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Catalase Synthesis in *Escherichia coli* Is Not Controlled by Catabolite Repression[1]

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Catabolite repression is not involved in the regulation of catalase gene expression. The presence of glucose in minimal salts media and LB medium did not affect the basal levels of catalase but did enhance catalase synthesis following induction with either hydrogen peroxide or ascorbate. The cofactor for catabolite gene activator protein, cAMP, did not affect either the basal levels or the rate or extent of catalase synthesis. Catalase synthesis occurred normally in an adenylate cyclase mutant where β-galactosidase, a catabolite-sensitive enzyme was not synthesized.

Catalase appears to be essential for the protection of aerobically growing organisms against the toxic effects of hydrogen peroxide generated in various cellular reactions. Unlike the study of catalase gene regulation in eucaryotes which is extensive (1), similar studies in procaryotes have been limited to suggesting: (a) a link between the synthesis of catalase and the synthesis of components of the respiratory chain in *Escherichia coli* (2), (b) the presence of two electrophoretically distinct catalases, one constitutive and the other repressible, and (c) the involvement of catabolite repression in catalase synthesis in yeast (3), *Bacteroides fragilis* (4), and *E. coli* (2, 5). These latter observations of catabolite repression affecting catalase levels in *E. coli* contradicted an earlier report (6) that showed that glucose did not affect catalase levels in *E. coli* growing in a tryptic casein digest. They were also inconsistent with the report that pyocyanine caused a 10-fold increase in catalase levels in a glucose-containing medium (7).

An additional level of control in catalase gene expression was suggested by the induction of catalase synthesis in response to H_2O_2 added directly to the medium (5, 8), and to H_2O_2 generated *in situ* from pyocyanine (7) and ascorbate (8). However, the direct role of H_2O_2 as an inducer was called into question, when it was found that catalase synthesis was induced in the absence of H_2O_2 during anaerobic growth in the presence of nitrate (2). Despite this confusion, the facile nature of the induction of catalase synthesis in response to either H_2O_2 or ascorbate represented a convenient tool to be used in the study of catalase gene expression. This report describes experiments which show that glucose did not inhibit and cAMP did not enhance either the basal or induced levels of catalase in *E. coli*, and it concludes that catabolite repression does not play a role in catalase gene expression.

MATERIALS AND METHODS

All chemicals and enzymes were obtained from Sigma; yeast extract and bacto-tryptone were obtained from Difco. The strains of *E. coli* used were:

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TABLE I

BASAL LEVELS OF CATALASE ACTIVITY IN MID-LOG PHASE *E. coli* B23 GROWING IN MINIMAL SALTS MEDIUM (A) AND LB MEDIUM (B) CONTAINING VARIOUS SUPPLEMENTS

Medium	Supplement[a]	Catalase activity (units/mg dry cell wt)
A	Glucose	13.98 (±0.82)
	Glucose + cAMP	14.23 (±0.43)
	Glucose + CA	9.08 (±0.62)
	Glucose + CA + cAMP	8.98 (±0.53)
	Glycerol	14.68 (±1.14)
	Glycerol + glucose	14.80 (±0.82)
	Glycerol + CA	10.02 (±0.72)
	Glycerol + glucose + CA	9.36 (±1.02)
	Succinate	14.94 (±1.22)
	Succinate + glucose	15.10 (±1.04)
	Succinate + CA	14.34 (±1.04)
	Succinate + glucose + CA	13.82 (±0.91)
B	—	5.52 (±0.43)
	Glucose	5.69 (±0.22)
	cAMP	5.43 (±0.21)

[a] The following concentrations of supplements were used: glucose, 16 mM; glycerol, 24 mM; succinate, 50 mM; casamino acids (CA), 0.04%; cAMP, 4 mM.

B23, B wild type; MP180, K12 wild type, *HfrH*; and MP259, *cya*, *HfrH*.

Cultures of *E. coli* were grown in volumes of 25 ml in 250-ml side-arm flasks at 37°C on shaker bed at 200 rpm following growth with a Klett-Summerson colorimeter using a blue filter. The LB medium contained 10 g bacto-tryptone, 5 g yeast extract, and 5 g sodium chloride in 1 liter of water (9). The minimal salts medium contained 0.1 M Tris, pH 7.6, 1.6 mM Na_2SO_4, 0.5 mM KH_2PO_4, 1 mM $MgSO_4$, 10 mM NaCl, 0.3 μM $FeCl_3$, 0.1 mM $CaCl_2$, 10 mM $(NH_4)_2SO_4$, and was supplemented with 16 mM glucose, 24 mM glycerol, 50 mM succinate, and 0.04% casamino acids as specified in individual experiments. Ascorbic acid was prepared fresh just prior to use. The relationship of 0.152 mg dry cell weight/ml at 100 Klett units in minimal salts medium and 0.165 mg dry cell weight/ml at 100 Klett units in LB medium was determined by drying culture aliquots and correcting for any weight contribution from the medium.

Culture aliquots were removed at various times and chilled on ice until they were assayed for catalase activity, a period of no more than 1 h. Catalase activity was assayed according to the procedure of Rørth and Jensen (10) using a Gilson oxygraph Model KM equipped with a Clark-type electrode to quantitate the production of O_2 from H_2O_2 at 37°C in a 2-ml reaction volume. The culture aliquots were diluted where appropriate and placed in the jacketed reaction vessel where they were stirred vigorously. When the temperature had equilibrated (approximately 1 min) and the oxygen concentration appeared constant, the reaction was initiated by the addition of 3 mM H_2O_2. The evolution of O_2 was followed for 1 min. One unit of catalase is defined as the amount of enzyme breaking down 1 μmol of H_2O_2 per minute. Enzyme activities are expressed as units per milligram dry cell weight where dry cell weight was determined from the Klett-Summerson colorimeter reading. All experiments were carried out a minimum of two times and the results were averaged. The levels of β-galactosidase were determined following the addition of 1 mM isopropylthiogalactoside to cultures following the published procedure (9).

The concentration of cAMP in LB medium was assayed using a kit purchased from Amersham following published procedures (11, 12). No assayable cAMP was present representing a concentration of less than 2 nM. The concentration of glucose in LB medium was assayed in a Glucostat kit purchased from Boehringer-Mannheim and was found to be 8.3 μM.

RESULTS

Effect of Fermentable and Nonfermentable Carbon Sources on Basal Catalase Levels in E. coli

The basal catalase levels in midlog phase *E. coli* which have been reported in the literature contain a discrepancy between the levels in rich media (4–5 units/mg dry cell wt in LB medium (8) and 5–6 units/mg dry cell weight in trypticase soy broth containing 8.3% glucose (2)) and in minimal medium (10–12 units/mg dry cell wt in glucose salts medium (7)). A more extensive comparison of basal catalase levels in midlog phase *E. coli* growing in various media is contained in Table I. The basal levels were essentially the same, 14–15 units/mg dry cell weight, regardless of whether glucose, glycerol, or succinate was the carbon source and a combination of glucose with either glycerol or succinate did not affect these levels. Therefore, whether or not the carbon source was fermentable had no effect on the basal catalase levels. By contrast, a 30% reduction, to 9–10 units/mg dry cell weight, in the basal levels of cultures growing in glycerol-salts and glucose-salts media but not in succinate-salts medium was caused by a casamino acid supplement. For compar-

ison, the levels of catalase in cultures of *E. coli* growing in LB medium with and without 16 mM glucose were the same, 5.5 units/mg dry cell weight (Table I). Even 50 mM glucose (data not shown) did not affect the basal level.

Effect of Fermentable and Nonfermentable Carbon Sources on the Induction of Catalase Synthesis in E. coli

The synthesis of catalase has been induced by H_2O_2 (5, 8), pyocyanine (7), and ascorbic acid (8), and in conjunction with the diauxie response (2). The composition of LB medium is complex and, while very little glucose was present (8.3 μM), other carbohydrates could be present in the yeast extract making a definition of carbon utilization difficult. However, a glucose supplement in LB medium caused a 15% increase in the final level of catalase reached after induction with 5.5 mM ascorbate (Fig. 1), just the opposite of what would be expected if catabolite repression affected the process.

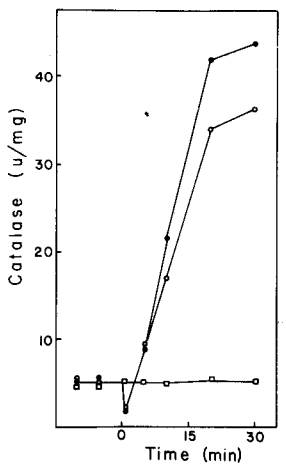

FIG. 1. Effect of glucose on the induction of catalase synthesis in *E. coli* B23 growing in LB medium. Ascorbic acid (2.8 mM) was added at time 0 to cultures of *E. coli* growing in LB medium (○) and LB medium supplemented with 16 mM glucose (●). No ascorbate was added to a culture growing LB medium with 16 mM glucose (□). Aliquots were removed at various times and chilled on ice until assayed for catalase activity which is expressed as units/mg dry cell weight.

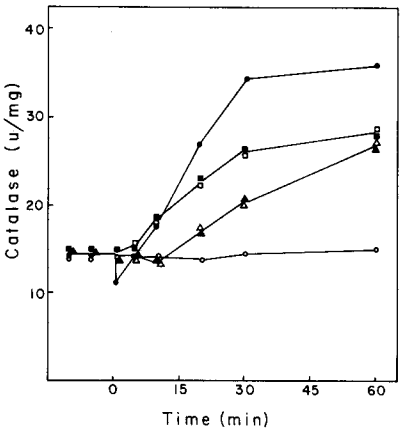

FIG. 2. Effect of different carbon sources in minimal salts medium on the induction of catalase synthesis in *E. coli* B23. Ascorbic acid (0.57 mM) was added at time 0 to cultures of *E. coli* growing in minimal salts medium supplemented with 16 mM glucose (●), 24 mM glycerol (■), 24 mM glycerol and 16 mM glucose (□), 50 mM succinate (▲), and 50 mM succinate and 16 mM glucose (△). No ascorbate was added to a culture growing in minimal salts medium with 16 mM glucose (○) and a similar lack of change was observed when glycerol and succinate were the carbon sources. Aliquots were removed at various times and chilled on ice until assayed for catalase activity which is expressed as units/mg dry cell weight.

Following the induction of catalase synthesis in cultures growing in clearly defined minimal media, the greatest increase occurred in glucose-salts medium and smaller increases were observed in glycerol-salts and succinate-salts media (Fig. 2). These latter two cultures also exhibited slower growth rates in approximate proportion to the appearance of catalase. Supplementing the glycerol and succinate cultures with glucose one-half generation prior to the addition of ascorbate did not affect the amount of induction (Fig. 2). If the glucose in these media was present for more than one generation time prior to the addition of ascorbate, the amount of induction increased so that the final levels were similar to levels in the glucose-salts culture.

When casamino acids were used as a supplement in the minimal media, the final induced level was enhanced about 15% in all three media (Fig. 3). As noted in

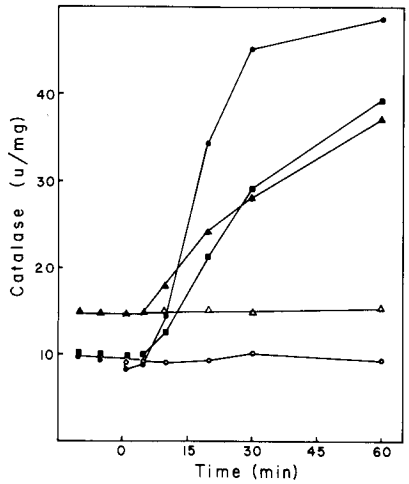

FIG. 3. Effect of different carbon sources in minimal salts medium containing 0.04% casamino acids on the induction of catalase synthesis in *E. coli* B23. Ascorbic acid (0.57 mM) was added at time 0 to cultures of *E. coli* growing in minimal salts medium supplemented with 16 mM glucose (●), 24 mM glycerol (■), and 50 mM succinate (▲). No ascorbate was added to cultures growing in minimal salts medium with casamino acids and glucose (○) or succinate (△). Aliquots were removed at various times and chilled on ice until assayed for catalase activity which is expressed as units/mg dry cell weight.

Table I, the basal levels in glycerol- and glucose-containing media were lower than the levels in succinate salts medium giving rise to an apparent greater induction although the final levels in glycerol and succinate cultures were similar. The final level in the casamino acid-supplemented glucose-salts medium culture was similar to the level reached in the culture grown in glucose-supplemented LB medium (Fig. 1). In all of these experiments, 0.62 mM H_2O_2 could replace ascorbic acid as the inducing agent but the levels of catalase were reduced by 10–15% in minimal salts medium.

Effect of cAMP on the Induction of Catalase Synthesis in E. coli.

The mechanism for catabolite repression utilizes cAMP as a cofactor for catabolite gene activator protein (CAP) activation of catabolite-sensitive genes (11). As compiled in Table I, cAMP did not affect the basal levels of catalase in cultures of *E. coli* growing in either LB medium or glucose-salts medium. This confirmed the earlier report in which cAMP was added to trypticase soy broth with no effect on the basal levels (2). Neither the rate nor the extent of catalase induced by H_2O_2 in a culture growing in glucose salts medium was affected by 1 mM cAMP (Fig. 4) or 4 mM cAMP (data not shown). Similarly, neither concentration of cAMP had any effect on catalase synthesis induced by ascorbate in LB medium (data not shown). To confirm that cAMP was entering the cell, the levels of β-galactosidase were assayed before and after the addition of isopropylthiogalactoside (IPTG)[3] to cultures growing in glycerol-salts and glucose-salts media with and without cAMP (Table II). The synthesis of β-galactosidase was inhibited only in the glucose culture and this was overcome by the addition of 1 mM cAMP.

For the expression of genes sensitive to catabolite repression in adenylate cyclase mutants, an external source of cAMP is required and if catalase gene expression were dependent upon cAMP, no induction should occur in a *cya* strain in the absence of cAMP. To the contrary, the same basal level, the same rate of synthesis, and the same fully induced level of catalase were observed in both a wild-type strain (MP180) and a *cya* strain (MP259) following induction with 2.8 mM ascorbate in LB medium (Fig. 5). No assayable cAMP was evident in LB medium, which represents a concentration of less than 2 nM. In order to confirm the genotype of the *cya* strain, β-galactosidase levels were assayed before and after induction with IPTG in both LB medium and glycerol-salts medium. The basal level of β-galactosidase was lower in the *cya* strain than in the wild-type strain and, in contrast to the wild-type strain, no induction occurred except in the presence of 1 mM cAMP (Table II).

DISCUSSION

Catabolite repression is not involved in the regulation of catalase gene expression

[3] Abbreviation used: IPTG, isopropylthiogalactoside.

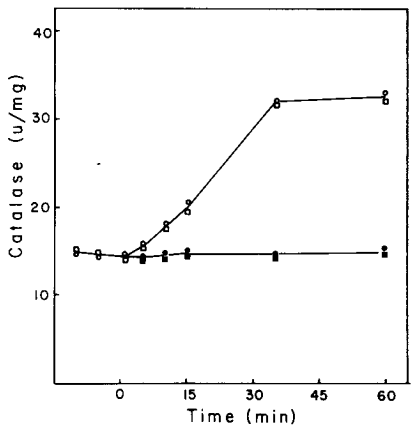

FIG. 4. Effect of cAMP in glucose-salts medium on the induction of catalase synthesis in *E. coli -B23*. H_2O_2 *(0.62 mM)* was added at time 0 to cultures of *E. coli* growing in minimal salts medium containing 16 mM glucose and either no cAMP (○) or 1 mM cAMP (□). No H_2O_2 was added to parallel cultures with no cAMP (●) or 1 mM cAMP (■). Aliquots were removed at various times and chilled on ice until assayed for catalase activity which is expressed as units/mg dry cell weight.

in *E. coli*. Supporting this conclusion are the following observations: (i) maximum induction of catalase synthesis required glucose in the medium, (ii) glucose added to media containing nonfermentable carbon sources did not reduce either the basal or induced levels of catalase, and (iii) cAMP, the cofactor for gene activation, played no role in the turn-on of catalase synthesis in either wild-type strains or adenylate cyclase mutants. This conclusion agrees with an earlier report which had shown that glucose added to a casein digest medium did not affect catalase activity even though the activities of other enzymes were reduced (6). A more recent report (2) disagreed with this conclusion, using as its basis the fact that cAMP speeded up the turn-on of catalase synthesis during the diauxie response, but this is subject to an alternate interpretation. Because catalase is normally induced during the diauxie response, its inducer, hydrogen peroxide or related

TABLE II

INDUCTION OF β-GALACTOSIDASE BY IPTG IN *E. coli* WITH AND WITHOUT 1 mM cAMP ADDED TO VARIOUS MEDIA

Strain	Medium	Time after IPTG addition (min)	β-Galactosidase activity (units/mg dry cell wt)	
			−cAMP	+cAMP
B23	Glycerol-salts	−2	25	24
		+5	153	149
		+20	329	366
B23	Glucose-salts	−2	20	18
		+5	32	138
		+20	38	322
MP180	Glycerol-salts	−2	32	39
		+5	189	172
		+20	374	376
MP259 (*cya*⁻)	Glycerol-salts	−2	20	41
		+5	12	178
		+20	13	381
MP180	LB medium	−2	117	112
		+5	368	292
		+20	651	654
MP259 (*cya*⁻)	LB medium	−2	49	109
		+5	46	295
		+20	38	667

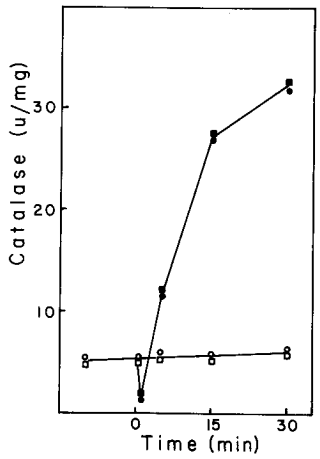

FIG. 5. Induction of catalase synthesis in cultures of wild-type and adenylate cyclase mutant strains of *E. coli*. Ascorbic acid (2.8 mM) was added at time 0 to a culture of MP180 (●) and a culture of MP259 (■) growing in LB medium. No ascorbate was added to parallel cultures of MP180 (○) and MP259 (□). Aliquots were removed at various times and chilled on ice until assayed for catalase activity which is expressed as units/mg dry cell weight.

peroxide, must be produced during secondary metabolism and a speed-up in the diauxie response by cAMP would result in a speed up in the production of the catalase inducer. Hence, the more rapid turn-on of catalase synthesis by cAMP could be an indirect rather than a direct effect.

The other observations of catabolite repression are also subject to alternate explanations. In *E. coli*, non-log phase cells were used (5). Therefore, the reported catalase levels would not necessarily represent levels during log phase growth and would be subject to metabolic changes occurring during entry to stationary phase. In yeast, high concentrations of succinate and citrate elicited the same effect on catalase as did the high concentration of glucose (3) casting doubt on the specificity of the observed inhibition. In *Bacteroides fragilis* (4), commencement of glucose metabolism could have caused a termination of other metabolic processes which were producing a peroxide inducer of catalase synthesis.

Despite the apparent noninvolvement of glucose, other metabolic changes clearly affect catalase synthesis in *E. coli*. An amino acid supplement caused a significant drop in basal catalase levels but facilitated protein synthesis such that greater catalase synthesis occurred after induction with H_2O_2 or ascorbate. Succinate metabolism resulted in slow growth and a corresponding slow induction of catalase even though basal levels of catalase were high. The concept of peroxide inducers of catalase synthesis being produced under some growth conditions and not under others may offer a better explanation for many of the observed changes in catalase activity.

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