CATABOLITE REPRESSION OF CHLORAMPHENICOL ACETYL TRANSFERASE SYNTHESIS IN *E. COLI* K_{12}

Jon Harwood and David H. Smith

Departments of Bacteriology and Immunology and Pediatrics
Harvard Medical School
Children's Hospital Medical Center
Boston, Massachusetts 02115

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SUMMARY

The specific activity of chloramphenicol acetyl transferase (CAT) in R^+ *E. coli* K_{12} strains grown on glucose was 3-5 fold lower than when grown on glycerol. This decrease was eliminated completely when the cells were grown in the presence of cyclic 3'5' AMP but not 5' AMP. When an adenyl cyclase-defective mutant and its parent strain, both harboring the R factor, were grown on glucose plus 5' AMP, the level of CAT was 3-4 fold lower in the mutant than in the parent. Replacing 5' AMP with cyclic AMP increased the CAT level 20-30 fold.

E. coli cells harboring resistance (R) factors which confer on the host resistance to chloramphenicol (Cml) synthesize constitutively an R factor-coded (1) enzyme that inactivates this drug via acetylation (2,3,4). Because the regulation of synthesis of enzymes produced constitutively has not been well defined and that of chloramphenicol acetyl transferase (CAT) and other R factor-coded enzymes has not been investigated, we undertook a study of CAT synthesis in R^+ strains of *E. coli* K_{12}. Our results indicate that CAT, and another R factor-coded enzyme, streptomycin adenylate transferase (SAT), are subject to cyclic 3'5' AMP-mediated, catabolite repression.

MATERIALS AND METHODS

E. coli K_{12} strains: AB1932-1 has been described (5). Strain 3000 (Hfr H, thi^-) was obtained from Dr. B. Magasanik. Strain 1100 and its adenyl cyclase-deficient mutant, 5336, (6) were the gifts of Dr. I. Pastan. Because of its very low levels of cyclic AMP, strain 5336 fails to grow on lactose, arabinose, maltose, mannitol or glycerol unless cyclic AMP is supplied in the medium.

R factors: The R factor JJ1 is a tetracycline-sensitive derivative of the R factor RE 130 (7), and confers resistance to Cml, streptomycin, spectinomycin, sulfadiazine, and mercuric chloride. The R factor RK5 has been described (8). JJ1 was crossed by conjugation into E. coli 1100 and 5336 from E. coli AB1932-1/JJ1 by selecting for recombinants on minimal glucose agar containing thiamin, 8μ g Cml/ml and 1mM cyclic AMP.

Preparation of Extracts: Extracts were prepared by growing the cells at least 4-5 generations in minimal medium (9) to mid- or late log phase, washing twice with cold phosphate buffer, resuspending in 0.1 M Tris, pH 7.8, and disrupting with 3 to 7 15-second bursts of sonification at an intensity of 65 units with a Bronson Sonifier. The temperature of the suspension was kept below 20°C during the sonification by suspending it in an ice-water bath. The "sonicate" was centrifuged at 4°C for 30 minutes at 30,000 x g, and the resulting supernatant fluid was used without further purification as a source of enzymes.

Enzyme Assays: β -galactosidase was measured in crude extracts of cells grown in the presence of 5×10^{-4} M isopropylthiogalactoside by determining spectrophotometrically the initial velocity of the release of o-nitrophenol from o-nitrophenylgalactoside using a Gilford Recorder Model 2000. CAT was assayed according to the method of Shaw and Brodsky (10). SAT was assayed by the method of Yamada et al. (11) modified according to Smith et al. (12). Protein concentrations in crude extract were measured according to Lowry et al. at 500 mμ (13).

RESULTS AND DISCUSSION

E. coli AB1932-1/JJ1 was grown in minimal media containing each of several carbon sources, some of which have been shown to cause catabolite repression of the synthesis of certain inducible enzymes. The specific activities (s.a.) of CAT and SAT in crude extracts of these cells (Table I) indicate that both enzymes are subject to catabolite repression. This phenomenon is not peculiar to JJ1-coded CAT because similar data were

obtained for AB1932-1/RK5 grown in minimal medium containing either glycerol, glucose, or glucose-6-phosphate as carbon source. A trivial explanation for these findings -- that the R factor segregates more readily when the cells are grown on glucose -- was ruled out by the observation that R-

TABLE I

RELATIONSHIP BETWEEN R FACTOR ENZYME LEVELS AND CARBON SOURCE IN MEDIUM[a]

Carbon Source (0.2%)	Enzyme Specific Activities	
	CAT[b]	SAT[c]
Glycerol	4560	3.54
Glucose	780	0.79
Glucose-6-phosphate	980	0.39
Arabinose	1460	1.08
Mannitol	1520	0.99
Rhamnose	3120	0.33

[a]The strain used was E. coli AB1932-1/JJ1.
[b]nmoles p-nitro-m-carboxythio-phenol produced per min. per mg. protein at 25°C.
[c]cpm of α-^{32}P-ATP per 10μg aliquot which adsorb to a 1 cm^2 piece of phosphocellulose paper per μg protein per min. at 37°C.

TABLE II

RELATIONSHIP BETWEEN ENZYME LEVELS AND ADENINE NUCLEOTIDES ADDED TO MEDIUM[a]

Carbon Source (0.4%)	Nucleotide (5mM)	Enzyme Specific Activities	
		β-gal[b]	CAT[c]
Glycerol	0	4500	1800
Glycerol	5' AMP	4800	2000
Glycerol	cyclic 3'5' AMP	5100	3600
Glucose	0	1300	460
Glucose	5' AMP	2600	580
Glucose	cyclic 3'5' AMP	5200	4200

[a]Overnight cultures of E. coli 3000/JJ1, grown in minimal medium with either glycerol or glucose, were diluted 1-to-50 into fresh medium containing IPTG at 7.5 x 10^{-4}M, grown and incubated for 1-2 generations after which aliquots of each culture were chilled and stored at 0°C; the remainder of each culture was divided, 5' AMP was added to one subculture and cyclic 3'5' AMP to the other. The 4 cultures were then incubated 2-3 more generations harvested and assayed. Neither nucleotide affected the growth rate of the cells.
[b]nmoles o-nitrophenol produced per min. per mg. protein at 25°C.
[c]units as in Table I.

segregant frequencies in minimal glycerol and minimal glucose cultures of AB1932-1/JJ1 were both less than 5%.

Since cyclic AMP has been implicated as a mediator of catabolite repression (14), its effect on the intracellular levels of CAT was investigated. Table II illustrates that 5mM cyclic AMP, but not 5mM 5' AMP, raises the s.a. of both CAT and β-galactosidase in 3000/JJ1 grown on glucose back to the basal, unrepressed level.

These findings prompted an examination of the effect of a low intracellular cyclic AMP concentration on the s.a. of CAT. Table III shows that when 1100/JJ1 and 5336/JJ1 (the cyclic AMP deficient mutant) were grown on glucose plus 5' AMP (as a control nucleotide), the s.a. of β-galactosidase was 30-fold, and that of CAT 4-fold lower in the mutant than in the parent strain. Substitution of cyclic AMP for 5' AMP increased the levels of both enzymes in both strains 30-50 fold (or essentially back to unrepressed basal levels). Comparable data (not shown) indicate that the s.a. of SAT in these strains is also approximately 30-fold higher when the cells are grown on glucose in the presence of cyclic AMP rather than 5' AMP.

TABLE III

RELATIONSHIP BETWEEN ENZYME LEVELS AND CYCLIC 3'5' AMP

E. coli K12 Strains	Medium Supplements		Enzyme Specific Activities[a]	
	Carbon Source (0.4%)	Nucleotide 3mM	β-gal	CAT
1100/JJ1	Glycerol	5' AMP	4950	3000
	Glycerol	cyclic AMP	6100	4300
	Glucose	5' AMP	2550	980
	Glucose	cyclic AMP	5150	4450
5336/JJ1	Glucose	5' AMP	84	260
	Glucose	cyclic AMP	4750	7000

[a]Expressed as described in Tables I and II.

The lower s.a. of CAT in extracts of glucose-grown cells is not due simply to an enzyme inhibitor that is not found in extracts of glycerol-grown cells because when two such extracts were mixed the resultant level of CAT activity was additive. Also, the addition to the assay mixture of glucose or glucose-6-phosphate at final concentrations of 0.2% or 5' AMP or cyclic AMP at 1mM, did not significantly alter the CAT activity. The carbon source and cyclic AMP must therefore affect the synthesis rather than the activity of the enzyme.

Because synthesis of two JJ1-coded enzymes was found to be catabolite repressible, the catabolite-sensitive target (responsive to cyclic AMP) might be a regulatory site affecting R factor replication. This explanation seems very unlikely, however. First, in an E. coli strain in which the CAT locus is part of a defective P1 phage genome (15), the s.a. of the CAT was several fold lower when the cells were grown on glucose than on glycerol despite the fact that there is only one copy of this extrachromosomal P1 genome per host chromosome (S. Falkow, personal communication). Second, the ratio of R factor copies to host chromosome copies is between 1 and 2 (in E. coli bearing either R factor R_1 or 144) whether the cells are grown on glucose or glycerol (D.K. Haapala, personal communication).

Recent evidence (16-18) suggests that cyclic AMP antagonizes the catabolite repression of β-galactosidase synthesis by interacting with the promoter region of the lactose operon. The data presented here suggest that the promoter region for the CAT structural gene(s) is apparently similar to that of the lactose operon in its responsiveness to cyclic AMP. The possibility that the structural loci for CAT and SAT have a common promoter region is being studied. Not all the R factor-coded enzymes are catabolite repressed, however, since the synthesis of R_1-coded penicillinase is similar in an E. coli K_{12} strain grown on glycerol or glucose (19).

Catabolite repression of the snythesis of CAT and SAT was not anticipated since, to our knowledge, this is the first report of

constitutive enzyme syntheses being subject to this form of control, and since neither of these enzymes has any known catabolic function. The data presented may, therefore, provoke a reexamination of these enzymes' evolutionary origins and/or role in the cell.

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