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Genetic Mapping of *katG*, a Locus That Affects Synthesis of the Bifunctional Catalase-Peroxidase Hydroperoxidase I in *Escherichia coli*

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A locus unlinked to either *katE* or *katF* that affected catalase levels in *Escherichia coli* was identified and localized between *metB* and *ppc* at 89.2 min on the genome. The locus was named *katG*. Mutations in *katG* which prevented the formation of both isoenzyme forms of the bifunctional catalase-peroxidase HPI were created both by nitrosoguanidine and by transposon Tn*10* insertions. All *katG*$^+$ recombinants and transductants contained both HPI isoenzymes. Despite the common feature of little or no catalase activity in four of the catalase-deficient strains, subtle differences in the phenotypes of each strain resulted from the different *katG* mutations. All three mutants caused by nitrosoguanidine produced a protein with little or no catalase activity but with the same subunit molecular weight and with similar antigenic properties to HPI, implying the presence of missense mutations rather than nonsense mutations in each strain. Indeed one mutant produced an HPI-like protein that retained peroxidase activity, whereas the HPI-like protein in a second mutant exhibited no catalase or peroxidase activity. The third mutant responded to ascorbate induction with the synthesis of near normal catalase levels, suggesting a regulatory defect. The Tn*10* insertion mutant produced no catalase and no protein that was antigenically similar to HPI.

Catalase activity in *Escherichia coli* was initially isolated and characterized in a bifunctional enzyme that also possessed peroxidase activity (3). Both enzymes use hydrogen peroxide as a substrate, but catalase uses a two-electron transfer in the dismutation of H_2O_2 to oxygen and water, whereas peroxidase uses a one-electron transfer to a hydroperoxide acceptor. Subsequently, two other monofunctional catalase species were identified, one, labeled hydroperoxidase II (HPII), with an electrophoretic mobility faster than HPI (4) and a second, labeled HPIII (23), with an electrophoretic mobility slower than HPI. Complicating these designations is the previous naming of what is now clearly an isoenzyme form of HPI as HPII (18, 23); consequently, in the future the isoenzyme forms of HPI will be called HPI-A and HPI-B.

A genetic analysis of catalase expression in *Escherichia coli* has been undertaken, and two classes of mutants that affect HPIII synthesis have been mapped and labeled *katE* (16) and *katF* (17). Because the synthesis of HPI-A and HPI-B was not affected by either of these loci, a search was initiated for another locus. This paper describes the mapping and partial characterization of *katG*, a locus that affected HPI synthesis.

MATERIALS AND METHODS

Bacterial strains. The strains used in this work and their characteristics are listed in Table 1. All genetic manipulations and strain constructions involving recombination and generalized phage-mediated transduction were carried out as described by Miller (19).

Media. LB medium (19) contained 10 g of tryptone (Difco Laboratories), 5 g of yeast extract (Difco), and 10 g of NaCl per liter. M9 minimal medium (19) contained 6 g of Na_2HPO_4, 3 g of KH_2PO_4, 0.5 g of NaCl, and 1 g of NH_4Cl per liter supplemented after autoclaving with 10 μM $CaCl_2$, 1 mM $MgSO_4$, 3 μM vitamin B_1, 0.16 mM L-amino acids as required, and glucose at 0.2% (wt/vol). Streptomycin (80 μg/ml), tetracycline (15 μg/ml), and kanamycin (25 μg/ml) were added as required. Solid media were prepared with 1.5% agar. Dilutions were made in SM buffer (0.05 M Tris-hydrochloride [pH 7.5], 0.01 M $MgSO_4$, 0.1% gelatin).

Transposon Tn*10* insertions. A *katG* catalase-deficient mutant of UM178 was selected after transposon Tn*10* insertion (12) with λ561 (*b*221 *c*I857::Tn*10* *O*am29 *P*am80) (8) as previously described (17).

Enzyme assays. Catalase activity was determined by the method of Rørth and Jensen (25) in a Gilson oxygraph equipped with a Clark electrode. One unit of catalase is defined as the amount that decomposes 1 μmol of H_2O_2 in 1 min at 37°C. Peroxidase activity was determined as previously described (26), and one unit is the amount of enzyme decomposing 1 μmol of peroxide per min at 25°C. Protein was estimated by the method described by Layne (14).

Screening of other genetic markers. *metA* and *metB* strains were scored by their requirement for 0.16 mM methionine. *argG* and *argH* strains were scored by their requirement for 0.16 mM arginine. *pfkA* strains were scored by their inability to grow on 0.2% mannitol (20). *ppc* strains were scored by their inability to grow on 0.2% glucose. *aceB* strains were scored by their poor growth on 0.2% acetate.

Visualization of catalase and peroxidase activity on polyacrylamide gels. Catalase and peroxidase activities were visualized by the method of Gregory and Fridovich (9) on 9.5% polyacrylamide gels as described by Davis (7) but with the separation gel prepared at pH 8.1 rather than pH 8.9. Staining was as previously described (9) except that 3 mM H_2O_2 was used for catalase. Similar gels were run in bicine-imidazole buffer (3), revealing that HPIII did migrate faster

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TABLE 1. *E. coli* K-12 strains used

Strain	Genotype	Source[a]
CSH7	*lacY rpsL thi-1*	C.S.H.C: (19)
CSH57a	*leuB6 proC83 purE42 trpE38 his-208 argG77 ilvA681 metA160 thi-1 ara-14 lacY1 galK2 xyl-5 mtl-1 azi-6 rpsL109 tonA23 tsx-67 supE44 malA38 xthA*	C.S.H.C. (19)
Ra2	*sup* Hfr	C.S.H.C. (19) CSH60
D7011	*trpR thi* HfrC	C.S.H.C. (19) CSH61
KL14	*thi* Hfr	C.S.H.C. (19) CSH64
KL16	*thi-1* Hfr	C.S.H.C. (19) CSH74
MP180	*thi-1* HfrH	M. L. Pearson (22)
JK84	*hisS glyA relA argH thi lacY* or *Z xyl mtl rpsL supE*	C.G.S.C.
SA53	*deoA upp udp metB argF relA* Hfr(R4)	C.G.S.C.
AM1	*pfkA relA tonA* T2^r *pit-10 spoT*	G.C.S.C.
DV21A05	*aceB6 ppc-2 glc-1 thi-1 relA1 lacZ43 spoT1*	C.G.S.C.
UM1	As CSH7 but *katE1 KatG14*	16; see text
UM2	As CSH57a but *KatE2 katG15*	16; see text
UM5	As UM1 but *his*	Nitrosoguanidine
UM56-64	As CSH57a but *katF3 katG16*	17, see text
UM178	As UM5 but *lac*$^+$ *katG*$^+$	CSH64 × UM5 → *lac*$^+$ *kat*$^+$
UM180	As UM56-64 but *lac*$^+$ *katG*$^+$	CSH64 × UM5 → *lac*$^+$ *kat*$^+$
UM181	As UM2 but *lac*$^+$ *katG*$^+$	CSH64 × UM2 → *lac*$^+$ *kat*$^+$
UM183	As UM2 but *arg*$^+$	CSH64 × UM2 → *arg*$^+$
UM185	As UM183 but *argH*	P1(JK84) × UM183 → *met*$^+$ *argH*
UM189	As UM185 but *metB*	P1(SA53) × UM185 → *arg*$^+$ *metB*
UM196	As UM178 but *katG17*::Tn*10*	UM178 × λ561 → Tet^r *kat*
UM197	As CSH57a but *katG17*::Tn*10*	P1(UM196) × CSH57a → Tet^r *katG*

[a] C.S.H.C., Cold Spring Harbor Collection; C.G.S.C., Coli Genetic Center (B. Bachmann, curator).

than the main band, HPI, but that HPI was not resolvable into its isoenzyme forms in that system.

Native-molecular-weight analysis. For native-molecular-weight analysis, the method of Hedrick and Smith (11) was used in which pure protein or extracts were electrophoresed as described by Davis (7) on a series of gels of various acrylamide concentrations from 6 to 14%. The R_f of the protein relative to bromphenol blue tracking dye was determined for each different-percentage gel. The retardation coefficient for each protein was determined from the slope of a graph of 100 log (100 R_f) against the polyacrylamide concentration. The logarithm of the retardation coefficient was then plotted against the logarithm of the protein molecular weight. A series of proteins of known molecular weights from 29,000 to 650,000 were obtained from Sigma Chemical Co. and run as standards. For the standards and for pure HPI, the gels were stained with Coomassie brilliant blue dye. For catalase and peroxidase in crude extracts, the gels were stained for those activities as described above, thereby allowing native-molecular-weight determination even in the crude state. In the case of HPI, whether the protein was pure did not affect the molecular weight.

Denatured-molecular-weight analysis. The protein samples were mixed with an equal volume of gel sample buffer containing 0.1 M sodium phosphate (pH 7.0), 1% sodium dodecyl sulfate, 0.14 M 2-mercapotethanol, and 7 M urea and boiled for 2 min. This solution was loaded on a discontinuous slab polyacrylamide gel composed of a 4% stacking gel at pH 6.8 and an 8% running gel at pH 8.8 with both gels containing 0.1% sodium dodecyl sulfate as described by Laemmli (13). The gels were stained with Coomasie brilliant blue. Molecular weight markers were obtained from Sigma.

Immunodiffusion and immunoprecipitate isolation and analysis. Cells from 2-liter cultures grown in LB medium were treated as if for catalase purification (17) up to analysis with the DEAE-Sephadex A50 column, which was eluted in one step with 0.5 M NaCl in 50 mM potassium phosphate (pH 7.0) rather than with a gradient. The fractions containing the main peak of eluted protein were pooled, concentrated with 80% ammonium sulfate, and dialyzed against 50 mM potassium phosphate (pH 7.0). This solution was subjected to double diffusion analysis, radial diffusion analysis, and immunoprecipitation. Double diffusion analysis was carried

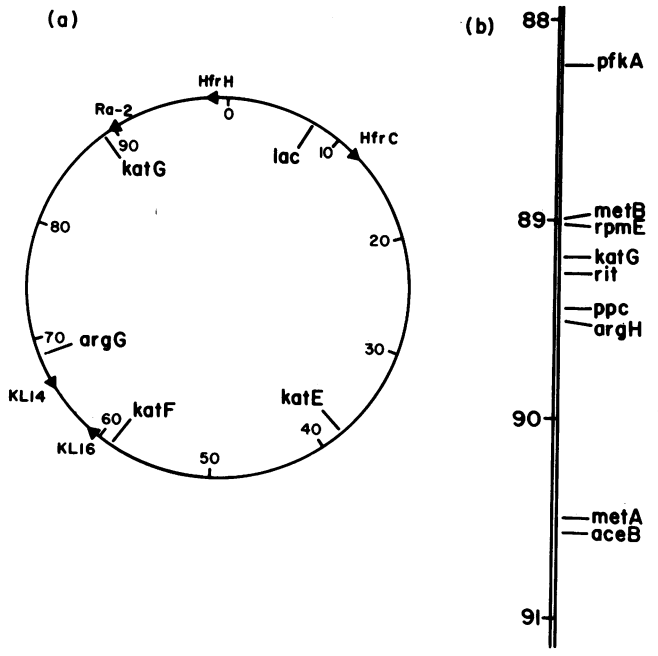

FIG. 1. (a) Genetic map of *E. coli* K-12 (1). The origins and directions of transfer from the Hfr strains used in this study are indicated by the arrows. The locations of *katE* (16), *katF* (17), and *katG* are indicated, as are the locations of *lac* and *argG* for reference. (b) Genetic map orienting *katG* relative to nearby genes in *E. coli* K-12. Gene locations (in minutes) are based on the 100-min *E. coli* map (1), with *metB* at 89.0 min as a reference point. The map distances were calculated by the equation of Wu (27) from the cotransduction frequencies in Tables 3 and 4.

TABLE 2. Appearance of *kat*$^+$ phenotype in *lac*$^+$ recombinants

Recipient	% *kat*$^+$ in *lac*$^+$ recombinants from:		
	HfrH	Ra2	KL14
UM1	0	0	42
UM2	0	0	21
UM56-64	0	0	58

out in plates containing 0.9% (wt/vol) NaCl and 1.0% (wt/vol) agar against an antiserum prepared with a purified mixture ofHPI (5). For radial diffusion analysis, the agar contained 0.25 ml of antiserum per 15 ml of agar. To isolate the immunoprecipitate, 0.1 ml of partially purified extract (from DEAE-Sephadex A50) was mixed with 0.05 ml of antiserum and incubated at room temperature for 2 h. The precipitate was collected by centrifugation, washed one time in SM buffer, and suspended in 0.02 ml of gel sample buffer which was then boiled for 2 min. This mixture was run on a discontinuous denaturing polyacrylamide gel.

Ascorbate effect on catalase and peroxidase. Cultures were grown at 37°C in LB medium on a shaker bed, and growth was monitored with a Klett-Summerson colorimeter with a blue filter (100 Klett units represented 0.14 mg [dry cell weight]/ml as determined by weighing culture samples after drying at 100°C and correcting for medium weight). Ascorbic acid was dissolved in water just before use and was added to cultures at a cell density of approximately 50 Klett units. The catalase activity could be measured directly in a 2-ml culture sample without having to lyse the cells. The peroxidase activity had to be measured in cell extracts which were prepared by suspending cells from 2 liters of LB medium in 2 ml of SM buffer and sonicating three times in 30-s pulses. After removal of the cell debris by centrifugation, the supernatant was assayed for peroxidase.

RESULTS

Identification of a second *kat* locus in *katE* and *katF* mutants. Both *katE* at 37.8 min and *katF* at 59.0 min were initially located by using time-of-entry data generated from

TABLE 3. Mapping of *kat-2* relative to adjacent genes by three-factor transductional crosses

Donor	Recipient	Selected marker	Unselected marker(s)		
			Class	No.	%
JK84 (*argH*)	UM183 (*kat metA*)	*met*$^+$	*argH kat*$^+$	1	1
			argH kat	9	5
			arg$^+$ *kat*$^+$	3	2
			arg$^+$ *kat*	167	92
SA53 (*metB*)	UM185 (*kat argH*)	*arg*$^+$	*metB kat*$^+$	106	19
			metB kat	7	1
			met$^+$ *kat*$^+$	106	19
			met$^+$ *kat*	351	61
CSH57a (*metA*)	UM185 (*kat argH*)	*arg*$^+$	*metA kat*$^+$	0	0
			metA kat	7	3
			met$^+$ *kat*$^+$	115	48
			met$^+$ *kat*	118	49
AM1 (*pfkA*)	UM185 (*kat argH*)	*arg*$^+$	*pfkA kat*$^+$	23	10
			pfkA kat	0	0
			pfkA$^+$ *kat*$^+$	90	37
			pfkA$^+$ *kat*	127	53
DV21A05 (*ppc*)	UM185 (*kat argH*)	*arg*$^+$	*ppc kat*$^+$	119	50
			ppc kat	52	21
			ppc$^+$ *kat*$^+$	14	6
			ppc$^+$ *kat*	65	23
JK84 (*argH*)	UM189 (*metB kat*)	*met*$^+$	*argH kat*$^+$	187	31
			argH kat	11	2
			arg$^+$ *kat*$^+$	332	55
			arg$^+$ *kat*	70	12
DV21A05 (*ppc*)	UM189 (*metB kat*)	*met*$^+$	*ppc kat*$^+$	38	16
			ppc kat	2	1
			ppc$^+$ *kat*$^+$	149	62
			ppc$^+$ *kat*	51	21
DV21A05 (*aceB*)	UM189 (*metB kat*)	*met*$^+$	*aceB kat*$^+$	6	3
			aceB kat	0	0
			aceB$^+$ *kat*$^+$	182	76
			aceB$^+$ *kat*	52	21

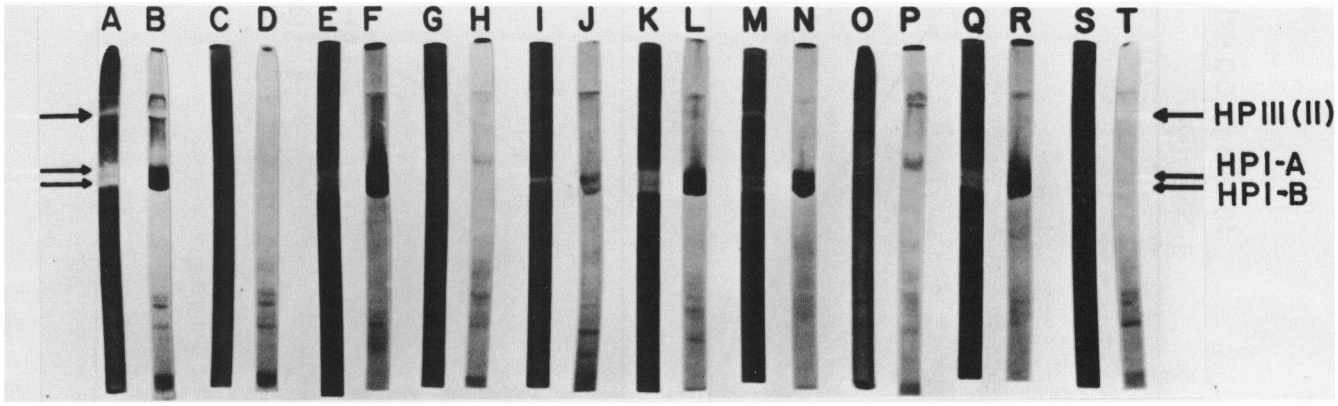

FIG. 2. Visualization of catalase and peroxidase in crude extracts of various strains after electrophoresis on native polyacrylamide gels. Lanes A, C, E, G, I, K, M, O, Q, and S were stained for catalase activity, which is evident as a light band(s), and Lanes B, D, F, H, J, L, N, P, R, and T were stained for peroxidase activity, which is evident as a dark band(s). The following samples were run: A and B, from CSH57a, 1.45 mg of protein; C and D, from UM2, 1.35 mg of protein; E and F, from UM181, 1.55 mg of protein; G and H, from UM56-64, 0.93 mg of protein; I and J, from UM56-64, 20 min after ascorbate addition, 0.89 mg of protein; K and L, from UM180, 0.72 mg of protein; M and N, from CSH7, 1.08 mg of protein; O and P, from UM1, 1.23 mg of protein; Q and R, from UM178, 1.93 mg of protein; S and T, from UM196, 1.08 mg of protein. All extracts were prepared from overnight cultures except those from UM56-64 which were prepared from mid-log-phase cells without (G and H) or with (I and J) 2.5 mM ascorbate added.

TABLE 4. Mapping of *kat17*::Tn*10* relative to adjacent genes by P1 phage transductional crosses

Donor	Recipient	Selected marker	Unselected marker(s)		
			Class	No.	%
UM196 (*kat17*::Tn*10*)	SA53 (*metB*)	Tet^r	*met*$^+$	188	78
			metB	52	22
UM196 (*kat17*::Tn*10*)	JK84 (*argH*)	Tet^r	*arg*$^+$	67	28
			argH	170	72
UM196 (*kat17*::Tn*10*)	JK84 (*argH*)	*arg*$^+$	Tet^r	107	45
			Tet^s	133	55
UM196 (*kat17*::Tn*10*)	DV21A05 (*ppC*)	Tet^r	*ppc*$^+$	155	65
			ppc	85	35
UM196 (*kat17*::Tn*10*)	DV21A05 (*ppc*)	*ppc*$^+$	Tet^r	173	72
			Tet^s	67	28

HfrH (origin at 97 min [Fig. 1a]) (2) and KL16 (origin at 62 min), which prevented screening of the chromosome segment between 62 and 97 min for loci affecting catalase expression. This latter screening was accomplished by using the additional Hfr strains Ra2 (origin at 89 min) and KL14 (origin at 67 min) and interrupting the conjugations with recipients UM1, UM2, and UM56-64 before either *katE* or *katF* could be introduced. When *lac*$^+$ recombinants were selected and scored for catalase, only recombinants produced by KL14 in all three recipients exhibited catalase activity (Table 2). The use of HfrC (origin at 13 min) as a donor caused *kat*$^+$ activity to appear in a significant number of *argG*$^+$ recombinants but not *met*$^+$, *leu*$^+$, or *lac*$^+$ recombinants. These data located a *kat* locus in all three mutant strains between 67 and 89 min, a location that was unlinked to either *katE* or *katF*.

The catalase mutants UM2 and UM56-64 contained the markers *argG*, *malA*, *xyl*, *mtl*, *ile*, and *metA* or *metB* in or near the 67 to 90 min region (1), and when positive phenotypes of each of these markers were selected after P1 transduction, the *kat*$^+$ phenotype was found only in *met*$^+$ strains and at a frequency of 17%. Because the identity of the *met-160* marker in these strains was unknown, being either *metA* or *metB*, *met*$^+$ strains containing the *argH* marker were constructed. P1 transductions of these strains were then carried out incorporating other markers in this region including *pfkA*, *metB*, *ppc*, and *metA*. For strain UM2, the results contained in Table 3 reveal the order *pfkA-metB-kat-ppc-argH-metA*. They also establish that the *met-160* locus is *metA160*. The analogous data obtained for UM1 and UM56-64 were consistent with the same map location of the new catalase locus and are not shown. Because this new locus is in a location quite distinct from *katE* and *katF*, we have named it *katG* (Fig. 1b). Furthermore, each of the three original catalase-deficient mutants are in fact double mutants for catalase expression as follows: UM1 is *katE1 katG14*; UM2 is *katE2 katG15*; UM56-64 is *katF3 katG16*.

The catalase species present in UM181, a *katG*$^+$ recombinant of UM2, were identified on polyacrylamide gels as being the bifunctional isoenzymes HPI-A and HPI-B (17).

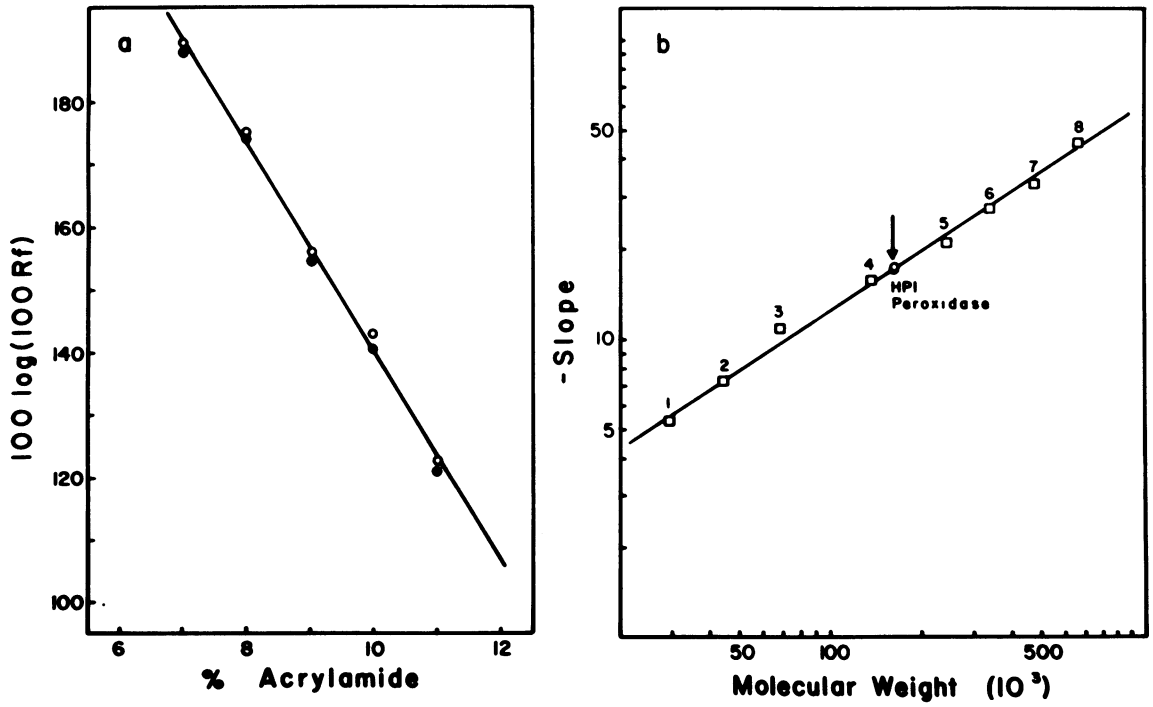

FIG. 3. (a) Effect of different acrylamide concentrations on the mobility of purified HPI (●) and of peroxidase from UM1 (○). (b) Determination of the molecular weight of HPI (●) and peroxidase from UM1 (○) from the slope determined in (a). The proteins (□) used as molecular weight standards from Sigma were as follows: 1, carbonic anhydrase, 29,000; 2, egg albumin, 45,000; 3, bovine serum albumin (monomer), 66,000; 4, bovine serum albumin (dimer), 132,000; 5, urease (dimer), 240,000; 6, thyroglobulin (dimer), 335,000; 7, urease (tetramer), 480,000; and 8, thyroglobulin (tetramer), 669,000.

The same species were also found in *katG*$^+$ recombinants of UM1 (UM178) and UM56-64 (UM180) (Fig. 2).

Transposon insertions in *katG*. Because of the presence of two independent catalase species in *Escherichia coli*, the identification of catalase mutants resulting from a single transposon insertion event was difficult. However, HPIII contributed slightly more than HPI to the evolution of oxygen in the plate assay, and the absence of HPIII was accompanied by an observable decrease in O_2 evolution which allowed the isolation of *katE12*::Tn*10* and *katF13*::Tn*10*. Unfortunately, the reverse selection of a strain lacking HPI in the presence of active HPIII was not possible. Therefore, a strain (UM178) lacking HPIII but containing the HPI isoenzymes was isolated and subjected to transposon mutagenesis selecting for mutants deficient in catalase. One such mutant was isolated (UM196) and characterized as having no assayable catalase or peroxidase activity in either the oxygraph assay or on gels. P1 transduction mapping experiments summarized in Table 4 confirmed that *kat-17*::Tn*10* was located in the order *metB-kat-17*::Tn*10-ppc-argH*, and it was therefore named *katG17*::Tn*10*.

Phenotypic differences among *katG* mutants. All three original catalase-deficient mutants, UM1, UM2, and UM56-64, were initially isolated because of the common phenotypic characteristic, low catalase levels. Upon further study, as follows, we found that each strain had a unique phenotype resulting from the different *katG* mutations. (i) Visualization of catalase and peroxidase activities after electrophoresis of cell extracts confirmed the absence of catalase in UM1, UM2, and UM56-64 but revealed a band of peroxidase activity in UM1 extracts. The peroxidase band had the same electrophoretic mobility (Fig. 2) and the same molecular weight of 165,000 ± 6,000 (Fig. 3) as HPI-A and -B. This molecular weight for HPI suggested a dimer structure, whereas the initial characterization of HPI had shown it to be a tetramer (3). This discrepancy could be explained in terms of aggregation which was dependent on the solvent and the analytical technique. In the presence of the chaotropic reagent Tris-glycine, the molecular weight of HPI was observed to be 165,000 ± 6,000 on gels or 340,000 on Sepharose 6B gel filtration. In the presence of phosphate buffer the molecular weight was observed to be 340,000 during centrifugation (3) and greater than 900,000 on Sepharose 6B gel filtration. The dimer form with a molecular weight of 165,000 was simply the smallest active species found. (ii) Immunodiffusion of partially purified cell extracts against an antiserum prepared with pure HPI-A and -B revealed that there was cross-reacting material in UM1, UM2, and UM56-64, all of which had been isolated after nitrosoguanidine mutagenesis. In contrast, no cross-reacting material was present in extracts of strain UM196 containing *katG17*::Tn*10* (Fig. 4a). Isolation of the immunoprecipitate and electrophoresis on sodium dodecyl sulfate-polyacrylamide gels revealed a predominant protein with a molecular weight of 84,000 in addition to the immunoglobulin bands in precipitates from CSH57a, UM1, UM2, and UM56-64 (Fig. 5). Only the precipitate from UM2 differed slightly in that there appeared to be two separate proteins resolved. Quantitation of the cross-reacting material in the extracts from stationary-phase cells by radial immunodiffusion revealed 67.8, 50.2, and 65.1% as much cross-reactivity per mg of protein in extracts of UM1, UM2, and UM56-64, respectively, as in extracts of CSH57a (Fig. 4b). The presence of inactive or partially active protein in all three mutants is most easily explained in terms of missense mutations. (iii) Each of the nitrosoguanidine-induced mutants responded differently to ascorbate, which in the parent strain, CSH57a, caused a three- to fourfold induction of catalase and peroxidase (Table 5). In strain UM1, ascorbate caused a threefold increase in peroxidase levels, indicating that the mutation that affected enzyme activity had not affected its inducibility. Strain UM2 differed in that only a small increase in the amount of immunoprecipitable protein was observed after ascorbate treatment (Fig. 4b), suggesting the presence of a second lesion affecting inducibility of HPI in addition to the one affecting enzyme activity. The two lesions were closely linked because the *katG*$^+$ recombinants of UM2 were normally inducible for catalase. Strain UM56-64 differed by responding to ascorbate with a 25-fold increase in HPI-A and -B (Fig. 2; Table 5) although the final induced level of catalase was still slightly lower than the induced level in CSH57a. This suggested the presence of two separate mutations as well, one that reduced the specific activity of the enzyme and a second that enhanced the level of catalase induction. Strain UM196 with the *katG*::Tn*10* insertion produced no catalase and did not respond to ascorbate.

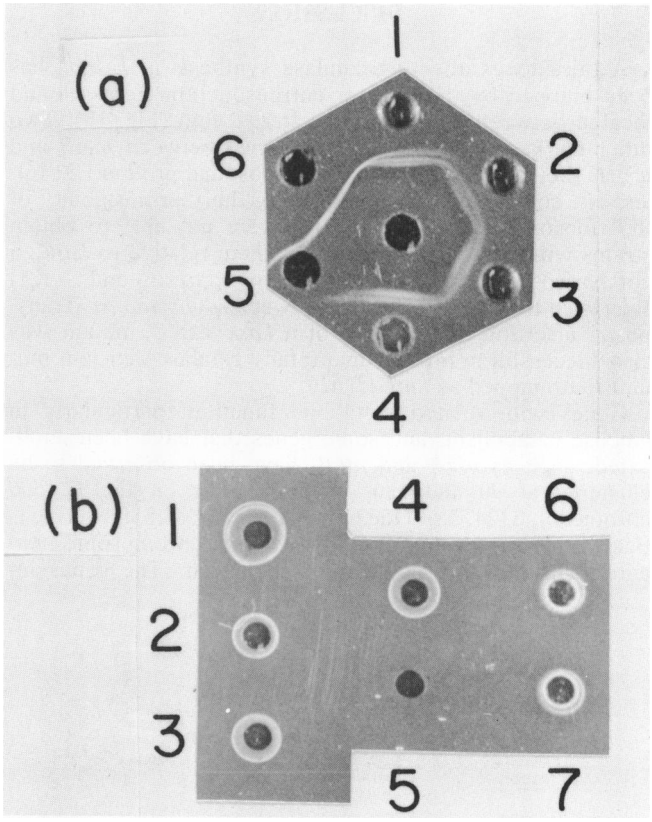

FIG. 4. (a) Double immunodiffusion analysis of various fractions eluted from DEAE-Sephadex A50. All cultures were isolated after growth into stationary phase. The center well contained antiserum prepared against a purified mixture of HPI-A and HPI-B. The following samples, by well number, were analyzed: 1, 269 μg of protein from CSH57a; 2, 270 μg of protein from UM1; 3, 275 μg of protein from UM2; 4, 260 μg of protein from UM56-64; 5, 266 μg of protein from UM196; and 6, 20 μg of purified HPI. (b) Radial immunodiffusion analysis of various fractions eluted from DEAE-Sephadex A50. The following samples, by well number, were analyzed: 1–5, as described in (a); 6, 526 μg of protein from UM2 isolated in mid-log phase; 7, 576 μg of protein from UM2 isolated 30 min after the addition of 2.5 mM ascorbate to a mid-log-phase culture.

DISCUSSION

A third locus affecting catalase synthesis in *E. coli* has been mapped by three-factor cotransductional crosses and located between *metB* and *ppc* at 89.2 min (Fig. 1b). Two other markers have also been mapped between *metB* and *argH*; they are *rpmE*, coding for ribosomal protein L31 (6), and *rit*, coding for a factor affecting the thermostability of the ribosome (21). Although we were not able to obtain strains with these markers to map them relative to *katG*, a comparison of our data with those for *rpmE* (6) and *rit* (21) suggested that *katG* might fall between *rpmE* and *rit*. Transposon insertion mutagenesis of a *katE katG*$^+$ mutant was also successful in producing a totally catalase-deficient mutant that mapped as *katG*::Tn*10*.

Genes with related metabolic function, particularly in glucose catabolism, and some genes that have been duplicated, such as *pfkA* and *pfkB*, have been observed to be clustered at 90 and 180° to each other on the *E. coli* chromosome (24, 28). One explanation for such clustering is that during the evolution of the existing chromosome, two entire chromosome duplications occurred. The genes involved in catalase synthesis also fall into this category, with *katE* and *katG* being located 50.4 min or 180° apart on the chromosome. An extension of this observation is that *katF* at 59 min may have a counterpart around 9 min and, in fact, *katC* in *Salmonella typhimurium* was located at 7 min (1, 15), although a mutant in this locus has not yet been isolated in *E. coli*. Indeed, it is surprising that not one of the three catalase loci so far mapped in *E. coli*, *katEFG*, corresponds to any of four loci, *katABCD*, that were identified and mapped near 7 min and 74 min on the *Salmonella typhimurium* genome.

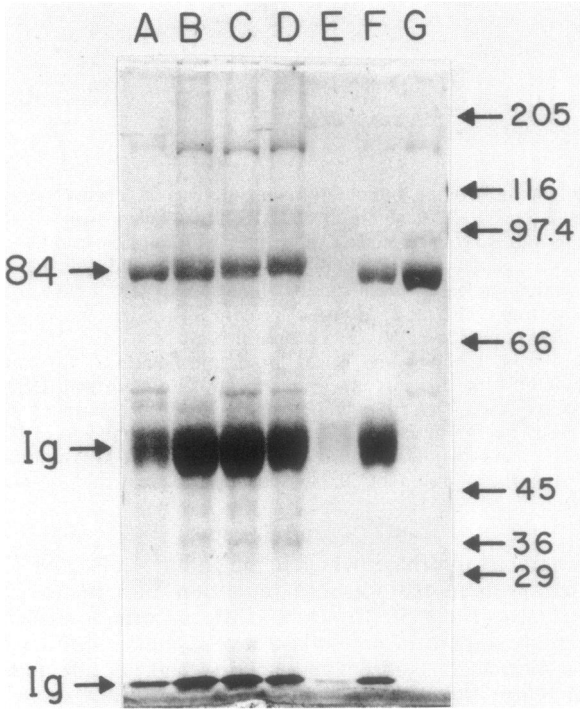

FIG. 5. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of immunoprecipitates formed between the HPI antiserum and various fractions eluted from DEAE-Sephadex A50. Precipitates from the following amounts of protein were electrophoresed: A, 0.42 mg of protein from CSH57a; B, 1.35 mg of protein from UM1; C, 1.53 mg of protein from UM2; D, 1.22 mg of protein from UM56-64; E, 1.33 mg of protein from UM196; F, 24 μg of purified HPI; G, 12 μg of purified HPI as a control without antiserum precipitation. The numbers on the right side indicate, in descending order, the location of the following molecular weight standards; myosin, 205,000; β-galactosidase, 116,000; phosphorylase B, 97,400; bovine plasma albumin, 66,000; egg albumin, 45,000; glyceraldehyde-3-phosphate dehydrogenase, 36,000; carbonic anhydrase, 29,000. On the left side, 84 indicates the location of the protein of 84,000 molecular weight that is characteristic of the subunit size of HPI. Ig, Locations of the two main immunoglobulin bands.

TABLE 5. Catalase and peroxidase activities in various strains before and 20 min after ascorbate addition[a]

Strain	Catalase activity (μ/mg [dry cell wt]		Peroxidase activity (μ/mg of protein)	
	Asc$^-$	Asc$^+$	Asc$^-$	Asc$^+$
CSH57A	2.76	9.65	1.54×10^{-4}	4.61×10^{-4}
UM1	ND[b]	ND	0.33×10^{-4}	2.43×10^{-4}
UM2	ND	ND	NA[c]	NA
UM56-64	0.35	7.59	NA	NA
UM196	ND	ND	NA	NA

[a] Asc$^-$, Before ascorbate addition; Asc$^+$, 20 min after ascorbate addition.
[b] ND, Not detectable.
[c] NA, Not assayed.

A comparison of the four quite different phenotypes in the four *katG* mutants, UM1, UM2, UM56-64, and UM196, suggested that *katG* is the structural gene for the two isoenzymes of HPI. All of the catalase mutants created by nitrosoguanidine that mapped at *katG* still produced a protein that had a subunit molecular weight of 84,000 and that was antigenically similar to HPI-A and -B. This HPI-like protein from UM2 possessed no assayable catalase activity and no assayable peroxidase activity. The same protein from UM1 retained nearly normal levels of peroxidase activity but not assayable catalase. Finally, UM56-64 produced slightly reduced levels of HPI-like protein with a lower than normal specific activity. In all three mutants, a missense mutation was most likely responsible for the modified or reduced enzymatic activity present in the full-length protein. The response of these HPI-like proteins to ascorbate suggested the presence of a further mutation affecting the regulation of HPI synthesis that was responsible for reduced induction in UM2 and enhanced induction in UM56-64. The Tn*10* insertion in UM196 prevented the formation of any HPI-like protein, and ascorbate had no effect. Clearly the transposon was inserted early in the coding sequence to prevent the formation of immunoprecipitable protein.

The nomenclature of the various hydroperoxidases in *E. coli* has required clarification of the confusion arising from the use of two different buffer systems for electrophoretic separation of the enzymes. Initially, HPII was reported to migrate faster than HPI on gels run in Tris-glycine buffer (10), and this made HPII different from HPIII(17, 23) which migrated slower from HPII in the same buffer system. In addition, there was no mention of isoenzyme forms of HPI (10), resulting in the fastest migrating catalase being mistakenly labeled HPII (23). It is now clear, however, that there are two isoenzyme forms of HPI, and we have named them HPI-A and HPI-B. Furthermore, electrophoresis of HPIII on gels run in bicine-imidazole buffer revealed that the monofunctional HPIII migrated faster than the single band of bifunctional HPI, making HPIII appear to be very similar to HPII in that system. Consequently, the catalase-labeled

HPIII, which is affected by *katE* and *katF*, has been labeled HPIII (HPII) in Fig. 2 pending the purification and characterization of HPIII to confirm its identity as HPII.

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