REQUIREMENT OF CYCLIC AMP FOR INDUCTION OF GMP REDUCTASE IN ESCHERICHIA COLI

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Summary: Guanosine-5'-monophosphate reductase is induced in Escherichia coli by growth of the bacteria in the presence of guanine or guanosine. In mutants that lack adenyl cyclase this induction does not occur unless cyclic 3',5'-AMP is present. GMP reductase is not subject to catabolite repression.

Guanosine-5'-monophosphate reductase (NADPH: GMP oxidoreductase EC 1.6.6.9) catalyzes the irreversible, NADPH-dependent conversion of GMP to IMP. Its occurrence and role in the interconversion of purine nucleotides in enteric bacteria was first described by Mager and Magasanik (1). They also reported that it was sensitive to feedback inhibition by ATP and was not influenced by other enzymes essential for the de novo biosynthesis of AMP or GMP. In this early report, there was no indication that the enzyme was inducible.

Nijkamp and DeHaan (2) mapped the gene (guaC) controlling GMP reductase in E. coli at approximately 88 minutes on the Taylor map (3). They found that the level of GMP reductase increased with the increasing concentration of guanine in the medium while IMP dehydrogenase (guaB) and XMP aminase (guaA) were coordinately repressed by the increased levels of guanine.

In this paper we present evidence that GMP reductase in E. coli is an inducible enzyme and that cyclic AMP is required for the induction. The reductase does not appear to be sensitive to catabolite repression.

MATERIALS AND METHODS

Bacteria: Strain GP-1 is a cya mutant deficient in adenyl cyclase derived from Escherichia coli K-12 (HfrH) by Yokota and Gots (4). Both strains require thiamine for growth and

GP-1 has additional requirements for methionine, isoleucine and valine.

Media: The minimal salts medium (E) of Vogel and Bonner (5) was supplemented with glucose (0.2%) unless otherwise indicated. All cultures were routinely supplemented with thiamine (0.5 µg/ml) and GP-1 cultures were further supplemented with casein hydrolysate (0.1%) and methionine, isoleucine and valine at 20 to 40 µg/ml as indicated in the individual experiments.

Preparation of Extracts: All cultures were grown in one liter flasks (50 to 100 ml medium) at 37°C on a New Brunswick rotatory shaker. Cultures were checked for gross reversion to prototrophy, harvested by centrifugation and washed three times (30 ml per wash) with 0.03 M sodium phosphate buffer, pH 7.5. The cell pellets were resuspended in 0.5 to 2.0 ml of fresh buffer containing 2 mM 2-mercaptoethanol. Cell-free extracts were prepared by sonic disruption, 3 to 4 bursts of 20 second duration, interrupted by brief cooling periods. The cell debris was removed by centrifugation at 29,000 x g for 1 hour. Protein was determined by the Lowry method (6).

Enzyme assay: GMP reductase was measured by a modification of the assay of Mager and Magasanik (1). Dithiothreitol was used in place of cysteine at 1.0 mM and the assay was measured in a Gilford recording spectrophotometer at 293 nm. Purine nucleoside phosphorylase was assayed according to Kalckar (7) and guanine uptake and incorporation was patterned after Kalle and Gots (8).

RESULTS AND DISCUSSION

The presence of either guanine or guanosine in the medium caused an increase in GMP reductase. In order to circumvent the problem of poor solubility of guanine, guanosine was routinely used as the inducing agent in these studies.

Table 1 shows the levels of GMP reductase in *E. coli* HfrH grown with and without guanosine. The amount of induction ranged from 2.5 to 7.8 fold increase over the noninduced levels. Strain GP-1, an adenyl cyclase deficient mutant, was examined for the possibility that induction of GMP reductase might be analogous to the cyclic AMP-facilitated induction

Table 1

GMP reductase induction in Escherichia coli

Additions to media	GMP reductase (nmoles/min/mg protein)	
	Wild type (strain HfrH)	cya mutant (strain GP-1)
none	0.17	0.40
guanosine	1.69	0.56
guanosine plus cyclic AMP	1.27	3.28
cyclic AMP	0.31	0.41

Bacteria were grown overnight in Medium E, 0.2% glucose, thiamine (0.5 μg/ml) guanosine (200 μg/ml) and cyclic AMP (1.0 mM) as indicated. Strain GP-1 was supplied with amino acids as indicated in the text.

of other inducible enzymes (9). GMP reductase is not induced in this mutant in the absence of cyclic AMP (Table 1). The cyclic AMP alone does not act as an inducer of GMP reductase in the wild type strain. The optimal conditions for induction in the cya mutant were high levels of the required amino acids (40 μg/ml) or amino acids at 20 μg/ml plus 0.1% casein hydrolysate.

One of the inherent problems involved in a system which is induced by a metabolizable compound is that the inducer is constantly being removed from the system. To partially alleviate this problem, the cells were induced for two hours instead of an overnight induction. Table 2 shows the induction of HfrH and GP-1. The addition of cyclic AMP to the inducing medium had no effect on the levels of GMP reductase in the parent strain, however, the definite requirement for cyclic AMP in the induction is clearly illustrated in the cya mutant. Analogues of guanine such as 8-azaguanine and mercaptoguan-

Table 2

Induction of GMP reductase in log phase cultures

Strain	Fold increase in GMP reductase	
	no addition	with cyclic AMP
HfrH	5.3	4.5
GP-1	1.2	4.4

GMP reductase was measured as described in the Materials and Methods. Samples were taken before guanosine was added to the cultures and after 2 hours induction. Cyclic AMP (1 mM) was added along with the guanosine where indicated. Activity at time zero was 0.46 and 0.79 nmoles/min/mg protein for HfrH and GP-1 respectively.

osine could not serve as inducers and as yet no gratuitous inducer for GMP reductase has been found.

The inducible enzymes which respond to cyclic AMP are usually those which are sensitive to catabolite repression. The possible effect of catabolite repression on the induction of GMP reductase was therefore examined. Log phase cultures were induced in the presence and absence of glucose-6-phosphate. As shown in Table 3, no catabolite repression of GMP reductase was observed. Under similar conditions, glucose-6-phosphate caused at least a 50% reduction of β-galactosidase.

The induction of purine nucleoside phosphorylase has been reported by several investigators (10, 11). The levels of this enzyme in both the noninduced and induced cultures of HfrH and GP-1 were comparable. This eliminates the possibility that the initial utilization of guanosine is the limiting factor in the induction of GMP reductase and also indicates that the induction of purine nucleoside phosphorylase is not cyclic AMP dependent. The uptake and incorporation of guanine-8-C-14 in HfrH and GP-1 was similar in the two strains as was the hypoxanthine-guanine phosphoribosyltransferase activity.

These findings are unique in that an inducible enzyme which is neither catabolic

Table 3

Effect of glucose-6-phosphate on the induction of GMP reductase

Induction time (min)	GMP reductase (nmoles/min/mg protein)	
	Guanosine	Guanosine + glucose-6-PO_4
0	0.503	
30	0.870	1.39
60	1.76	2.26

An overnight culture of HfrH grown in medium E plus glycerol (0.5%) was collected by centrifugation, and resuspended in 5 times the original volume with medium E plus glycerol (0.5%). The cells were incubated with shaking at 37°C for 3 1/2 hours, a sample taken and the culture divided equally between 2 flasks. Both flasks contained guanosine (0.5 mM); glucose-6-phosphate (1×10^{-2} M) was added to one flask and samples were taken at 30 and 60 minutes. GMP reductase was assayed as described in the Materials and Methods.

nor subject to catabolite repression requires the participation of cyclic AMP for its induction. GMP reductase is not an obligate biosynthetic enzyme as indicated by the prototrophic properties of mutants lacking the enzyme (2, 12, 13). It serves primarily in the interconversions of purine nucleotides and would be required when there is an excess of GMP and a deficiency of AMP. Under these conditions control by substrate induction would be desirable. Previous reports have indicated that the composition of the media does not influence the GMP reductase activity in Salmonella (1), Aerobacter (1), the B strain of E. coli (1) and B. subtilis (13). However, with the K-12 (2) and the Harvard (12) strains of E. coli addition of guanine to the medium was necessary for demonstrable activity. Our findings confirm this with the K-12 strain except that constitutive levels are detectable. We attribute this to the synthesis of endogenous inducers in the prototrophic strains used. The constitutive activity can be completely abolished by decreasing the endogenous GMP level with the GMP inhibitor,

psicofuranine (unpublished observation). In contrast to the above report (1) we find that the level of GMP reductase in Salmonella typhimurium is also markedly influenced by the addition of guanosine to the medium. However, in an adenyl cyclase mutant of Salmonella, induction of GMP reductase does not require the addition of cyclic AMP.

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