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Catalases HPI and HPII in *Escherichia coli* are Induced Independently

PETER C. LOEWEN,[1] JACEK SWITALA, AND BARBARA L. TRIGGS-RAINE

Department of Microbiology, University of Manitoba, Winnipeg, Manitoba R3T 2N2, Canada

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Three strains of *Escherichia coli* differing only in the catalase locus mutated by transposon Tn10 were constructed. These strains produced only catalase HPI (*katE*::Tn10 and *katF*::Tn10 strains) or catalase HPII (*katG*::Tn10). HPI levels increased gradually about twofold during logarithmic growth but did not increase during growth into stationary phase in rich medium. HPII levels, which were initially threefold lower than HPI levels, did not change during logarithmic growth but did increase tenfold during growth into stationary phase. HPI levels increased in response to ascorbate or H_2O_2 being added to the medium but HPII levels did not. In minimal medium, any carbon source derived from the tricarboxylic acid cycle caused five- to tenfold higher HPII levels during logarithmic growth but had very little effect on HPI levels. Active electron transport did not affect either HPI or HPII levels.

Genetic mapping studies have confirmed the presence of two independent species of catalase in *Escherichia coli*. The loci *katE* (1) and *katF* (2) affect the synthesis of an apparently monofunctional catalase (or hydroperoxidase) HPII (2, 3), previously also named HPIII (3). The locus *katG* affects the synthesis of the bifunctional isoenzyme pair labeled HPI-A and -B (4) that possess both catalase and peroxidase activities (5).

Because it is not possible to accurately differentiate between HPI and HPII in a standard catalase assay, this multiplicity of enzymes has complicated the interpretation of data pertaining to changes in catalase levels that arose as a result of changes in metabolism. For example, it has been shown that catalase activity increases in response to H_2O_2 or ascorbate (6, 7, 8) and also during growth into stationary phase but a definition of whether the increase occurred in both catalases or just in one was not made. In order to clarify how the individual catalases responded to different growth conditions, strains that produced just one of the two catalases were required. This paper describes how such strains were used to identify significantly different responses of the two catalases to metabolic changes.

MATERIALS AND METHODS

Bacterial strains. The strains used in this work were all derived from HfrH strain MP180 (HrfH thi-1) (2). UM120 (as MP180 but *katE12*::Tn10) and UM122 (as MP180 but *katF13*::Tn10) were selected by the direct transposon mutagenesis of MP180 using λ561 (b221, CI857::Tn10, Oam29, Pam80) (9) and screening tetracycline-resistant colonies for catalase deficiency (2). The related *katG*::Tn10 mutant of MP180 was isolated in a different fashion. First a *katE katG*$^+$ derivative of UM1, UM178, was selected following recombination between KL14 and UM1 (4). This strain was then mutagenized using λ561 (9), and the resulting tetracycline-resisitant colonies were screened for catalase deficiency. A strain UM196 was isolated as *katG17*::Tn10 (4) and the Tn10 was transferred by P1 transduction (10) to MP180 to generate UM202 (as MP180 but *katG17*::Tn10).

Media. LB medium (10) contained 10 g tryptone (Difco Laboratories, Detroit, Mich.), 5 g yeast extract (Difco), and 10 g NaCl per liter. M9 minimal medium (10) contained 6 g Na_2HPO_4, 3 g KH_2PO_4, 0.5 g NaCl, and 1 g NH_4Cl per liter supplemented after autoclav-

[1] To whom correspondence should be addressed.

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ing with 10 μM $CaCl_2$, 1 mM $MgSO_4$, 3 μM vitamin B1, and various carbon sources as specified.

Tetracycline (15 μg/ml) was added as required. Solid media were prepared with 1.5% agar. Dilutions were made in SM buffer: 0.5 M Tris-hydrochloride (pH 7.6), 0.01 M $MgSO_4$, and 0.1% gelatin.

Visualization of catalase and peroxidase activities on polyacrylamide gels. Catalase and peroxidase activities were visualized by the method of Gregory and Fridovich (11) on 9.5% polyacrylamide gels which were run as described by Davis (12), except that Tris-HCl, pH 8.1, was used in place of Tris-HCl, pH 8.8. Staining was as described (11) except that 3 mM H_2O_2 was used for catalase.

Effect of ascorbate and growth phase on catalase. Cultures were grown at 37°C, in LB or supplemented minimal medium, on a shaker bed monitoring growth with a Klett-Summerson colorimeter with a blue filter [100 Klett units represented 0.14 mg/ml (dry cell weight) as determined by weighing culture samples after drying at 100°C and correcting for medium weight]. Anaerobic cultures were grown in sealed flasks which were flushed with nitrogen before use. Ascorbic acid was dissolved in water just before use and was added to the cultures at a cell density of 50 Klett units.

Enzyme assays. Catalase activity was determined by the method of Rorth and Jensen (13) on a Gilson oxygraph equipped with a Clark electrode. One unit of catalase was defined as the amount that decomposes 1 μmol of H_2O_2 in 1 min at 37°C. Citrate synthase was assayed by the method of Weitzman (14), and 1 unit was defined as the amount that catalyzes the formation of 1 μmol CoASH/min. Malate dehydrogenase was assayed by the method of Kitto (15) and 1 unit was defined as the amount required to reduce 1 μmol NAD^+/min.

RESULTS

Isolation of catalase mutants. A mutation in each of the three catalase loci so far identified was isolated individually in a wild-type HfrH strain, MP180. Following the direct insertion of Tn10 from λ561 into MP180, all tetracycline-resistant colonies were screened for catalase deficiency. As described (2), HPII contributes more to the apparent catalase levels on plates than the isoenzymes of HPI, and this made it possible to isolate strains deficient in HPII directly, even with HPI present. This resulted in the generation of UM120 (*katE*::Tn10) and UM122 (*katF*::Tn10) (2). The reciprocal selection of HPI-deficient strains in the presence of HPII was not possible, however, because of the high background of catalase generated by HPII. In order to create a *katG*::Tn10 mutant, a $katG^+$ recombinant of UM1 was selected using KL14 and characterized as UM178 containing only the isoenzymes of HPI (4). UM178 was then infected with λ561 and all tetracycline-resistant strains were scored for catalase activity, revealing catalase-deficient UM196, a *katG*::Tn10 mutant. This mutation was then transferred to the wild-type strain MP180 by P1 transduction, giving rise to UM202. The series of gels stained for either catalase or peroxidase shown in Fig. 1 confirmed that UM120 and UM122 produced only HPI while UM202 produced only HPII.

Changes in catalase during growth. An increase in catalase during growth of a wild-type strain of *E. coli* B into stationary phase had been observed (16), and this was confirmed using the K12 strain MP180 as shown in Fig. 2. Unfortunately, the species of catalase responsible for the changes, whether HPI or HPII or both, could not be identified. By using strains lacking one or the other catalase, it was possible to differentiate between changes in the two enzymes. The levels of HPI in the *katE*::Tn10 strain UM122 were similar to those observed in UM120 (data not shown). In contrast to these changes in HPI, the levels of HPII in UM202 remained very low during logarithmic growth. Approximately coincident with the slowdown in growth associated with stationary phase, the catalase levels commenced a very rapid increase to a level eight times higher than the basal levels. This increase essentially paralleled the increase in catalase observed in late logarithmic phase MP180. Clearly the increases in catalase levels in MP180 were a combination of the changes in both HPI and HPII with HPI rising slightly during logarithmic growth and HPII rising extensively during growth into stationary phase.

Induction of catalase synthesis by ascorbate. Ascorbate, which when oxidized yields H_2O_2, has been shown to be an effective inducer of catalase synthesis in *E. coli* (7). The species of catalase induced was tentatively identified as HPI but this was based on a qualitative assessment of catalase bands visualized following electro-

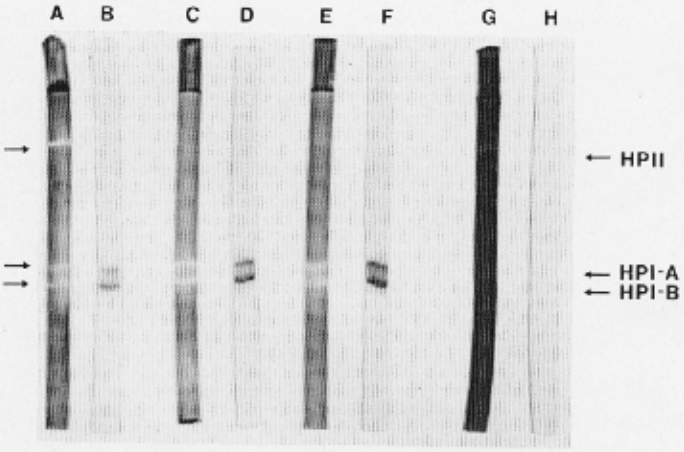

FIG. 1. Visualization of catalase and peroxidase in crude extracts of various strains after electrophoresis on native polyacrylamide gels. Lanes A, C, E, and G were stained for catalase activity, which is evident as a light band(s); and lanes B, D, F, and H were stained for peroxidase activity, which is evident as a dark band(s). The following samples were run: A and B from MP180, 540 μg protein; C and D from UM120, 535 μg protein; E and F from UM122, 545 μg protein; G and H from UM202, 560 μg protein. All extracts were prepared from overnight cultures.

phoresis on polyacrylamide gels (7). In order to confirm this observation, the same mutants that individually produced just one of the catalase species were employed. The addition of ascorbate to cultures of MP180 and UM120 caused a rapid increase in the levels of catalase (Fig. 3) confirming that HPI, the only catalase present in UM120, was induced by ascorbate or H_2O_2. HPI in UM122 responded similarly (data not shown). In contrast to this response, there was no increase in the levels of HPII when ascorbate was added to a culture of UM202 (Fig. 3). This result confirmed that only HPI responded to ascorbate or H_2O_2 induction.

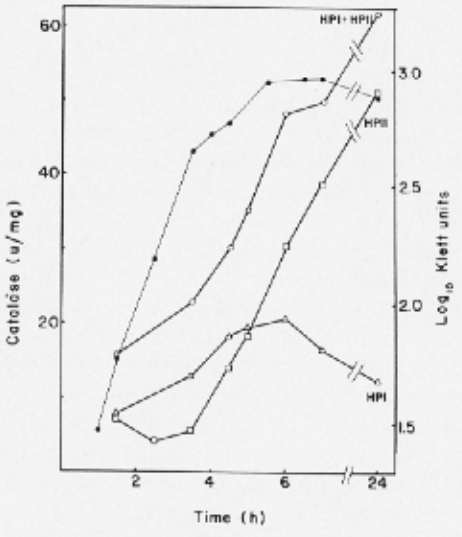

FIG. 2. Changes in catalase levels during growth in LB medium. Strains were grown with aeration on a shaker bed in LB medium and growth was monitored (●). Samples were removed at various times and assayed for catalase (○, HPI and HPII in MP180; △, HPI in UM120; □, HPII in UM202). Catalase is expressed as units per milligram dry cell weight.

Effect of growth medium on HPII levels. The induction of HPII has been tentatively correlated to the turn-on of synthesis of certain electron transport intermediates (16). That data and the data in Fig. 2 could also be explained as resulting from undefined metabolic changes caused by changes in carbon source during late logarithmic phase growth. In order to distinguish between these two possible explanations, UM202 was grown on various carbon sources some of which would support fermentative growth while others would require active electron transport (17) for growth (Table I). Glucose resulted in a low level of HPII in mid-log phase cultures, an observation made by other workers using

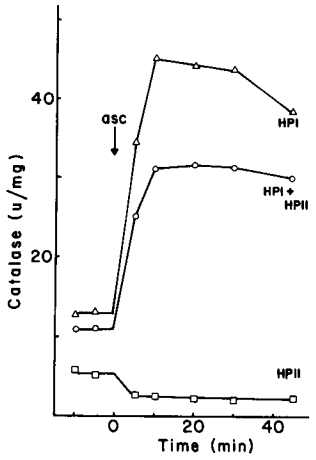

FIG. 3. Changes in catalase levels following the addition of 2.5 mM ascorbate to cultures growing in LB medium. Catalase was assayed in MP180 (○, HPI and HPII), UM120 (△, HPI), and UM202 (□, HPII). Catalase is expressed as units per milligram dry cell weight.

wild-type cells containing HPI and HPII. The presence of cAMP in the medium did not alter the level of HPII, and lactose, gluconate, and glycerol all resulted in similar low levels of HPII, confirming that a catabolite repression mechanism mediated by glucose transport and the cAMP-catabolite gene activator complex was not involved (18). Furthermore, the terminal electron acceptor for growth on glycerol, whether oxygen, nitrate, or fumarate, did not affect the levels of HPII in mid-log phase cells. Clearly, the presence of an active electron transport system was not sufficient to cause an increase in HPII levels.

Elevated levels of HPII were found in mid-log phase cells only when carbon sources from the tricarboxylic cycle were used (Table I). Fumarate, malate and succinate all resulted in HPII levels 5- to 10-fold higher than the basal levels observed in glucose grown cells. Acetate, citrate, and glutamate also resulted in higher levels of HPII but only 3- to 5-fold higher. Improved aeration did not affect HPII levels in mid-log cultures.

No consistent pattern was evident in the HPII levels in cells grown into stationary phase. Significantly, anaerobic growth with fumarate, but not nitrate as terminal electron acceptor, resulted in an increase in HPII levels during growth into stationary phase. This is another indication that the route of carbon metabolism is more important than the occurrence of electron transport in determining the levels of HPII.

Effect of growth medium on HPI levels. The levels of HPI varied over only a twofold range during growth on LB medium (Fig. 2). Consistent with this observation was the lack of any significant differences in HPI levels among mid-log cultures growing on glucose, lactose, or glycerol. Along with the absence of any response to cAMP, this confirmed the absence of catabolite repression in HPI synthesis. Even with tricarboxylic acid cycle carbon sources, HPI levels in mid-log phase cells were at most twofold higher than in cells growing on glucose medium. However, growth into

TABLE I

LEVELS OF HPI AND HPII IN MID-LOGARITHMIC AND STATIONARY PHASE CULTURES OF UM122 AND UM202 GROWN ON DIFFERENT CARBON SOURCES

	Catalase (u/mg dry cell weight)			
	UM202 (HPII)		UM122 (HPI)	
Carbon Source	ML[a]	SP[a]	ML	SP
Glucose	3.6	24.1	12.3	10.3
Glucose + cAMP	2.0	12.9	11.5	14.4
Gluconate	1.6	18.2	12.7	13.1
Lactose	1.2	12.5	11.7	9.6
Glycerol	3.5	5.9	14.3	13.2
Lactate	5.2	4.5	13.1	10.7
Acetate	8.9	11.7	ND[b]	ND[b]
Citrate	11.4	18.0	23.2	35.3
Glutamate	16.6	36.5	21.8	48.9
Succinate	18.6	59.7	16.7	49.3
Fumarate	30.5	66.3	18.0	48.6
Malate	26.7	47.9	18.1	64.6
Glucose-nitrate[c]	1.1	0.2	13.9	20.8
Glycerol-nitrate[c]	1.6	0.3	17.5	27.5
Glycerol-fumarate[c]	1.9	5.2	14.4	12.1
LB	1.9	44.9	12.8	41.8
LB + bubbled air	1.8	85.8	9.1	40.2

[a] ML, mid-log phase cells; SP, stationary phase cells.
[b] Not determined.
[c] Anaerobic growth.

TABLE II

LEVELS OF MALATE DEHYDROGENASE, CITRATE SYNTHASE, AND CATALASE (HPII) IN CRUDE EXTRACTS OF UM202, AND CATALASE (HPI) IN CRUDE EXTRACTS OF UM122[a]

Carbon source	Growth phase	Units/mg protein		Catalase	
		Malate dehydrogenase	Citrate synthase	HPII (UM202)	HPI (UM122)
Glucose	Mid-log	1.9	0.025	1.9	5.2
Glucose	Stationary	2.7	0.073	5.4	3.1
Succinate	Mid-log	4.3	0.16	28.2	6.4
Succinate	Stationary	3.2	0.13	22.2	17.6
Lactose	Mid-log	0.4	0.016	0.7	5.5
Lactose	Stationary	1.4	0.053	2.4	4.2
LB	Mid-log	0.7	0.076	3.6	1.9
LB	Stationary	3.7	0.150	22.2	2.2

[a] Levels of malate dehydrogenase and citrate synthase were also determined for UM122, and were found to be similar to those listed for UM202.

stationary phase on the tricarboxylic acid (TCA)[2] cycle carbon sources did promote a three- to fourfold increase in HPI levels. The levels of HPI in UM120, the katE mutant, were similar to those shown for UM122, the katF mutant, and are not shown.

Levels of the tricarboxylic acid cycle enzymes. In order to confirm that enzymes of the TCA cycle were induced during growth into stationary phase or during growth on TCA cycle intermediates, extracts from mid-log and stationary phase cells grown on glucose, lactose, succinate, or LB medium were prepared and assayed for citrate synthase, malate dehydrogenase, and catalase. The results are contained in Table II and reveal that the levels of the two TCA cycle enzymes are higher during mid-log growth on succinate. The levels of the three enzymes did not change uniformly however except in LB medium. The discrepancy between apparently comparable catalase activities in Tables I and II arose because activities were determined in whole cells for Table I (units per mg dry cell weight) and in cell extracts for Table II (units per mg protein).

DISCUSSION

The levels of the two main catalases in *E. coli*, HPI and HPII, exhibited different responses to metabolic changes. The bifunctional catalase-peroxidase HPI was induced by ascorbate or H_2O_2 added to the medium whereas HPII was not. Conversely, HPII levels were 5- to 10-fold higher during mid-log phase growth on TCA cycle intermediates as compared to non-TCA cycle intermediates whereas HPI levels were not. In a presumably related response, HPII levels increased significantly during growth into stationary phase on most carbon sources whereas HPI levels usually did not. Therefore, the synthesis of both enzymes could be induced, albeit under different conditions, contrary to an earlier report that suggested that only HPII was inducible (16).

The mechanisms involved in inducing the synthesis of the two enzymes remain unclear. The response of HPI to H_2O_2 is clearly a protective response and could involve H_2O_2 causing, directly or indirectly, either the activation of a positive regulator or the inactivation of a negative regulator. The increases in HPI observed during growth into stationary phase on some TCA cycle intermediates could also be the result of induction by H_2O_2 generated by enzymes involved in the metabolism of these compounds. The increases in HPII caused by growth on different carbon sources or by growth into stationary phase cannot be explained in terms of H_2O_2 induction because there was no direct effect of H_2O_2 on HPII

[2] Abbreviation used: TCA, tricarboxylic acid.

synthesis. Because changes in HPII levels occurred independent of whether or not active electron transport was occurring, it was concluded that HPII synthesis was not linked to the synthesis of electron transport intermediates contrary to an earlier suggestion (16). In fact the data in the earlier report could have been interpreted in terms of HPII synthesis being linked to some other metabolic change occurring during growth into stationary phase. For example, there is an apparent correlation between the enhanced synthesis of HPII and of TCA cycle enzymes brought on by growth on TCA cycle intermediates. Whether or not the two processes are directly linked by a common induction mechanism is unknown and is the target for further studies.

Recent results from a study of cellular response to metabolic stress have also suggested that the syntheses of HPI and HPII are regulated independently. HPI is controlled by a newly discovered global regulatory locus *oxyR* that is involved in defense against oxidative stress and in the synthesis of some heat shock proteins (19). In contrast, HPII does not respond to *oxyR*.

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