Inducible and constitutive nucleoside-binding sites in *Escherichia coli*:
differential inhibition by nucleoside analogues

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ABSTRACT

Constitutive and inducible nucleoside-binding sites on the surface of the cells of *E. coli* B have different patterns of inhibition by nucleoside analogues. 1(β -D-Ribofuranosyl)-4-aminopyrimidine-6-one and isoguanosine preferentially inhibit the inducible binding sites while showdomycin and N⁴-dimethylcytidine interfere more strongly with the constitutive function.

INTRODUCTION

Binding sites are important components of nucleoside-transporting systems in bacteria¹. In *E. coli* B two kinds of nucleoside-binding sites appear to be present; those with high substrate affinity are constitutive while those with lower affinity are inducible by 2'-deoxythymidine² concurrently with the enzymes of 2'-deoxyribonucleoside catabolism (the deo-enzymes³). Therefore, the two binding proteins appear to be coded by two independent genes and may be expected to be non-identical. Using a group of nucleoside analogues previously found to interfere with nucleoside-binding sites⁴, the patterns of inhibition of nucleoside transport were determined in *E. coli* B and in a mutant resistant to showdomycin and 5-azacytidine, both with and without previous induction with 2'-deoxythymidine. The *zCyd*^r *Shm*^r mutants appear to be deficient in constitutive nucleoside-binding sites; altered kinetics of several types of nucleoside conversion by whole cells of these mutants were described earlier⁵. The present experiments show that the inhibition pattern of the mutant is different from that of wild type strain; several inhibitors showing preference for either constitutive or inducible transporting system were discovered.

METHODS

1(β -D-Ribofuranosyl)-4-aminopyrimidine-6-one (I) was prepared⁶ and kindly donated by Dr M. Prystaš from this Institute. N⁴-Dimethyleytidine (II) was obtained as described by Yano and Hayatsu⁷. Isoguanosine (III) was synthesized according to Montgomery and Hewson⁸. Showdomycin was prepared synthetically⁹ and kindly donated to us by Dr L. Kalveda from this Institute. 5-Azacytidine was synthesized according to Pískala and Šerm¹⁰. 2 [¹⁴C] 2'-Deoxythymidine (50 mCi/mole) was purchased from the Institute for Research and Application of Radioisotopes, Praha; this preparation was diluted with non-radioactive 2'-deoxythymidine, Australanal, Wien.

E. coli B and a mutant resistant to both 5-azacytidine and showdomycin^{2,5} (α Cyd^r Shm^r) were used in this study. The conditions of cultivating the bacteria and of induction with thymidine were described earlier^{2,5}. The efficiency of induction of the deo-enzymes³ was checked by determining the activity of thymidine phosphorylase¹¹ in cell-free extracts; at least ten-times more of this enzyme was found in induced bacteria as compared with non-induced cultures.

The techniques of sampling and analysis of culture media by paper chromatography were described earlier^{5,12}. To obtain data for the Lineweaver-Burk plots the bacteria were preincubated with nucleoside analogues for 10 min before adding labelled 2'-deoxythymidine; this precaution was adopted in order to detect any irreversible inactivation of nucleoside-binding sites by the analogue, which might need some time to develop.

The interference of the nucleoside analogues with 2'-deoxycytidine uptake was detected indirectly by measuring the growth inhibition in bacteria cultivated in the presence of 6-azauracil and 2'-deoxycytidine; since *de novo* biosynthesis of pyrimidines is blocked by 6-azauracil¹³, the growth rate of the bacteria becomes dependent on an external pyrimidine source and is limited by the rate of its uptake.

RESULTS

The inhibition patterns of 2'-deoxythymidine phosphorolysis by whole cells of *E. coli* B and the α Cyd^r Shm^r mutant, both

Fig.1. Patterns of in vivo inhibition of 2'-deoxythymidine phosphorolysis by nucleoside analogues. A, B, *E.coli* B; C, D, *E.coli* zCyd⁺ Shm⁻; A, C, non-induced cultures; B, D, cultures previously induced with thymidine (2 mM). 1, 2'-deoxy-2'-chloro-5-methyluridine; 2, purine riboside; 3, N⁶-methyl-adenine; 4, 4-methoxypurine riboside; 5, N³-hydroxyethyluridine; 6, 1(β-D-ribofuranosyl)-4-aminopyrimidine-6-one; 7, showdomycin; 8, isoguanosine; 9, N⁴-dimethylcytidine. The concentration of 2-deoxythymidine was 80 μM, that of the analogues 400 μM.

non-induced and previously induced with 2'-deoxythymidine, are presented in Fig. 1. It may be seen that both strains produce different patterns. 1(β -D-Ribofuranosyl)-4-aminopyrimidine-6-one (I) and isoguanosine (II) very strongly inhibit the phosphorolysis of 2'-deoxythymidine in the mutant but are slightly effective in wild-type strain. On the other hand, showdomycin and N⁴-dimethylcytidine (III) are relatively strong inhibitors in wild-type cells but have hardly any effect on the mutant. Lineweaver-Burk plots of the kinetics of inhibitions of 2'-deoxythymidine phosphorolysis by whole cells show that the difference between wild-type and mutant strains is very striking (Fig. 2); the K_m/K_i ratios (Table I) differ by a factor of 40 with 1(β -D-ribofuranosyl)-4-aminopyrimidine-6-one, by 20 with isoguanosine, and by 2.6 with showdomycin. Inhibitors of the type of 2'-halogeno-2'-deoxyuridine derivatives⁴, e.g. 2'-chloro-2'-deoxy-5-methyluridine, strongly inhibit 2'-deoxythymidine phosphorolysis in both strains.

Table I. K_m/K_i values for the inhibition of 2'-deoxythymidine phosphorolysis by whole cells of *E. coli*

Inhibitor	K_m/K_i	
	α Cyd ^r Shm ^r	wild-type
1(β -D-Ribofuranosyl)-4-aminopyrimidine-6-one (I)	1.4	0.03
Isoguanosine (II)	1.3	0.06
Showdomycin	0.09	0.24
N ⁴ -Dimethylcytidine (III)	0.035	0.13
2'-Deoxy-2'-chloro-5-methyluridine	2.3	1.2

Upon induction with thymidine the inhibition pattern of the wild-type strain changes distinctly, becoming more similar to that of the mutant (Fig. 1); it appears that in induced wild-type bacteria the phosphorolysis of 2'-deoxythymidine is highly susceptible to both wild-type-specific and mutant-specific inhibitors. In the mutant the pattern does not change on induction.

Table II shows that 1(β -D-ribofuranosyl)-4-aminopyrimidine-

Fig. 2. Lineweaver-Burk plots of in vivo inhibition of 2'-deoxythymidine phosphorylation by nucleoside analogues.

A, *E. coli* zCyd^RShm^R; B, *E. coli* B; ⊖, no inhibitor; O, 2'-chloro-2'-deoxy-5-methyluridine; ⊙, 1(β-D-ribofuranosyl)-4-aminopyrimidine-6-one; ⊖, isoguanosine; ●, showdomycin.

The concentration of the inhibitors was 400 μM.

-6-one (I) and isoguanosine (II) efficiently inhibit the uptake of 2'-deoxycytidine in mutant strain but are devoid of this ability in wild-type strain. The same compounds antagonize the inhibitory effect of 5-azacytidine showing the same strain specificity (Table III). On the other hand, N⁴-dimethyleytidine (III) is effective in wild-type strain but has no effect in the mutant. Therefore the differential effect is not limited to phosphorolysis of 2'-deoxythymidine but affects a broad range of nucleoside conversion types. This observation is compatible with previous findings^{1,2,4}, indicating that a single type of nucleoside-binding sites may participate in transporting various ribo- and 2'-deoxyribonucleosides.

Table II. Interference of the analogues with 2'-deoxycytidine uptake by bacteria inhibited with 6-azauracil. The components, i.e. 6-azauracil (90 μ M), 2'-deoxycytidine (45 μ M) and the analogues (400 μ M) were added to bacterial cultures (1×10^8 cells/ml) at zero time and the increase of turbidity was measured at 560 nm 90 min later; during this period the bacteria were incubated at 37°C with shaking.

Analogue added	Inhibitor of pyrimidine biosynthesis	External pyrimidine source	Rate of growth % control sCyd ^r Shm ^r	wild type
none	none	none	100	100
none	z ⁶ Ura	none	28	17
none	z ⁶ Ura	dCyd	92	93
1(β -D-ribofuranosyl)-4-aminopyrimidine-6-one	z ⁶ Ura	dCyd	43	87
isoguanosine	z ⁶ Ura	dCyd	74	92
N ⁴ -dimethyleytidine	z ⁶ Ura	dCyd	92	84
2'-deoxy-2'-chloro-5-methyluridine	z ⁶ Ura	dCyd	54	69
1(β -D-ribofuranosyl)-4-aminopyrimidine-6-one	none	dCyd	92	92
2'-deoxy-2'-chloro-5-methyluridine	none	dCyd	90	90

Table III. Detoxication of 5-azacytidine by nucleoside analogues in *E. coli*. The conc. of 5-azacytidine was 40 μ M, that of the analogues 400 μ M.

Analogue added	Percent detoxication ^a			
	zCyd ^r Shm ^r		wild-type	
	non-induced	induced	non-induced	induced
1(β -D-ribofuranosyl)-4-aminopyrimidine-6-one (I)	46	73	18	15
isoguanosine (II)	43	44	0	0
N ⁴ -dimethyl-cytidine (III)	0	0	42	13
purine riboside	20	46	58	48
2'-chloro-2'-deoxy-5-methyluridine	46	57	80	76

^a Calculated using the formula $(v - v_0 / v_{\max} - v_0) \times 100$, where $v_{\max}$, v_0 and v indicate the growth rates of uninhibited culture, of the culture with 5-azacytidine alone, and of the culture with both 5-azacytidine and the analogue, respectively. The values of $v_0 / v_{\max}$ were 0.62 for non-induced zCyd^rShm^r bacteria; 0.38 for these bacteria induced with thymidine: 0.18 for non-induced *E. coli* B and 0.15 for induced wild-type bacteria.

DISCUSSION

The results confirm the non-identity of nucleoside-binding sites in wild-type non-induced *E. coli* and in a mutant resistant to both 5-azacytidine and showdomycin. Moreover, the change of the inhibition pattern upon induction of wild-type strains with 2'-deoxythymidine indicates that binding sites similar to those found in the mutant are augmented in wild-type bacteria concurrently with the enzymes of the deo-group.

The behaviour of the mutant strain shows that the inducible binding sites may function independently of the constitutive sites, being accessible directly to the nucleoside molecules from the medium; nevertheless, the inhibition pattern of wild-type induced bacteria, showing susceptibility to both classes of inhibitors, suggests a partly cooperative function of the two kinds of binding sites.

The above examples indicate that specific inhibitors of nucleoside-binding sites could be used for selective growth regulation in mixed populations of cells, controlling the growth either by the addition of a toxic nucleoside analogue or of a natural nucleoside under conditions of blocked de novo pyrimidine or purine biosynthesis.

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