UREA, A SPECIFIC INHIBITOR OF CATABOLITE SENSITIVE OPERONS

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SUMMARY : Low concentrations of urea inhibit specifically the expression of operons sensitive to catabolite repression. This inhibition is not relieved by cyclic AMP. It is suggested that the promoter of catabolite sensitive operons may be the target of the urea effect.

Many inducible systems responsible for an early step in the metabolism of carbon and nitrogen sources are subject to catabolite repression (1). *In vivo* and *in vitro* studies show that cyclic AMP and its receptor, the CAP protein, are the main elements of this positive regulation, the target of which is the promoter of catabolite-sensitive operons (for ref. see 2). These promoters no doubt possess structural analogies that permit specific recognition by the regulatory elements. In fact certain compounds such as nalidixic acid (3) or acridine orange (4) inhibit preferentially the transcription of catabolite-sensitive operons. In the present paper we show that addition of low concentrations of urea (0.5 to 0.75 M) to bacterial cultures inhibits selectively the expression of operons sensitive to catabolite repression.

MATERIALS AND METHODS

Strains and media. The following *E. coli* strains were used : wild-type strain 3000; Lac promoter mutant L_8UV_5 (a gift from J. Beckwith); and 71-56-14 a mutant carrying an i-z fusion (a gift from B. Müller-Hill).

Bacteria were grown at 37°C in 63 minimal medium with glycerol (0.4%) as carbon source and supplemented with vitamin B_1.

Induction. β-galactosidase was induced with 1 mM isopropyl-B-D-thiogalactoside, galactokinase with 0.2 mM D-fucose, tryptophanase with 10 mM tryptophan and phage λ receptor with 10 mM maltose.

Enzymatic assays. β-galactosidase was assayed according to (5), galactokinase according to (6), tryptophanase according to (7) phage λ receptor according to (8), glucose-6-phosphate dehydrogenase according to (9) and homoserine dehydrogenase according to (10). Enzyme units are expressed in nmoles of substrate converted per min at 28°C.

RESULTS AND DISCUSSION

When urea is added to an exponentially growing culture of wild-type strain 3000, the differential rate of β-galactosidase synthesis decreases. The extent of inhibition is a function of urea concentration (Fig. 1). It can be also seen

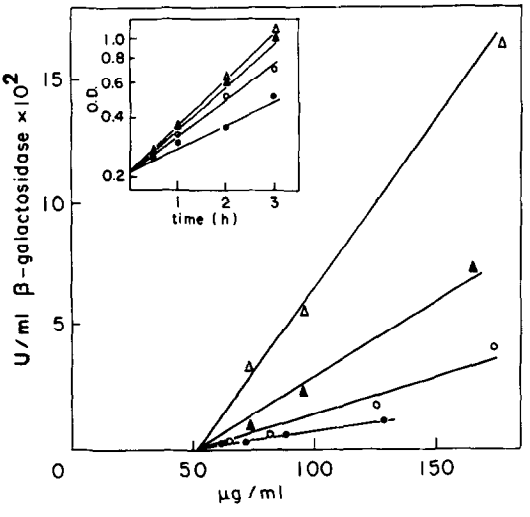

Fig. 1 Effect of different urea concentrations on the differential rate of β-galactosidase synthesis.

To exponential cultures of strain 3000 different concentrations of urea and inducer were added concomitantly. At different time intervals the amount of β-galactosidase was determined. Units of enzyme are plotted as a function of bacterial dry weight. Δ—Δ without urea, ▲—▲ with 0.5 M urea, o—o with 0.65 M urea, and ●—● with 0.72 M urea. The growth curves of the cultures are shown in the insert.

in Fig. 1 (insert) that urea concentrations that inhibit enzyme synthesis by as much as 75% have no effect on bacterial growth; accordingly urea does not significantly affect overall biosynthesis. The effect of urea is reversible : as soon as it is eliminated from the bacterial culture, β-galactosidase synthesis returns to its normal rate (data not shown).

Table I

Effect of urea on catabolite sensitive and insensitive systems

Activity	Differential rate of synthesis		
	- urea	+ urea 0.63 M	% inhibition
β-galactosidase	14000	3700	74
Tryptophanase	800	31.5	96
Galactokinase	12350	1670	86
Phage λ receptor	5600	600	89
G-6-P dehydrogenase	51.5	64.5	0
Homoserine dehydrogenase	96.5	101	0
Ileu C^{14} incorporation	370	370	0

Exponential cultures of strain 3000 were separately induced for β-galactosidase, for galactokinase, for tryptophanase and for phage λ receptor in the absence and in the presence of urea (added at instant of induction). Activities were assayed as described in Materials and Methods during 2-3 generations of growth. The results are expressed in units per mg dry weight bacteria for enzymes, in number of molecules per bacteria for phage λ receptor, and in nmoles of Ileu incorporated per mg dry weight bacteria for total incorporation.

Urea reduces specifically the differential rate of synthesis of systems known to be sensitive to catabolite repression, while the syntheses of catabolite-insensitive enzymes are unaffected. Table I shows that in the presence of 0.63 M urea, the rates of synthesis of β-galactosidase, galactokinase, tryptophanase and phage λ receptor (all catabolite sensitive) are strongly reduced. The total amino acid incorporation as well as the synthesis of the two biosynthetic enzymes assayed are not affected by urea. These results strongly suggest that at low concentrations, urea specifically inhibits the expression of catabolite sensitive operons.

If urea acts by enhancing catabolite repression, it seems likely that cyclic AMP would antagonize its effect. But surprizingly, as can be seen in Fig. 2, cyclic AMP does not reverse the repression by urea even at a urea concentration

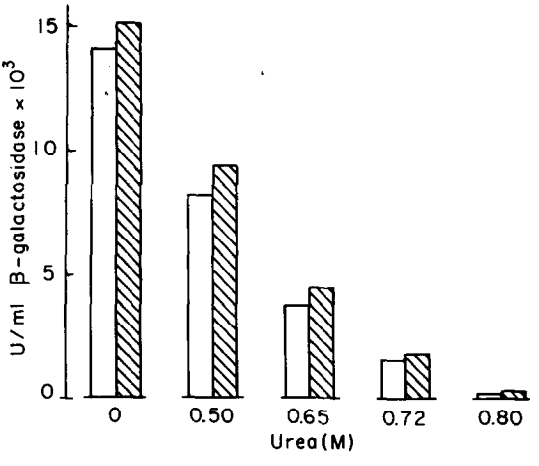

Fig. 2 Effect of cyclic AMP on the differential rate of β-galactosidase synthesis in the presence or absence of urea.

Conditions are similar as described in Fig. 1

▭ without cyclic AMP

▨ in the presence of 5.10^{-3}M cyclic AMP.

(0.5 M) which only moderately inhibits β-galactosidase synthesis.

Experiments have shown that the effect of urea on β-galactosidase synthesis in cryptic (y^-) or constitutive (i^- and o^c) mutants is similar to that in the wild-type (data not shown). Therefore, the urea effect seems to be independent of inducer permeation or inducer-repressor-operator interaction. Results presented in Table II strongly suggest that the target

Table II

Effect of urea on the differential rate of β-galactosidase and galactokinase synthesis in different strains

Strain	β-galactosidase U/mg			galactokinase U/mg		
	- urea	+ urea	% inhibition	- urea	+ urea	% inhibition
3000	10400	2600	75	9340	1500	84
L_8UV_5	8700	5000	43	10300	2500	76
71-56-14	330	290	12	7340	1670	77

Experimental conditions are as described in Fig. 1; urea concentration was 0.65 M. A parallel experiment on β-galactosidase induction in the three strains was conducted using glucose instead of glycerol. Under these conditions the values obtained for glucose were : 5000 U/mg for strain 3000; 6000 for strain L_8UV_5, and 300 for strain 71-56-14.

of urea action is the promoter. Urea has little effect on β-galactosidase synthesis in a strain carrying a mutation in the Lac promoter that renders the Lac operon largely insensitive to catabolite repression (11). Furthermore, when the z gene is fused to the i gene in a strain producing a hybrid repressor - β-galactosidase protein (12) under the control of a catabolite insensitive promoter (i^q), the rate of synthesis of the hybrid molecule is not affected by urea.

It should be noted that urea is not accumulated by the bacteria, since its intracellular concentration is not significantly different from the external one (data not shown). Therefore, it seems unlikely that urea acts as a protein denaturing agent.

In conclusion, at concentrations sufficiently low not to affect bacterial growth or enzymatic activity, urea specifically inhibits the expression of catabolite sensitive operons.

One possible explanation which would account for our results is that urea interacts directly with some specific sequences of a catabolite-sensitive promoter, thus "freezing" it in a particular conformation which does not allow CAP protein binging and/or RNA polymerase recognition. This interpretation would account for the inability of cyclic AMP to reverse the effect of urea. *In vitro* experiments to verify the hypothesis are now in progress.

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