coli B.
—o———o— control; —●———● 5-azacytidine (20 /ug/ml).

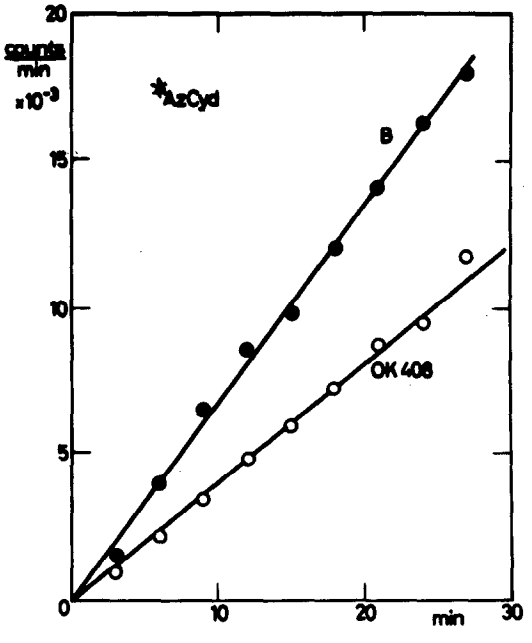

Fig.2. Incorporation of 5-azacytidine by cultures of E.coli. The concentration of /^{14}C/5-azacytidine (specific radioactivity 4 mC/mmole) was 5 /ug/ml. The optical density (A_{650}) of the culture of E.coli B was 0.221, that of strain OK 408, 0.221.

due to the inability of taking up the inhibitor, /^{14}C/ 5-azacytidine was used to compare the incorporation rates in wild-type and deaminaseless strains. Fig.2 shows that the mutant does incorporate 5-azacytidine, though at a somewhat reduced rate.

These results show that 5-azacytidine itself, though extensively incorporated into RNA, is a weak inhibitor of protein synthesis. Deamination to 5-azauridine is essential for the inhibition. In accord with this conclusion, 5′,6-anhydro-5-azauridine, known to produce 5-azauridine in an aqueous medium, inhibits protein synthesis completely even in the deaminaseless strain (Fig.3).

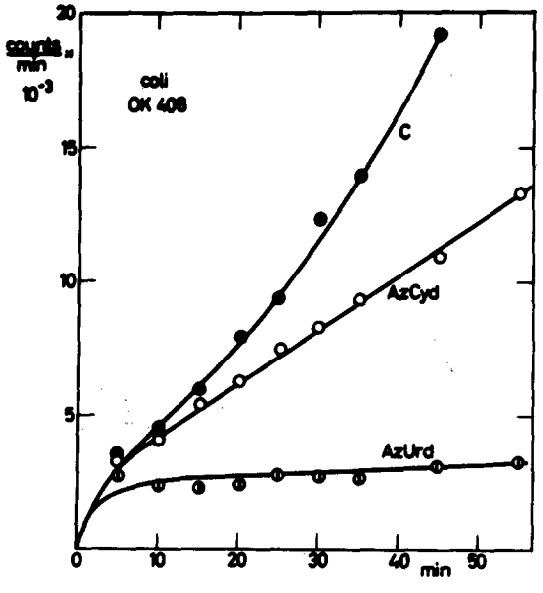

Fig.3. Incorporation of leucine by a culture of E.coli OK 408 in the presence of 5-azacytidine (AzCyd) and 5-azauridine (AzUrd). The conditions were similar to those in Fig.1.

On the other hand, the replication of phage T4 is strongly inhibited by 5-azacytidine even in the deaminase-deficient

TABLE I

Production of phage T4 by wild-type and cytidine deaminase-deficient strains of E.coli in the presence of 5-azacytidine. The bacteria were grown to $3x10^8$ cells/ml and L-tryptophan (20 µg/ml) was added. The cultures were infected with 10 viable phages per bacterial cell. After 75 min lysis was completed by adding a few drops of chloroform. The concentration of 5-azacytidine was 20 µg/ml.

Additions	Phage titre		% control	
	E.coli B	OK 408	E.coli B	OK 408
none	$6.1x10^{10}$	$1.3x10^{10}$	100	100
5-azacytidine	$5.6x10^7$	$5.1x10^7$	0.08	0.39

strains (Tab.I). Therefore 5-azacytidine itself, without prior deamination, is responsible for blocking the replication of phage T4. Since the inhibition of replication of phage DNA by 5-azacytidine is limited to T-even phages, it seems likely that 5-azacytidine, or its phosphorylated derivatives, interfere with the formation of 5-hydroxymethyldeoxycytidylate.

The present findings show that total inhibition of protein synthesis is not a general function of 5-azapyrimidines incorporated into mRNA, but is specific for 5-azauridine. According to quantum-chemical calculations (Piťhová et al., 1965b,c) the hydrogen-bonding properties of 5-azacytosine should not be different from cytosine, so that 5-azacytosine could be incorporated into mRNA instead of cytosine and recognized as such by the correct anticodon of tRNA. However, 5-azauracil easily undergoes hydration (Piťhová et al., 1965a) of the double bond 5,6, followed by ring opening to yield N-formylbiuret. If this reaction is accelerated by incorporation of 5-azauridine into a polynucleotide structure, e.g. by orbital overlap of stacked bases,

the inactivity of mRNA in protein synthesis could be understood.

The details of this work will be published in Collection of Czechoslovak Chemical Communications (Doskočil and Šorm, in press).

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