INDUCIBLE NUCLEOSIDE PERMEASE IN ESCHERICHIA COLI.

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SUMMARY

Mutants of E. coli resistant to both 5-azacytidine and showdomycin assume a partially sensitive phenotype when grown in the presence of thymidine. The kinetics of several other types of nucleoside conversion, known to depend on nucleoside-transporting system, is also altered toward the behavior of wild-type cells. It appears that a nucleoside-transporting component, distinct from the constitutive permease of non-induced wild-type cells, and coded by a different gene, is induced concurrently with the enzymes of the deo group.

Bacterial enzymes of nucleoside metabolism are usually constitutive, but their levels may be increased many times by growing the cells in the presence of a suitable inducer (1, 2). A single nucleoside may induce a number of enzymes required for a whole metabolic pathway (3). On the other hand special transporting systems participate in many types of nucleoside conversion (4, 5, 6) and it is not clear how the increased demand for substrate supply is met in induced cells; we may presume either that the transporting components are present in large excess in non-induced cells or that they could be induced concurrently with the enzymes of the corresponding metabolic pathway. This investigation confirms the latter proposition; the induced permease appears to be coded by a different gene than the constitutive permease and seems to differ from it by its lower affinity for showdomycin.

MATERIALS AND METHODS

E. coli B was mutagenized with N-nitro-N'-nitrosoguanidine (7) and mutants resistant to 5-azacytidine were selected as

described (5); one resistant colony was taken from each batch of mutagenized bacteria to prevent repeated isolation of the same mutant clone. The strains resistant to 5-azacytidine were tested for their ability to grow on agar containing showdomycin (1, 5 and 25 ug/ml).

For measurements of growth rates and kinetics of nucleoside conversion the bacteria were grown in shaken flasks on mineral salts medium (8) either with glucose or with glycerol, both supplemented with 0.25% casamino acids. For induction of nucleoside-catabolizing enzymes thymidine or cytidine (total dose 1 mg/ml) were added in four equal portions in intervals of 30 min. The induced cultures as well as the non-induced controls were filtered on nitrocellulose membrane filters, the bacteria were washed with fresh medium and resuspended in the medium without the inducer. After shaking for 15 min at 37 °C the labeled nucleosides or inhibitors were added and the measurements of metabolic conversion were started. Growth of bacteria was measured by determining the turbidity at 560 nm. The rates of incorporation of 5-azacytidine (5) and the conversion of deoxycytidine (9) were followed as described, using 2 [^{14}C]deoxycytidine (43 mCi/mmole) and 4 [^{14}C]5-azacytidine (30 mCi/mmole). Thymidine (A grade) was from Calbiochem; cytidine was obtained from Lachema, Brno. Showdomycin was synthesized (10) by Dr. L. Kalvoda from this Institute.

RESULTS

Although both 5-azacytidine and showdomycin are analogs of nucleosides and their transport is mediated by nucleoside-transporting system, the mechanisms of their inhibitory

effects are quite different (11, 12); we may expect that mutants cross-resistant to both these inhibitors should be deficient in nucleoside-transporting system. Of 20 mutants resistant to 5-azacytidine 12 were found capable of growing normally in the presence of 25/ug/ml showdomycin (AzCyd^r Shm^r), while the rest of them was completely inhibited at 1/ug/ml showdomycin (AzCyd^r Shm^s), similarly as wild-type bacteria. Both groups of mutants were found to incorporate 5-azacytidine at a reduced rate (5). In AzCyd^r Shm^r mutants the rate of 5-azacytidine incorporation increased several times upon induction with thymidine, while in AzCyd^r Shm^s mutants no such change was observed; a slight increase of the rate of 5-azacytidine incorporation was usually noted with wild-type cells. Cytidine in glycerol medium (13) was an efficient inducer of thymidine phosphorylase, but, unlike thymidine, failed to cause a proportional increase of the rate of incorporation of 5-azacytidine (Table I).

The AzCyd^r Shm^r cells became considerably more sensitive to 5-azacytidine and slightly more sensitive to showdomycin when induced with thymidine; on the other hand the AzCyd^r Shm^s mutants did not lose their resistance to 5-azacytidine (Table II). When induced cells were grown for several generations in thymidine-free medium, their original resistance to 5-azacytidine reappeared; similarly when plated on agar with 5-azacytidine their colony-forming ability was found unaffected by previous induction, indicating the stability of the resistant genotype.

The AzCyd^r Shm^r (but not the AzCyd^r Shm^s) mutants were found to metabolize deoxycytidine at a severely reduced rate while the activity of (deoxy)cytidine de-

Table I

Rates of Incorporation of 5-Azacytidine by Whole Cells and Activity of Thymidine Phosphorylase in Cell-Free Extracts

Bacterial strain	Carbon source	Induction	Rate of 5-azacytidine Incorporation nmoles/min	Activity of thymidine phosphorylase nmoles/min
E.coli B ($AzCyd^{S}Shm^{S}$)	glucose	none	2.8	10.1
		dThd	3.1	115
		Cyd	2.8	10.3
026 ($AzCyd^{r}Shm^{r}$)	glucose	none	0.22	9.2
		dThd	1.20	94
		Cyd	0.20	9.3
026 ($AzCyd^{r}Shm^{r}$)	glycerol	none	0.22	9.3
		dThd	0.80	89
		Cyd	0.30	52
018 ($AzCyd^{r}Shm^{s}$)	glucose	none	0.20	-
		dThd	0.18	-
018 ($AzCyd^{r}Shm^{s}$)	glycerol	none	0.24	9.0
		dThd	0.24	140
		Cyd	0.19	47

The rates of conversion were normalized to 10^{9} bacterial cells.

Table II

Effect of Induction with Thymidine on Growth Rates in the Presence of Inhibitors

Strain of E. coli	Induction	Growth rates in the presence of AzCyd 20/uM	Shm 20/uM	Shm 100/uM
B ($AzCyd^{S}Shm^{S}$)	none	14	0	0
026 ($AzCyd^{r}Shm^{r}$)	none	62	99	87
026 ($AzCyd^{r}Shm^{r}$)	dThd	34	92	55
018 ($AzCyd^{r}Shm^{s}$)	none	64	0	0
018 ($AzCyd^{r}Shm^{s}$)	dThd	62	0	0

Growth rates were calculated from semilogarithmic plots of turbidity measured between 20 and 100 min after the addition of the inhibitor and expressed in % control. Mean doubling times of all control cultures in the absence of inhibitors were 34 min.

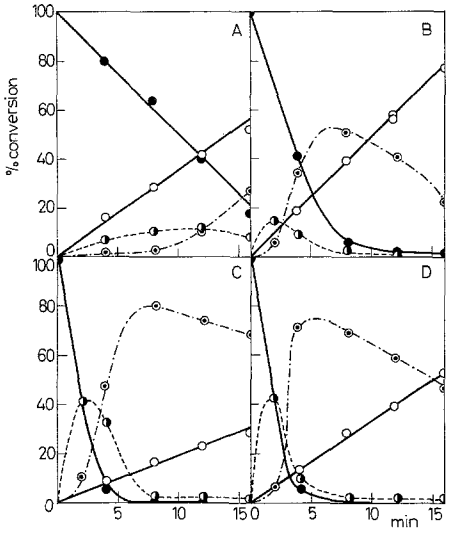

Figure 1. Metabolic conversion of deoxycytidine by strains of E. coli resistant to 5-azacytidine. The initial conc. of 2 [^{14}C]deoxycytidine was 40 uM and the density of bacterial cultures was approx. 10^9 cells/ml. A,B, strain 026 (AzCyd^r Shm^r); C,D, strain 018 (AzCyd^r Shm^s); A,C, non-induced cultures; B,D, cultures induced with thymidine; ●——● deoxycytidine; ◑ - - - -◑ deoxyuridine; ◎ - - -◎ uracil; ○——○ material incorporated into fraction insoluble in 5% trichloroacetic acid.

aminase as measured in cell free extract was equal to that of wild-type cells. Upon induction with thymidine their capacity of metabolizing deoxycytidine was restored to levels approaching those observed in wild-type cells (Fig. 1). The rate of deoxycytidine conversion has been reported to indicate the efficiency of nucleoside-transporting system (6).

To summarize, all AzCyd^r Shm^r mutants are defective in several types of nucleoside conversion (cf. 14); all these defects may be partially removed by growth in the presence of thymidine. In contrast the AzCyd^r Shm^s mutants do not show any defect of nucleoside metabolism besides the impairment of incorporation of 5-azacytidine; the identification of the genetic defect of these mutants will be reported later.

DISCUSSION

These results indicate that a nucleoside-transporting component (shortly called permease) may be induced in cells deficient in the constitutive nucleoside-transporting system. The inducible component appears to belong to the deo operon as defined by Hammer-Jespersen et al. (3), being inducible with thymidine in the presence of any carbon source or, though much less efficiently, with cytidine in the absence of glucose. Although we were unable to demonstrate the induction of permease in wild-type cells, it appears that it does take place, accounting for the increase of the overall cellular capacity of catabolizing thymidine with the same high affinity for substrate as in non-induced cells; no parallel increase of the rate of 5-azacytidine incorporation can be observed, because the constitutive permease alone may be sufficient for saturating uridine kinase responsible for 5-azacytidine incorporation. Since the induction takes place in all AzCyd^r Shm^r mutants isolated so far, it appears that the inducible permease is coded by a different gene than the constitutive component;while the former seems to be a part of the deo operon, the latter may belong to a different control group. Although sharing the affinity for 5-azacytidine and deoxycytidine, the constitutive and inducible components seem to differ in their relative affinity for 5-azacytidine and showdomycin; this is indicated by the fact that the induced sensitization is very pronounced with 5-azacytidine but very slight with showdomycin.

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