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Genetic Mapping of *katF*, a Locus That with *katE* Affects the Synthesis of a Second Catalase Species in *Escherichia coli*

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A class of catalase-deficient mutants that was unlinked to *katE* was localized between *mutS* and *cys* at 59.0 min on the *Escherichia coli* genome. This locus was named *katF*. Transposon Tn*10* insertions were isolated that mapped in both *katE* and *katF* loci. The catalase species present in *katE*$^+$ and *katF*$^+$ recombinants was found to be different from the main catalase activities, HPI and HPII, in several respects. It did not have an associated peroxidase activity; it was electrophoretically slower on native polyacrylamide gels; it eluted from DEAE-Sephadex A50 at a higher salt concentration; its K_m for H_2O_2 was 30.9 mM as compared with 3.7 mM for HPI and HPII; its synthesis was not induced by ascorbate; and it did not cross react with HPI-HPII antisera. This new catalase was labeled HPIII.

In *Escherichia coli* cells, catalase activity, which employs a two-electron transfer in the dismutation of hydrogen peroxide to oxyen and water, and peroxidase activity, which employs a one-electron transfer to hydroperoxide acceptor, reside in a bifunctional enzyme complex. Two such hydroperoxidases, HPI and HPII, have been separated electrophoretically (4, 12), and it has been suggested that HPII is derived from HPI (4). Recently it was shown that both HPI and HPII are encoded by a single hybrid ColE1 *E. coli* plasmid (20). The main hydroperoxidase HPI has been purified from *E. coli* B as a tetramer with a molecular weight of 337,000 containing two protoheme groups (4). A third electrophoretically slower catalase has been separated from HPI and HPII in crude extracts of *E. coli* K-12 (24), and it differed significantly from HPI-HPII in that it did not contain an associated peroxidase activity and was not encoded by the same plasmid (20).

In a first attempt to define the genetics of catalase in *E. coli*, a number of catalase-deficient mutants were isolated, and one class of these mutants named *katE* mapped at 37.8 min between *pfkB* and *xthA* (19). A second class of catalase mutants unlinked to *katE* was also isolated but was not mapped, and the function of the *katE* gene product was not defined. This paper describes the mapping of the locus affecting catalase synthesis in the second class of catalase mutants. This newly defined locus is shown to be involved, along with *katE*, in the synthesis of the third electrophoretically slowest catalase which is labeled HPIII.

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 (21).

Media. LB medium (21) contained 10 g of tryptone (Difco Laboratories, Detroit, Mich.), 5 g of yeast extract (Difco), and 10 g of NaCl per liter. M9 minimal medium (21) 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 B1, 0.16 mM L-amino acids, thymine and uracil as required, and glucose at 0.2% (wt/vol). Streptomycin (80 μg/ml) and tetracycline (15 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 $MgSO_4$, and 0.1% gelatin.

Transposon Tn*10* insertions. Catalase-deficient strains of MP180 were selected after transposon Tn*10* insertion (15) with λ561 (b221, C1857::Tn*10*, Oam29, Pam80) (9). Tetracycline-resistant colonies resulting from the insertion were picked onto LB plates and screened for catalase by applying a drop of 30% H_2O_2 with a syringe to the edge of each colony. Catalase-deficient strains failed to evolve bubbles of oxygen and were immediately streaked on a new LB plate.

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 was defined as the amount that decomposes 1 μmol of H_2O_2 in 1 min at 37°C. Peroxidase activity was assayed as described in the *Worthington Enzyme Manual* (30), and 1 U is the amount of enzyme decomposing 1 μmol of peroxide per min at 25°C. Protein was estimated by the method of Layne (17).

Screening of other genetic markers. *pyrG* strains were scored by their requirement for 25 μg of cytidine per ml. *cysH* strains were scored by their requirement for 50 μg of cysteine per ml. *mutS* strains were scored by selecting for the appearance of a high frequency of spontaneously streptomycin-resistant colonies in 0.1-ml samples of 2-ml overnight cultures grown in LB medium (6).

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 (7). Staining was as described before (11), except that 3 mM H_2O_2 was used for catalase.

Analysis of catalase on DEAE-Sephadex A50. The procedure followed is essentially that described by Claiborne and Fridovich (4), with some modifications. Cultures were grown to late logarithmic phase, collected by centrifugation, and frozen until use. Frozen cells, 15 to 25 g, were thawed and suspended in 80 ml of 50 mM potassium phosphate (pH 7.0) by stirring for 1 h. The cell suspension was passed through an Aminco French press at 20,000 lb/in^2. After centrifugation to remove cell debris, streptomycin sulfate was added to a final concentration of 2.5%. After stirring for 1 h, the precipitate was removed by centrifugation, and the

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

Strain	Genotype	Reference or source
CSH57A	*leuB6 proC83 purE42 trpE28 his-208 argG77 ilvA681 met-160 thi-1 ara-14 lacY1 galK2 xyl-5 mtl-1 azi-6 rpsL109 tonA23 tsx-67 supE44 malA38 xthA*	C.S.H.C.[a] (21)
CSH7	*lacY rpsL thi-1*	C.S.H.C. (21)
UMI	As CSH7 but *katE1 kat-1*[b]	Nitrosoguanidine (19)
UM2	As CSH57A but *katE2 katG2*	Nitosoguanidine (19)
UM4	As UM2 but *his*$^+$ *katE*$^+$	CSH74 × UM2 → *his*$^+$ *katE*$^+$
UM56-64	As CSH57A but *katF3 kat-3*[b]	Nitrosoguanidine
UM62	As CSH57A but *katF4*	Nitrosoguanidine
UM85	As CSH57A but *katF10*	Nitrosoguanidine
UM167	As UM56-64 but *his*$^+$ *katF*$^+$	CSH74 × UM56-64 → *his*$^+$ *katF*$^+$
UM181	As UM2 but *lac*$^+$ *katG*$^+$	CSH64 × UM2 → *lac*$^+$ *katG*$^+$
CSH60 (Ra-2)	Hfr *sup*	C.S.H.C.
CSH64 (KL14)	Hfr *thi-1*	C.S.H.C.
CSH74 (KL16)	Hfr *thi-1*	C.S.H.C.
MP180	*thi-1* HfrH	M. L. Pearson (23)
UM120	As MP180 but *katE12*::Tn*10*	MP180 × λ561
UM122	As MP180 but *katF13*::Tn*10*	MP180 × λ561
NP37	*pheS5 relA1 tonA22 T2*^{r} *pit-10 spoT1* Hfr	C.G.S.C.[c]
ES455	*mutS3 argA21 lysA22 thi-1 mtl-2 xyl-7 tonA21 tsx-1 supE44*	C.G.S.C.
JM1703	*cysI*::Tn*10 fda*(Ts) Δ(*his-gnd*)	M. C. Jones-Mortimer
PB11	*uvrA recA srl*::Tn*10*	M. C. Jones-Mortimer (3)
UM156	As UM56-64 but *cysI*::Tn*10*	Pl(JM1703) × UM56-64 → Tet^r *kat*
UM162	As UM56-64 but *srl*::Tn*10*	Pl(PB11) × UM56-64 → Tet^r *kat*
UM84	As UM56-64 but *thyA*	UM56-64 · aminopterin
JM96	*cysH thr leu trp his argH thi lac xyl gal mal rpsL katF*	M. C. Jones-Mortimer (13, 14)
JF627	*thi-1 pyrE60 pyrG cdd relA1 argE3 his-4 proA2 thr-1 leu-6 mtl-1 xyl-5 ara-14 galK2 lacY1 rpsL31 supE44*	J. D. Friesen (10)

[a] C.S.H.C., Cold Spring Harbor Collection.
[b] The second locus in these strains maps between 67.5 and 89.5 min, which is similar to *katG2* in UM2.
[c] C.G.S.C., Coli Genetic Stock Center (B. Bachmann, Curator).

supernatant was dialyzed overnight against 4 liters of 50 mM potassium phosphate (pH 7.0). After dialysis, the solution was clarified by centrifugation and brought to 25% of saturation with solid $(NH_4)_2SO_4$. After being stirred for 1 h, the precipitate was collected by centrifugation, and the supernatant was brought to 40% of saturation with $(NH_4)_2SO_4$. After being stirred for 1 h, the precipitate was collected by centrifugation, and the supernatant brought to 55% saturation with $(NH_4)_2SO_4$. After being stirred for 1 h, the precipitate was collected by centrifugation. All three pellets were suspended in a 1/10 volume of 50 mM potassium phosphate (pH 7.0) and dialyzed overnight against 4 liters of the same buffer. The resulting solutions were assayed for catalase, revealing usually 80% of the catalase in the 25 to 40% pellet and the remainder in the 40 to 55% pellet. Both solutions were pooled, diluted twofold, and charged onto a column of DEAE-Sephadex A50 (2.5 by 60 cm). The column was washed with 500 ml of 50 mM potassium phosphate (pH 7.0), and a 3-liter gradient from 0 to 0.5 M sodium chloride in the same buffer was applied. Fractions of 15 ml were collected and assayed for catalase, peroxidase, and total protein. The subsequent steps (4) of chromatography on agarose (Bio-Gel A-1.5m) and hydroxylapatite (Bio-Gel HTP) were carried out to prepare pure HPI and HPII. These two isoenzymes could not be separated, but sodium dodecyl sulfate-polyacrylamide gels revealed only a single band of 84,000 molecular weight when 25 μg of protein was loaded. The peak of HPIII was taken through the same two chromatographic steps but was only 75 to 80% pure as determined by native and sodium dodecyl sulfate-polyacrylamide gels.

Ascorbate and oxygen effect on catalase. Cultures were grown at 37°C in LB 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.

Double diffusion analysis. Samples of catalase, partially purified on DEAE-Sephadex A50, were subjected to double diffusion analysis in plates containing 0.9% (wt/vol) NaCl and 1.0% agar (wt/vol) against an antiserum prepared with a purified mixture of HPI and HPII (5).

RESULTS

Genetic analysis of *kat-3*. The lesions in catalase-deficient mutants labeled *kat-3*, *kat-4*, and *kat-10* were found to be unlinked to *katE* in that the *kat*$^+$ phenotype did not cotransduce with *pfkB*::Tn*10* (19). This suggested that there was at least one other locus besides *katE* that was involved in catalase synthesis. Strain UM56-64 containing the *kat-3* locus was identified on plates by its inability to evolve oxygen when brought in contact with 30% hydrogen peroxide, and the oxygraph assay revealed less than 0.2 U of

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

Donor	Recipient	Selected marker	Unselected marker(s)	
			Class	No. (%)
JM1703 (*cysI*::Tn*10*)	UM56-64 (*kat-3*)	Tet^r	*kat*$^+$	38 (43)
			kat	50 (57)
PB11 (*srl*::Tn*10*)	UM56-64 (*kat-3*)	Tet^r	*kat*$^+$	38 (9)
			kat	398 (91)
UM156 (*cysI*::Tn*10 kat-3*)	JF627 (*pyrG*)	Tet^r	*pyrG*$^+$ *kat*	67 (18)
			pyrG$^+$ *kat*$^+$	164 (44)
			pyrG kat	137 (37)
			pyrG kat$^+$	6 (1)
UM156 (*cysI*::Tn*10 kat-3*)	JF627 (*pyrG*)	*pyrG*$^+$	Tet^r *kat*	77 (17)
			Tet^r *kat*$^+$	104 (23)
			Tet^s *kat*	5 (1)
			Tet^s *kat*$^+$	274 (59)
UM156 (*cysI*::Tn*10 kat-3*)	ES455 (*mutS*)	Tet^r	*mut*$^+$ *kat*	124 (55)
			mut$^+$ *kat*$^+$	18 (8)
			mutS kat	27 (12)
			mutS kat$^+$	55 (25)
UM162 (*srl*::Tn*10 kat-3*)	ES455 (*mutS*)	Tet^r	*mut*$^+$ *kat*	11 (5)
			mut$^+$ *kat*$^+$	55 (27)
			mutS kat	0 (0)
			mutS kat$^+$	139 (68)
PB11 (*srl*::Tn*10*)	JM96 (*cysH kat*)	Tet^r	*cys*$^+$ *kat*$^+$	9 (2)
			cys$^+$ *kat*	1 (1)
			cysH kat$^+$	24 (5)
			cysH kat	446 (92)
PB11 (*srl*::Tn*10*)	JM96 (*cysH kat*)	*cys*$^+$	Tet^r *kat*$^+$	3 (1)
			Tet^r *kat*	4 (2)
			Tet^s *kat*$^+$	41 (17)
			Tet^s *kat*	192 (80)

catalase per mg (dry cell weight) as compared with 4.9 U per mg (dry cell weight) in the parent strain CSH57A. The strain UM56-64 was also 13-fold more sensitive than CSH57A was to killing by 1 mM hydrogen peroxide.

The location of *kat-3* on the chromosome was investigated first by conjugative crosses with MP180 and CSH74 and was found to be between the origin of transfer of CSH74 (61.5 min [1]) and the *his* operon (44 min). A *thyA* marker was introduced into UM56-64, creating strain UM84 into which the *kat*$^+$ phenotype was transferred within 5 min after *thy*$^+$ with CSH74 as donor. This placed *kat-3* in the 55 to 60 min region of the chromosome.

The gene order between 58 and 60 min on the *E. coli* map includes *srl mutS cys pyrG* (1). These markers were employed in three factor transduction crosses (Table 2) which established the order *srl mutS kat-3 cys pyrG* (Fig. 1). Because *kat-3* maps at a location distinct from *katE* and because *katA* through *katD* have been named in *Salmonella typhimurium* (18), the locus *kat-3* was named *katF3*. A similar map location was determined for *kat-4* in UM62 and *kat-10* in UM85. Coincidentally, the strain JM96 was found to be partially deficient in catalase when it was received. The lesion was mapped and found to be linked to *srl* at the same frequency as was *katF3* (Table 2), making JM96 a *katF* mutant as well.

Both *katE* and *katF* mutants were initially identified in recombination experiments with the Hfr strains MP180 (HfrH; clockwise from 98.7 min) and CSH74 (KL16; counter clockwise from 61.5 min) (2). To check the region of the chromosome between 68 and 98 min for the presence of a catalase gene, the strains UM1 (*katE1*), UM2 (*katE2*), and UM56-64 (*katF3*) were also crossed with the Hfr strains CSH64 (KL14; clockwise from 67.5 min) and CSH60 (Ra-2; clockwise from 89.5 min). *lac*$^+$ recombinants were selected in crosses that were interrupted before either *katE* or *katF* could enter and were then scored for *kat*$^+$ recombinants. Only CSH64 gave rise to *lac*$^+$ *kat*$^+$ recombinants and did so for all three catalase mutants, indicating the presence of another catalase locus between 67.5 min (origin of CSH64) and 89.5 min (origin of CSH60) in all three strains. This locus in UM2 was named *katG2*, and one strain carrying *katG2*$^+$ was picked as UM181 for further characterization below. It was not clear whether the *kat* loci between 67.5 and 89.5 min are the same in UM1, UM2, and UM56-64; consequently,

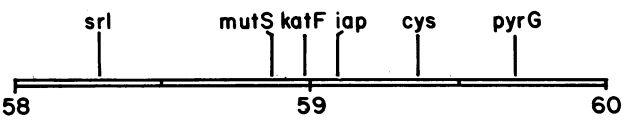

FIG. 1. Genetic map orienting *katF* relative to nearby genes in *E. coli* K-12. Gene locations (in minutes) are based on the 100-min *E. coli* map (1), with *cys* at 59.3 min as a reference point. The map distances were calculated by the equation of Wu (31) from the cotransduction frequencies in Tables 2 and 3.

TABLE 3. Mapping of catalase mutants created by transposon Tn*10* insertions

Donor	Recipient	Selected marker	Unselected markers	
			Class	No. (%)
UM120 (*kat-11*::Tn*10*)	NP37 (*pheS*)	Tet^r	*pheS*$^+$	148 (62)
			pheS	92 (38)
UM122 (*kat-12*::Tn*10*)	JF627 (*pyrG*)	Tet^r	*pyrG*$^+$	21 (11)
			pyrG	163 (89)
UM122 (*kat-12*::Tn*10*)	JF627 (*pyrG*)	*pyrG*$^+$	Tet^r	6 (3)
			Tet^s	234 (97)
UM122 (*kat-12*::Tn*10*)	JM96 (*cysH*)	Tet^r	*cysH*$^+$	185 (51)
			cysH	175 (49)
UM122 (*kat-12*::Tn*10*)	JM96 (*cysH*)	*cysH*$^+$	Tet^r	95 (26)
			Tet^s	265 (74)

the loci in UM1 and UM56-64 were not named. The mapping of these loci will be reported separately, but it was clear that the nitrosoguanidine mutagenesis used to create the catalase-deficient strains caused mutations in two separate loci.

Transposon insertion mutagenesis. Two mutants of MP180, UM120 and UM122, that evolved oxygen more slowly on plates when treated with H_2O_2 were isolated after Tn*10* mutagenesis. Despite low apparent catalase levels on plates, these strains exhibited normal catalase levels in the oxygraph assay. An explanation for this discrepancy will be presented below. Electrophoresis of crude extracts on native polyacrylamide gels revealed HPI and HPII but no HPIII, the electrophoretically slower catalase, in either UM120 or UM122. The time of entry of the Tn*10* marker into a recipient, CSH57A, occurred between 35 and 40 min for UM120 and between 55 and 60 min for UM122. All of the Tn*10*-containing recombinants exhibited low catalase when assayed on plates. P1 phage transduction crosses (Table 3) revealed that the Tn*10* in UM120 was linked to *pheS*, and the Tn*10* in UM122 was linked to *cys* and *pyrG*. Consequently, UM120 contained *katE12*::Tn*10*, and UM122 contained *katF13*::Tn*10*.

Identification of the catalase species in *katE*$^+$ and *katF*$^+$ strains. Extracts of both a *katE*$^+$ strain, UM4, derived from UM2 (*katE2*) and a *katF*$^+$ strain, UM167, derived from UM56-64 (*katF3*) were prepared and analyzed on native polyacrylamide gels. In both cases, only HPIII, the electrophoretically slower catalase without the associated peroxidase activity, was present, revealing that both *katE* and *katF* were involved in HPIII synthesis, although the precise roles remain undefined.

In a further attempt to investigate the difference between HPI-HPII and HPIII, the eluate from DEAE-Sephadex during catalase purification from CSH57A was investigated more closely and revealed a shoulder on the main peak of catalase activity but not on the corresponding peak of peroxidase activity (Fig. 2a). This suggested the presence of a second catalase activity that lacked the peroxidase activity characteristic of HPI-HPII. Indeed, visualization of the peroxidase and catalase after electrophoresis on polyacrylamide gels revealed only HPI-HPII in peak A (fractions 105 to 125), and the shoulder or peak B (fractions 135 to 155) contained HPIII in addition to HPI-HPII (Fig. 3). The eluate from an extract of UM4, the *katE*$^+$ recombinant of UM2, contained only a single peak of catalase with no coincident peroxidase activity (Fig. 2c); this peak corresponded to peak B in Fig. 2a. In addition, an extract of UM181, the *katG*$^+$ recombinant of UM2, revealed a single peak of catalase-peroxidase (Fig. 2d) that corresponded to peak A in Fig. 2a. Visualization on polyacrylamide gels (Fig. 3) revealed only HPI-HPII in this peak, and no HPIII was evident in the portion of the UM181 eluate corresponding to peak B. Two conclusions can be drawn: (i) HPIII exists in extracts of the parent strain CSH57A and can be separated from HPI-HPII by either ion-exchange chromatography or gel electrophoresis; and (ii) HPIII activity and HPI-HPII activity can be recombined into the same strain independently, involving widely separated loci.

Determination of K_m values of HPI-HPII and HPIII for H_2O_2. The elution patterns in Fig. 2 suggested that there was considerably more HPI and HPII than HPIII in cell extracts. This observation was consistent with oxygraph assays of whole cells in which *katE*$^+$ and *katF*$^+$ recombinants exhibited 1/10 to 1/4 as much catalase (0.3 to 1.0 U/mg [dry cell weight]) as did the parental strain (4 to 5 U/mg [dry cell weight]). However, this observation was inconsistent with the catalase levels observed in the plate assay in which *katE*$^+$ and *katF*$^+$ recombinants exhibited catalase levels equivalent to the parental strain. The hydrogen peroxide concentrations in the two assays were significantly different, with 1.1 mM being used in the standard oxygraph assay (25) and 8.8 M being used for the plate assay. When a higher concentration of H_2O_2 was used in the oxygraph assay, the level of catalase increased, indicating that 1.1 mM was not a saturating concentration. As a result, the K_m values for H_2O_2 of the catalase in whole cells and in crude extracts were determined for HPIII from UM4 (30.9 mM) and HPI-HPII from UM181 (3.7 mM). The K_m of pure HPI-HPII from CSH57A was determined to be 4.1 mM which was in close agreement with the reported value of 3.9 mM for pure HPI from *E. coli* B. Regardless of whether the assay was carried out in whole cells or cell extracts, the K_m values were the same for a given strain, and the K_m of HPIII was eight- to ninefold higher than the K_m of HPI-HPII was. The K_m of the peroxidase activity of HPI-HPII for H_2O_2 was determined to be 48 μM.

The column effluent shown in Fig. 2a was reassayed with 60 mM H_2O_2 in the oxygraph assay. The resulting elution profile (Fig. 2b) revealed that HPIII (peak B) made up a more significant fraction of the total catalase pool, which was consistent with the relative amounts of HPIII and HPI-HPII evident on native polyacrylamide gels and with the activity exhibited on plates.

Differential responses of HPI-HPII and HPIII to ascorbate. *E. coli* responds to both ascorbate and hydrogen peroxide with the rapid synthesis of catalase (8, 24, 32). Analysis on polyacrylamide gels revealed that the increase took place exclusively in HPI and HPII, with no change being evident in HPIII (24). This observation was confirmed by adding ascorbate to cultures of UM4 and UM181, which caused no change in HPIII in UM4 but caused a three- to fourfold increase in HPI-HPII in UM181 (Table 4). The catalase levels in both UM4 and UM181 did increase during growth into stationary phase to approximately the same level when assayed with 60 mM H_2O_2 (Table 4). There was no difference in catalase levels between cultures grown aerobically and anaerobically for either UM4 or UM181. Both pure HPI-HPII from CSH57A and 75% pure HPIII from UM4 were inhibited more than 90% by 5 mM sodium cyanide or 5 mM sodium azide.

Immunodiffusion analysis. Another confirmation that HPIII was distinct from HPI and HPII was obtained from double diffusion analysis against an antiserum prepared against purified HPI-HPII. Only fractions of peak A from

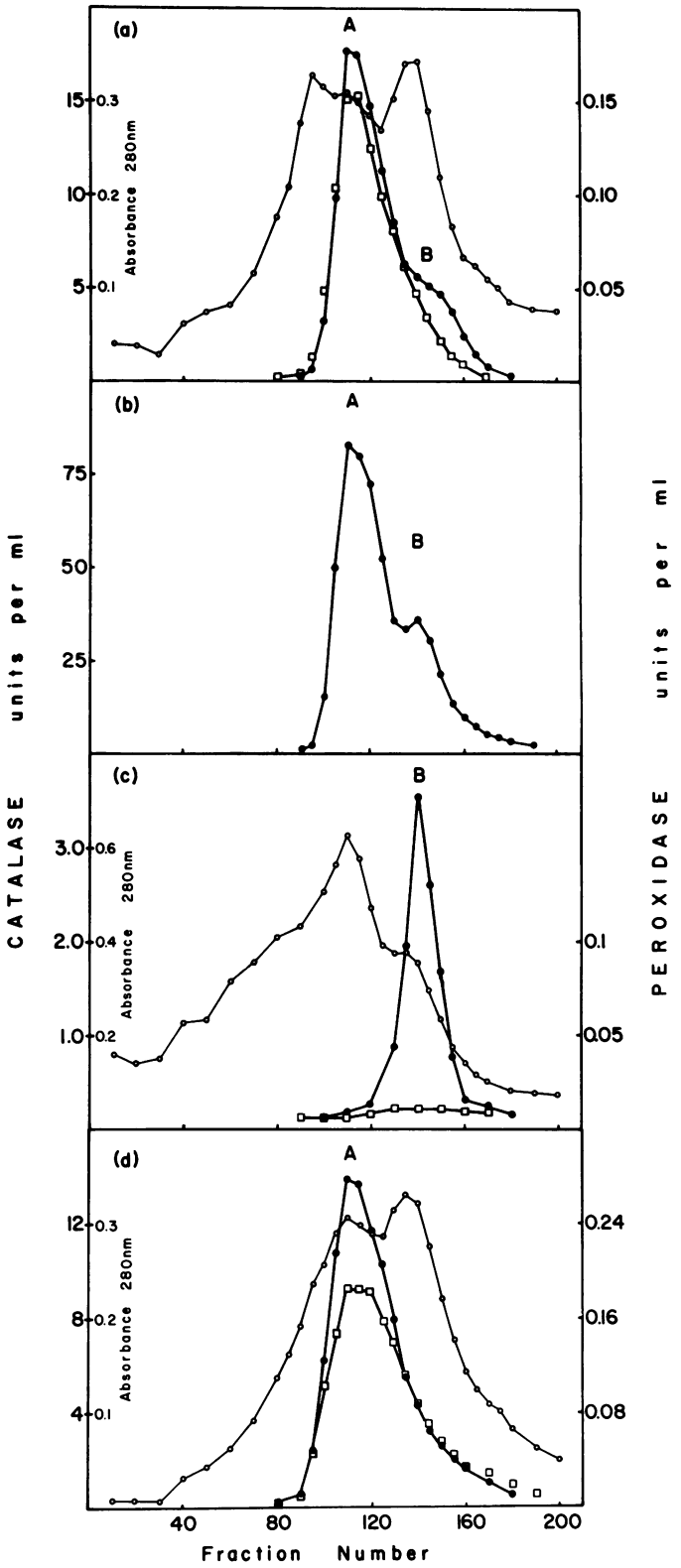

FIG. 2. Fractionation of various *E. coli* extracts on DEAE-Sephadex A50. After ammonium sulfate precipitation, extracts from CSH57A (a and b), UM4 (c), and UM181 (d), were fractionated on a column of DEAE-Sephadex A50 as described in the text. The absorbance at 280 nm (○) was determined as were catalase activity (●) and peroxidase activity (□). The elution profiles of catalase in a, c, and d were obtained with 1.1 mM H_2O_2 in the oxygraph assay. The elution profile of catalase in b was obtained from the same eluate as in a, but with 60 mM H_2O_2 as substrate. Peak A (in a, b, and d), Main peak centered at fraction 110, peak B (in a, b, and c), shoulder or peak centered at fraction 140.

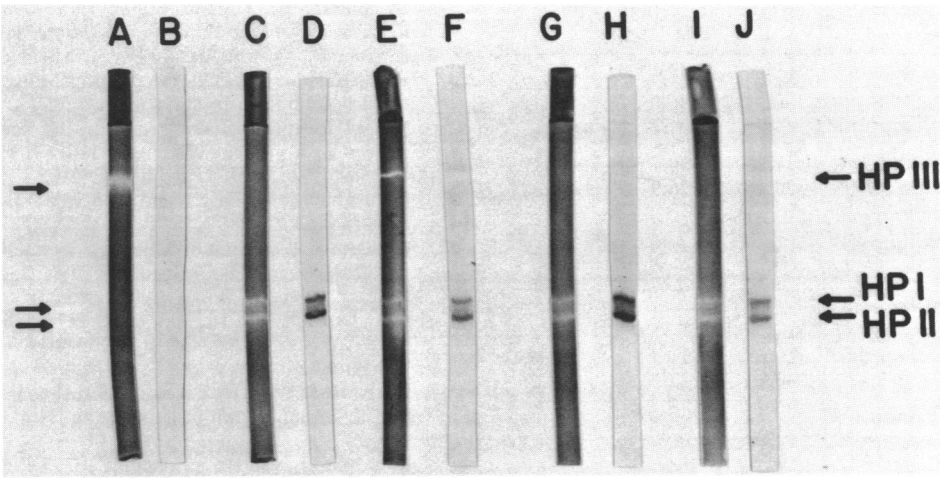

FIG. 3. Visualization of various fractions, eluted from DEAE-Sephadex A50, on native polyacrylamide gels. A, C, E, G, and I were stained for catalase activity which is evident as a colorless band(s), and B, D, F, H, and J were stained for peroxidase activity which is evident as a dark band(s). The following samples were run: (A and B) peak B (Fig. 2c) of UM4, 20.9 U of catalase or 158 μg of protein; (C and D) peak A (Fig. 2a) of CSH57A, 9.6 U of catalase or 50 μg of protein; (E and F) peak B (Fig. 2a) of CSH57A, 12.5 U of catalase or 78 μg of protein; (G and H) peak A (Fig. 2d) of UM181, 17.8 U of catalase or 65 μg of protein; (I and J) fractions 135 to 155 (Fig. 2d) of UM181, 6.3 U of catalase or 102 μg of protein. All catalase activities were determined with 60 mM H_2O_2.

CSH57A or UM181 (Fig. 2) containing HPI and HPII formed an immunoprecipitin band. Fractions of peak B from either UM4 or CSH57A in Fig. 2 that contained predominantly HPIII showed little or no reaction despite having an equivalent amount of protein and catalase in the well (Fig. 4).

DISCUSSION

A second locus unlinked to *katE* but still affecting catalase expression in *E. coli* was mapped in three factor-cotransductional crosses and was located between *mutS* and *cys* at 59 min on the chromosome. This new locus is designated *katF*. The *iap* locus is also located in this region and cotransduces 50 to 73% with *cys* markers (22), suggesting that *iap* is more tightly linked to *cys* than is *katF*. Consequently, we have placed *iap* between *katF* and *cys* (Fig. 1) even though we were not able to obtain the *iap* marker to verify the assignment. Transposon insertion mutagenesis was also successful in producing catalase-deficient mutants; these mutations mapped with both *katF*, which was linked to *pyrG* and *cysH*, and *katE*, which was linked to *pheS*.

The catalase activity identified in $katE^+$ and $katF^+$ recombinants has previously been reported only in a preliminary fashion as a faint catalase band on a native polyacrylamide gel (24). The slower electrophoretic mobility, higher affinity for DEAE-Sephadex, higher K_m for H_2O_2, lack of peroxidase activity, lack of reaction with an HPI-HPII antiserum, and absence of any response to ascorbate as compared with HPI and HPII all indicate that this is a new type of catalase in *E. coli*. We have named it HPIII which is consistent with the previously named hydroperoxidases HPI and HPII. HPI from *E. coli* B has been purified of HPII and characterized (4), but when the same procedure was used to purify catalase from *E. coli* K-12 (CSH57A), a mixture of nearly equal amounts of HPI and HPII resulted. Despite the mixture, only one subunit of 84,000 molecular weight was evident on sodium dodecyl sulfate-polyacrylamide gels. This fact, coupled with the fact that both HPI and HPII are encoded on the same plasmid (20), agrees with the conclusion (12) that they are encoded by the same gene, after which a minor modification occurs to create the second species. As a result, the isoenzyme pair of HPI-HPII can be considered as one species of catalase, and HPIII is a different species. In fact, *E. coli* is not alone in possessing two species of catalase; yeasts (27, 28), maize (26), and *Micrococcus radiodurans* (11) have all been shown to possess two species as well. In addition, a nonheme pseudocatalase has been isolated from *Lactobacillus plantarum* (16). Any similarity between it and HPIII seem unlikely, however, because the latter is inhibited by azide and cyanide, whereas the former is not.

The *katE1*, *katE2*, and *katF3* mutations were all caused by nitrosoguanidine which frequently causes more than one mutagenic event per chromosome. The absence of both catalase species in the three mutants UM1, UM2, and UM56-64 suggested that two distinct mutagenic events had occurred in each mutant. This was confirmed by the identification of the *katG2* locus in UM2 and possibly related loci in

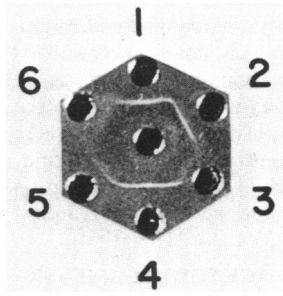

FIG. 4. Double immunodiffusion analysis of various fractions eluted from DEAE-Sephadex A50. The center well contained antiserum prepared against a purified mixture of HPI and HPII. The following samples were analyzed: (1) purified HPI-HPII, 22.3 U of catalase or 7 μg of protein; (2) peak A (Fig. 2a) of CSH57A, 19.6 U of catalase or 100 μg of protein; (3) peak B (Fig. 2a) of CSH57A, 12.5 U of catalase or 78 μg of protein; (4) peak A (Fig. 2d) of UM181, 35.6 U of catalase or 130 μg of protein; (5) fractions 135 to 155 (Fig. 2d) of UM181, 6.3 U of catalase or 102 μg of protein; (6) peak B (Fig. 2c) of UM4, 10.4 U of catalase or 79 μg of protein. All catalase activities were determined with 60 mM H_2O_2.

TABLE 4. Effect of ascorbate or growth into stationary phase on catalase levels in CSH57A, UM4, and UM181

Strain	Catalase (U/mg [dry cell wt]) at time (min)[a]				
	−10	−5	+15	+30	Overnight[b]
CSH57A					
(1.1 mM)[c]	3.0	2.7	11.2	10.7	10.2
(60 mM)	8.2	8.9	30.4	26.9	46.8
UM4					
(1.1 mM)	0.7	0.5	ND[d]	N.D.	1.7
(60 mM)	3.5	3.5	3.3	2.9	34.6
UM181					
(1.1 mM)	3.0	3.0	10.6	11.5	13.5
(60 mM)	7.0	7.4	40.2	42.7	44.2

[a] Ascorbate was added at 0 min.
[b] A separate culture was grown in LB medium for 16 h.
[c] H_2O_2 used in assay.
[d] N.D., Not detectable.

both UM1 and UM56-64 mapping between 67.5 and 89.5 min. These new loci affected HPI-HPII synthesis, whereas *katE* and *katF* affected HPIII synthesis. Consequently, either a *katE katG* or a *katF katG* genotype gives rise to a complete absence of catalase.

The presence of two catalases in *E. coli* which differ in their affinities for H_2O_2 provides an explanation for the discrepancy observed between catalase levels determined on solid and in liquid medium (19). Two classes of strains have been observed that give rise to discrepancies. One class, which includes the strains with Tn*10* insertions in *katE* and *katF*, evolves oxygen slowly on plates but exhibits normal catalase activity in the oxygraph. The second class, which includes the $katE^+$ and $katF^+$ recombinants, evolves oxygen normally on plates but exhibits very low levels of catalase in the oxygraph. Strains in the first class contain only HPI-HPII, whereas strains in the second class contain just HPIII; because of the low concentration of H_2O_2 used in the oxygraph assay relative to the K_m values, HPI-HPII gives rise to a 4- to 10-fold higher catalase level. On plates, however, 30% H_2O_2 is used, which is well above the K_m values of both catalases for H_2O_2, and strains with just HPIII present give rise to an equivalent or slightly more rapid oxygen evolution than do strains with just HPI-HPII present. The isolation of a yeast clone that was catalase positive on plates but inactive when assayed might also be explained by a change in the K_m of the enzyme for H_2O_2 (29).

There are three logical extensions of this work that must be addressed. The first is the complete purification and characterization of HPIII. The second is to identify the gene products of *katE* and *katF* and to define their roles in the synthesis of HPIII. The third is to precisely map *katG* in UM2 and the related genes in UM1 and UM56-64 which affect the synthesis of the HPI-HPII.

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