Molecular characterization of three mutations in *katG* affecting the activity of hydroperoxidase I of *Escherichia coli*

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Hydroperoxidase I (HPI) of *Escherichia coli* is a bifunctional enzyme exhibiting both catalase and peroxidase activities. Mutants lacking appreciable HPI have been generated using nitrosoguanidine and the gene encoding HPI, *katG*, has been cloned from three of these mutants using either classical probing methods or polymerase chain reaction amplification. The mutant genes were sequenced and the changes from wild-type sequence identified. Two mutants contained G to A changes in the coding strand, resulting in glycine to aspartate changes at residues 119 (*katG15*) and 314 (*katG16*) in the deduced amino acid sequence of the protein. A third mutant contained a C to T change resulting in a leucine to phenylalanine change at residue 139 (*katG14*). The Phe139-, Asp119-, and Asp314-containing mutants exhibited 13, <1, and 18%, respectively, of the wild-type catalase specific activity and 43, 4, and 45% of the wild-type peroxidase specific activity. All mutant enzymes bound less protoheme IX than the wild-type enzyme. The sensitivities of the mutant enzymes to the inhibitors hydroxylamine, azide, and cyanide and the activators imidazole and Tris were similar to those of the wild-type enzyme. The mutant enzymes were more sensitive to high temperature and to β-mercaptoethanol than the wild-type enzyme. The pH profiles of the mutant catalases were unchanged from the wild-type enzyme.

Key words: catalase, hydroperoxidase I, mutants, sequence analysis.

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L'hydroperoxydase I (HPI) d'*Escherichia coli* est une enzyme bifonctionnelle possédant deux activités : catalase et peroxydase. Utilisant la nitrosoguanidine, nous avons généré des mutants privés de façon appréciable de l'HPI et, à partir de trois de ces mutants, nous avons cloné le gène *katG* codant pour l'HPI à l'aide des méthodes classiques avec sonde ou par amplification de la réaction polymérasique. Le séquençage des gènes mutants nous a permis d'identifier les changements par rapport à la séquence de type sauvage. Dans la chaîne codante de deux mutants, des A remplacent des G et dans la séquence déduite des acides aminés de la protéine, l'aspartate remplace la glycine aux résidus 119 (*katG15*) et 314 (*katG16*). Dans un troisième mutant, T remplace C, d'où substitution d'une phénylalanine à la leucine au résidu 139 (*katG14*). Les mutants contenant Phe139, Asp119 et Asp314 possèdent une activité catalasique spécifique respectivement égale à 13%, moins de 1% et 18% de celle de type sauvage et une activité peroxydasique spécifique égale à 43, 4 et 45% de celle de type sauvage. Toutes les enzymes mutantes fixent moins le protohème IX que l'enzyme de type sauvage. La sensibilité des enzymes mutantes aux inhibiteurs tels que l'hydroxylamine, l'azide et la cyanure et aux activateurs comme l'imidazole et le Tris est semblable à celle de l'enzyme de type sauvage. Les enzymes mutantes sont plus sensibles aux températures élevées et au β-mercaptoéthanol que l'enzyme de type sauvage. Le pH optimum des enzymes mutantes est le même que celui de l'enzyme de type sauvage.

Mots clés : catalase, hydroperoxydase I, mutants, analyse de séquence.

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Introduction

Escherichia coli produces two catalases which have been named HPI and HPII (Claiborne and Fridovich 1979; Claiborne *et al.* 1979; Loewen and Switala 1986). HPI was characterized as a tetramer of 81 000 Da monomers with two associated protoheme IX groups and it was found to have a broad-spectrum peroxidase activity allowing it to use organic electron donors to reduce hydrogen peroxide (Claiborne and Fridovich 1979). HPII was shown to be a monofunctional catalase (Claiborne *et al.* 1979; Loewen and Switala 1986) existing as a hexamer of 93 000 Da subunits with one heme-*d*-like group per subunit. A 25.4-kb plasmid, pLC36-19, encoding HPI was identified in the Clark and Carbon library and characterized (Loewen *et al.* 1983). The gene was subsequently localized on a 2.8-kb segment and confirmed to be the same as *katG* (Triggs-Raine and Loewen 1987), which had been mapped at 89.2 min on the *E. coli* chromosome (Loewen *et al.* 1985*b*). Addition of H_2O_2 or ascorbate to cultures of *E. coli* caused an induction in HPI synthesis of up to 10-fold (Loewen *et al.* 1985*a*; Finn and Condon 1975; Richter and Loewen 1981; Yoshpe-Purer *et al.* 1977), which was shown to be positively regulated by OxyR (Christman *et al.* 1985, Morgan *et al.* 1986), a protein responsible for the positive regulation of a regulon involved in protection against oxidative stress.

The complete nucleotide sequence of *katG* has been determined (Triggs-Raine *et al.* 1988), revealing a 2181-bp open reading frame which predicts a sequence for the HPI subunit of 726 amino acids and a size of 80 049 Da. The predicted sequence of HPI revealed very little homology with any of the known catalase sequences. Consequently, it was not possible to draw any conclusions about the three-dimensional structure of the enzyme or direct a study of structure–function relationships using site-directed mutagenesis as a tool to change individual amino acids. An alternate approach to commencing such a study would be to identify the locations and effects of randomly generated mutations, to identify possible regions of the protein involved in enzyme action. A number of such mutants, generated using nitrosoguanidine, had been isolated for use in the mapping of *katG*. This

ABBREVIATIONS: HPI and HPII, hydroperoxidases I and II; Da, dalton(s); kb, kilobase(s); bp, base pair(s); SDS, sodium dodecyl sulfate.

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TABLE 1. Purification of wild-type and mutant HPIs

Fraction	Wild type	KatG14	KatG15	KatG16
Catalase (U/mg protein)				
Crude extract	160	19	4.2	5.4
Streptomycin sulfate	315	34	7.9	8.7
Ammonium sulfate	1320	140	15	150
DEAE-Sephadex	2480	320	16	450
Peroxidase (U/mg protein)				
DEAE-Sephadex	1.97	0.84	0.08	0.88

paper describes the cloning of three mutant *katG* genes, the characterization of the mutations by DNA sequence analysis, and the characterization of the mutant enzymes.

Experimental procedures

Bacterial strains and plasmids

Bacterial strains used included UM1 (*katG14*) (Loewen 1984), UM2 (*katG15*) (Loewen 1984), UM56-64 (*katG16*) (Loewen and Triggs 1984), UM262 (*recA katG*::Tn*10 pro leu rpsL hsdM hsdR endI lacY*), NM522 (*supE thi* Δ(*lac-proAB*) *hsd5* F′ (*proAB*$^+$ *lacZ*) (Mead *et al.* 1985), and HB101 (*pro leu rpsL hsdM hsdR endI recA lacY*) (Boyer and Rolland-Dusoix 1969) and were grown in LB medium (Miller 1972). Plasmids used included pAT153 (Twigg and Sheratt 1980), Bluescript KS M13+ (Stratagene Cloning Systems), and pBT22 (Triggs-Raine and Loewen 1987).

Subcloning of mutant katG *genes*

For *katG15* and *katG16*, genomic DNA from UM2 and UM56-64, respectively, was digested with restriction nuclease *Hin*dIII, followed by ligation into pAT153 (Miniatis *et al.* 1982). Following transformation into HB101, clones containing *katG* were identified by probing with nick-translated pBT22. For *katG14*, genomic DNA from UM1 was amplified in a polymerase chain reaction using primers 5′-AAGCTTAATTAAGATCAATTT (nucleotides 1–21 in Triggs-Raine *et al.* 1988) and 5′-AAGCTTCATGAAATCCAGCTA (complementary to nucleotides 2510–2489 in Triggs-Raine *et al.* 1988) in a Perkin Elmer DNA thermal cycler employing 30 cycles of 1 min at 93°C, 1 min at 50°C, and 2 min at 72°C. The 2.5-kb fragment was cloned into the *Eco*RV site of the Bluescript M13+ phagemid, followed by selection in NM522.

Sequencing of mutant katG *genes*

Eight oligonucleotides corresponding to segments of the *katG* sequence at 270-bp intervals were synthesized by the DNA Synthesis Laboratory, University of Calgary, and included 5′-TCAATTTGATCTACATC (15–31), 5′-ATTCTAATCGTTCTAAC (291–307); 5′-GTCGCCTGTTGTGGCCA (573–589), 5′-ACCGCTTTCTGCGGCAG (856–872), 5′-TTCAAGTTTGAGTGGGT (1145–1161), 5′-AAGATCTGATCTGGCAA (1434–1450), 5′-CATAGTGCTGGCTGGTG (1729–1745), and 5′-CAAAAACGGCGTCTTCA (2023–2039). The numbers in parentheses indicate the location in the *katG* sequence (Triggs-Raine *et al.* 1988). These oligonucleotides were used to prime elongation in the Sanger sequencing method (Sanger *et al.* 1977), to sequence the whole of *katG* and the adjacent control regions.

Purification of the mutant KatG proteins

Plasmids containing the mutant *katG* genes were transformed into UM262 containing a transposon in *katG* from which the only KatG protein produced would be expressed from the plasmid. Cells were grown in the presence of 250 μg ampicillin/mL LB medium to stationary phase at 37°C. The procedure for purifying the protein was essentially that of Claiborne and Fridovich (1979), as applied to the purification of HPII (Loewen and Switala 1986). Because the plasmid-encoded proteins were produced in such large amounts, it was usually only necessary to proceed as far as the first DEAE-Sephadex ion-exchange column to obtain pure protein. A summary of the purification data is contained in Table 1. Catalase activity was determined by the method of Rørth and Jensen (1967) using a Gilson oxygraph equipped with a Clark electrode. One unit of catalase is defined as the amount that decomposes 1 μmol H_2O_2/min at 37°C. Peroxidase activity was assayed as described by Worthington Biochemical Corp. (1969) and 1 unit is the amount of enzyme decomposing 1 μmol peroxide/min at 25°C. Protein was estimated by the method of Layne (1957).

Gel electrophoresis and staining

Gel electrophoresis of the purified proteins was carried out under denaturing conditions on SDS–polyacrylamide gels as previously described (Laemmli 1970; Weber *et al.* 1972), after which the protein bands were located by staining with Coomassie brilliant blue. Gel electrophoresis was also carried out under nondenaturing conditions as described by Davis (1964), except in pH 8.1 Tris–HCl and on 15 × 15 cm slab gels. Following electrophoresis on nondenaturing gels, peroxidase activity was visualized by the method of Gregory and Fridovich (1974) and catalase was stained as described (Clare *et al.* 1984) but using 20 mM H_2O_2 for better contrast.

Results

Isolation and sequencing of mutant katG *clones*

Both *katG15* and *katG16* were cloned on 3.8-kb fragments with restriction patterns identical to wild-type *katG*, whereas *katG14* was cloned on a 2.5-kb fragment resulting from polymerase chain reaction amplification. The sequence of *katG* has recently been determined (Triggs-Raine *et al.* 1988), allowing the synthesis of a set of oligonucleotide primers corresponding to portions of the *katG* gene for direct sequencing of the complete mutant genes without the need to subclone fragments. The primers were located at approximately 270-bp intervals such that elongation of each primer overlapped the succeeding primer, ensuring sequence analysis of the complete gene. All three mutants were found to contain a single base change from the wild-type sequence (Fig. 1). In *katG14* there was a C to T change in the coding strand at base 560 (Triggs-Raine *et al.* 1988), which would result in a leucine to phenylalanine change at amino acid 139 (Phe139) in the deduced amino acid sequence (Fig. 1*a*). In *katG15* and *katG16*, the nucleotide change was from A to G at bases 501 and 1056 respectively (Figs. 1*b* and *c*). These changes resulted in glycine to aspartate changes at amino acids 119 (Asp119) and 314 (Asp314) of the deduced protein sequence.

Characterization of mutant KatG *proteins*

The mutant proteins were purified from cells transformed with plasmids containing the mutant genes. The protocol employed was similar to that used to purify wild-type HPI, except that because of the large amount of protein expressed from the multicopy plasmids it was possible to eliminate the final two columns, thus stopping after one DEAE-Sephadex

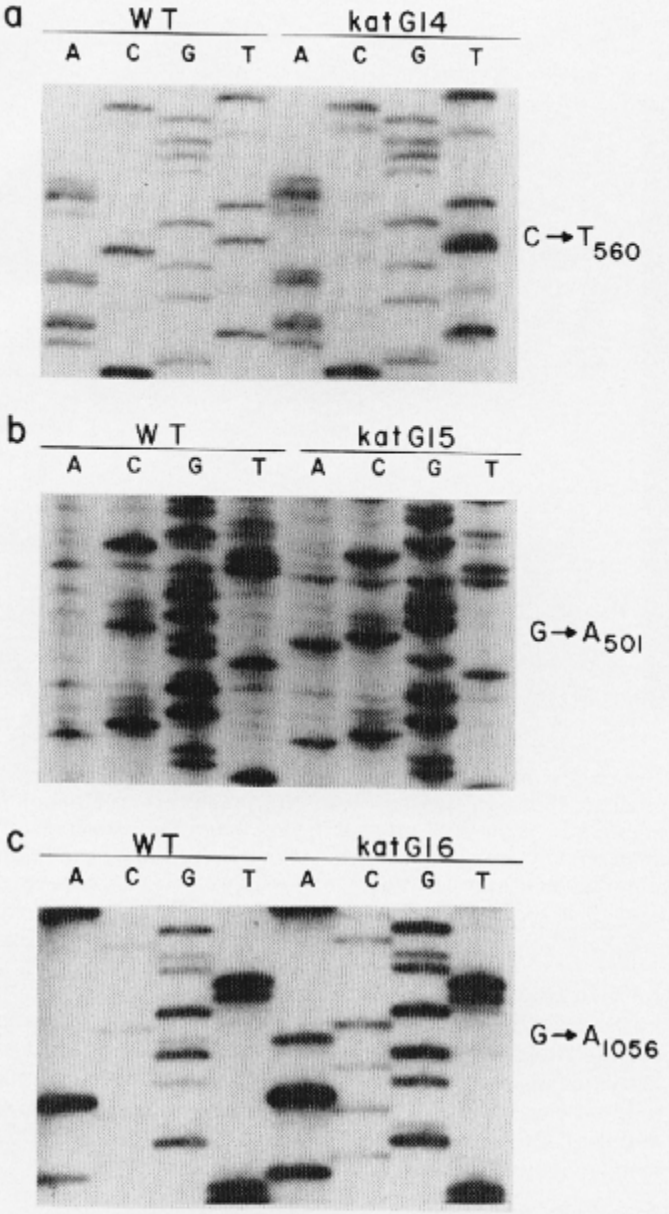


FIG. 1. Autoradiograms of sequencing gels revealing base changes in the three mutant *katG* genes. (*a*) The sequence of wild-type *katG* between bases 545 and 580 (CCGGATAACGTAAGCC$_{560}$TCGATAAAGCGCGTCGCCT) is shown on the left and the sequence on the right covers the same region for the mutant *katG14*. The C to T change at base 560 of *katG14* is indicated on the right side. (*b*) The sequence of wild-type *katG* between bases 490 and 515 (TGGACGCGGTGG$_{501}$CGCGGGTCGTGGT) is shown on the left and the sequence on the right covers the same region for the mutant *katG15*. The G to A change at base 501 of *katG15* is indicated on the right side. (*c*) The sequence of wild-type *katG* between the bases 1045 to 1067 (TTACGGCAGCGG$_{1056}$CGTTGGCGCA) is shown on the left and the sequence on the right covers the same region for the mutant *katG16*. The G to A change at base 1056 of *katG16* is indicated on the right side.

column. All three mutant enzymes exhibited reduced catalase activity as compared with the wild-type enzyme throughout the purification protocol, with purified KatG14, KatG15, and KatG16 exhibiting 13, <1, and 18%, respectively, of the wild-type catalase-specific activity. The peroxidase activity of the mutant enzymes was 43, 4, and 45% (KatG14, KatG15, and KatG16, respectively) of the wild-type peroxidase activity, indicating that all three changes had a greater effect on the catalase activity than on the peroxidase activity.

Electrophoretic analysis of wild-type HPI has always presented some problems of interpretation because the protein migrates as a single band on denaturing gels and as a double band on nondenaturing gels (Loewen *et al.* 1985*b*). Possible posttranslational modification affecting the charge but not the size of the protein or differing heme contents would explain this phenomenon, but has not been proven. Electrophoresis of the purified mutant proteins was similarly confusing. SDS–polyacrylamide gels revealed differences from the wild type in apparent size, as well as the presence of more than one subunit size in KatG14 and KatG16 (Fig. 2*a*). On nondenaturing gels all three mutant proteins differed from wild type by migrating as a single band of protein coincident with the single band of catalase and peroxidase activities (Fig. 2*b*). A change in conformation or charge resulting from the exchanged amino acids may be affecting both electrophoretic migration and posttranslational modification, but in the absence of more detailed chemical analysis of the protein a complete explanation of these data is not possible. KatG15, the least active of the three mutants, was also prone to precipitation upon storage, suggesting that its conformation was less stable and susceptible to disruption.

Effect of H_2O_2 and pH

Kinetic analysis of the effect of increasing H_2O_2 concentration on catalase activity revealed that 50% of apparent maximal activity was attained at the values of 6.3 and 2.8 mM for KatG15 and KatG16, respectively, which are quite similar to the value of 4.4 mM for the wild-type enzyme. KatG14 required a much higher H_2O_2 concentration of 20.2 mM to reach 50% of apparent maximum activity, indicating that the Phe139 mutation may be affecting the binding of H_2O_2 or the interchange of various reactive intermediates.

Wild-type HPI has a narrow optimum pH range between 6 and 7, as compared with other catalases (Goldberg and Hochman 1989; Loewen and Switala 1988) which retain activity from pH 4 to 10. The optimum pH range of the mutant enzymes was found to be very similar to that of the wild-type enzyme (Fig. 3). Only KatG14 was slightly different, with the curve being shifted to higher pH.

Spectral characteristics and heme content

The UV and visible spectra were determined for all of the mutant proteins and found to be similar to the spectrum of wild-type enzyme in terms of the location of the absorbance peaks, indicating that protoheme IX was bound to all of the mutants. However, the relative intensities of the various absorbance peaks varied, indicating that different amounts of heme were bound to the mutant proteins (Table 2). Using the peak at 280 nm as a measure of the protein concentration and the Soret peak at 407 nm as a measure of the protoheme IX content, 0.8, 0.4, and 0.8 heme groups per tetramer were determined to be present in KatG14, and KatG16 respectively. For comparison, the wild-type enzyme contained 2.2 heme groups per tetramer.

Sensitivity to activators

Tris and imidazole are known activators of the catalase, but not the peroxidase activity of HPI (Claiborne and Fridovich 1979). The catalase activities of both KatG14 and

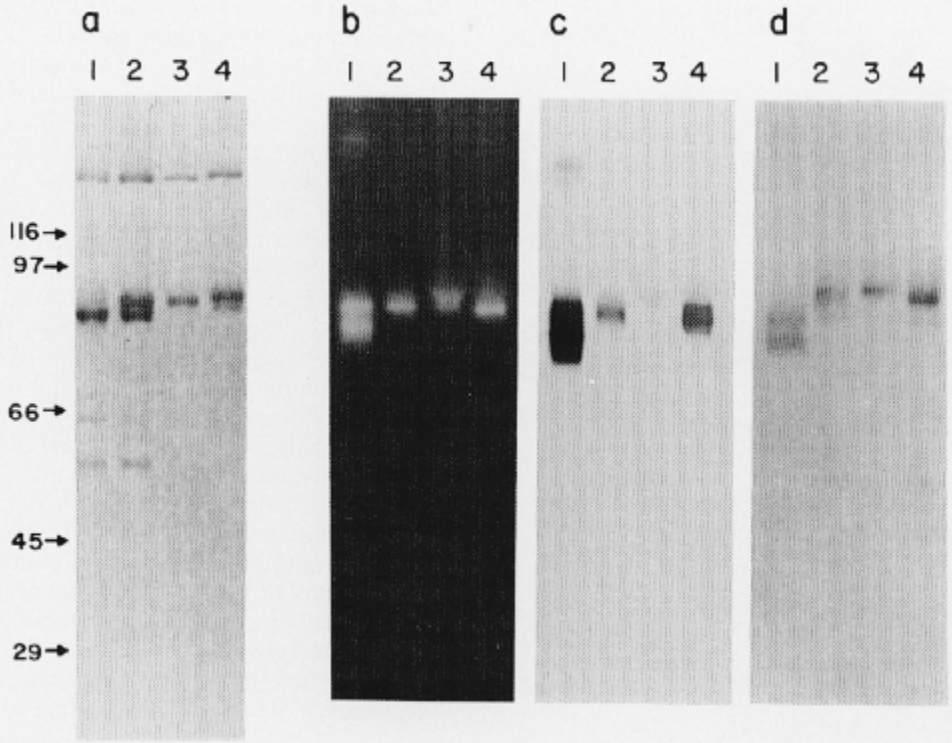
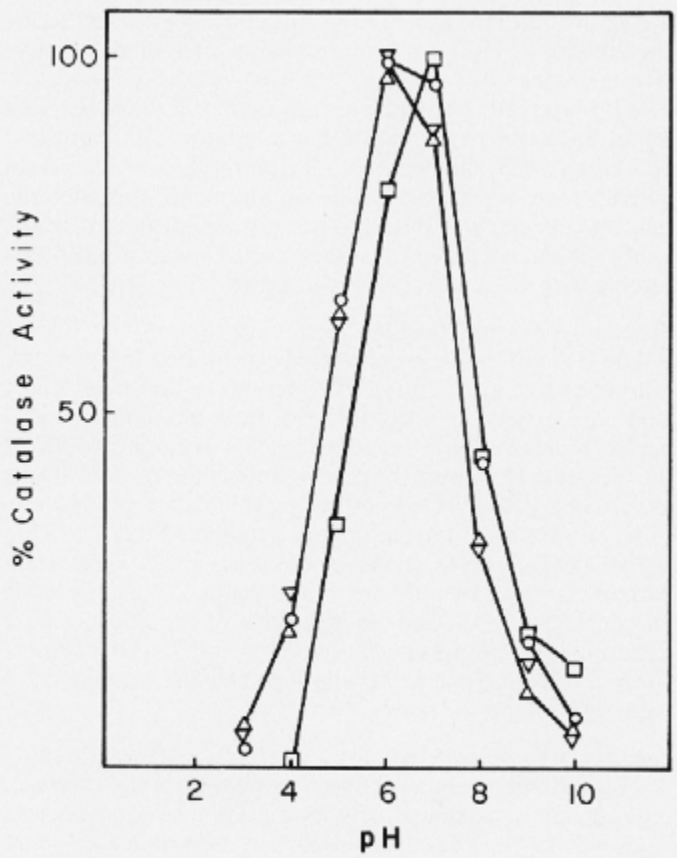


FIG. 2. Electrophoretic separation of purified KatG proteins. (*a*) Separation on an 8% SDS–polyacrylamide gel run as previously described (Laemmli 1970; Weber *et al.* 1972). The numbers at the left indicate the molecular weights ($\times 10^{-3}$) used for size estimation. The single large band in all samples was from incompletely denatured multimers and the smaller bands in lanes 1 and 2 were degradation products that appeared after storage for several months. (*b–d*) Separation on polyacrylamide gels run under nondenaturing conditions as previously described (Davis 1964). Gel *b* was stained for catalase, gel *c* was stained for peroxidase, and gel *d* was stained for protein. The following proteins were run: wild-type KatG (lane 1), KatG14 (lane 2), KatG15 (lane 3), and KatG16 (lane 4).



KatG16 were activated less than the wild-type catalase by both reagents, whereas KatG15 was activated to a greater extent (Table 3). The effect of the reagents on the peroxidase activities was more complex with imidazole inhibiting the wild-type and KatG14 activities and activating the KatG15 and KatG16 activities, while Tris inhibited KatG14 and KatG15 but didn't affect the other two.

Sensitivity to inhibitors

Azide, cyanide, and hydroxylamine are known inhibitors of catalase and their effect on the mutant and wild-type enzymes was compared, revealing few differences. Azide was effective in inhibiting the catalase activity but not the peroxidase activity of all three mutants (Figs. 4*a* and 4*b*). Cyanide inhibited both the catalase and peroxidase activities at about the same rate, although the peroxidase activity of KatG14 appeared to be slightly less sensitive than the other enzymes (Figs. 4*c* and 4*d*). Hydroxylamine inhibited the catalase activities more effectively than the peroxidase activities and again KatG14 differed from the others in being slightly more sensitive to the reagent (Figs. 4*e* and 4*f*).

β-Mercaptoethanol at 1 mM exerted a strong inhibitory effect on the catalase activity of all three mutants (Fig. 5*a*), but had little effect on the wild-type enzyme. The peroxidase activity of all of the enzymes including wild type was more

FIG. 3. Effect of pH on the catalase activity of the mutant KatG proteins. Wild-type KatG (○), KatG14 (□), KatG15 (△), and KatG16 (▽), were assayed at the various pHs indicated. The values were normalized to the maximum specific activity.

TABLE 2. Spectral characteristics of the wild-wype and mutant HPIs

	Wild type	KatG14	KatG15	KatG16
Wavelength (nm)				
280	1.8	4.4	10.3	5.4
407	1.0	1.0	1.0	1.0
497	0.20	0.15	0.18	0.17
637	0.09	0.08	0.09	0.09
Calculated values				
Ratio 407/280	0.55	0.23	0.10	0.19
Heme/tetramer	2.2	0.9	0.4	0.8

NOTE: The spectral values are all normalized to 407 nm.

TABLE 3. Effect of Tris and imidazole on wild-type and mutant HPIs

	Wild type		KatG14		KatG15		KatG16	
Reagent	Cat	Per	Cat	Per	Cat	Per	Cat	Per
10 mM KP_i (pH 7.5)	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
10 mM Tris (pH 7.6)	1.60	0.98	1.29	0.72	1.81	0.81	1.06	1.00
10 mM Imidazole (pH 7.5)	4.64	0.78	1.61	0.77	5.06	1.15	2.51	1.13

NOTE: Cat, catalase; Per, peroxidase.

sensitive, being inhibited 50% or more by just 0.1 mM β-mercaptoethanol (Fig. 5*b*). Because the single cysteine residue present in wild-type HPI was not changed in any of the mutants, the change in sensitivity to β-mercaptoethanol must arise from a changed location of the sulfhydryl portion of the amino acid, allowing its reduced form to interfere with the catalase reaction as well as the peroxidase reaction.

Temperature sensitivity

The mutant enzymes were all more temperature sensitive than the wild-type enzyme. At 50°C, the wild-type enzyme had lost only 40% of both catalase and peroxidase activities after 20 min, whereas the catalase activity of KatG15 was completely inactivated within 1 min and the other two mutants retained activity for only 10 min (Fig. 6). The peroxidase activities of the three mutants were significantly more heat stable than the corresponding catalase activities, with KatG14, KatG15, and KatG16 retaining 31, 14, and 46% of peroxidase activity, respectively, after 20 min at 50°C. Once again, KatG15 exhibited the greatest conformational instability.

Discussion

Three mutant strains of *E. coli* that exhibit low levels of catalase *in vivo* have been used as sources of *katG* genes that encode modified HPI protein. Nonsense mutations were not involved and each mutation resulted in an amino acid substitution in the predicted protein sequence, Gly to Asp119, Gly to Asp304, and Leu to Phe139. The primary identifiable physical effects of these changes were a conformational change and reduction in the amount of heme bound. The amino acid substitutions could interfere with heme binding by direct steric interaction in the heme binding site or through a conformational change which modifies the heme binding site. For the Asp304- and Phe139-containing mutants, there was a fairly close correlation between heme content and the peroxidase activity. However, the catalase activity of the same two mutants and both peroxidase and catalase activities of the Asp119 mutant were much lower than would be dictated by the reduced heme content. The unchanged response of the mutant enzymes to various inhibitors and activators could also be explained by the observed reduction in heme content inactivating the enzyme without heme, but allowing enzyme with heme to retain normal properties. The greater temperature sensitivity of the mutant enzymes and their enhanced sensitivity to β-mercaptoethanol indicated that the amino acid substitutions had caused a conformational change modifying the environment of the single cysteine residue and reducing the stability of the active tetramer. This was particularly evident in the case of the Asp119 mutant where freezing and thawing resulted in rapid denaturation and precipitation of the protein.

The properties of the mutant enzymes were consistent with the phenotypes of the original three mutants (Loewen *et al.* 1985*b*). UM2 (*katG15*) appeared to produce an HPI protein, detectable by immunoprecipitation, with very low catalase and peroxidase activity, as determined by staining polyacrylamide gels. UM1 (*katG14*) produced an immunoprecipitable HPI with nearly normal peroxidase activity but low catalase activity and UM56-64 (*katG16*) produced an HPI with slightly reduced enzyme activity. There appeared to be differences in expression of the mutant genes based on the immunoprecipitation experiments, but sequence analysis did not reveal any changes in the promoter region of *katG*. Consequently, the apparent differences in expression may have been a reflection of the varying stabilities of the different proteins.

In the absence of crystallographic data for HPI, the location of the heme has been the subject of conjecture. The heme of peroxidases, like cytochrome *c* peroxidase and other plant peroxidases, is bound close to the surface of the enzyme, satisfying the need for steric access by organic electron donors to the heme (Keilin and Hartree 1951; Welinder 1985). Bovine catalase, on the other hand, has the heme bound quite deep within the protein where it can still be

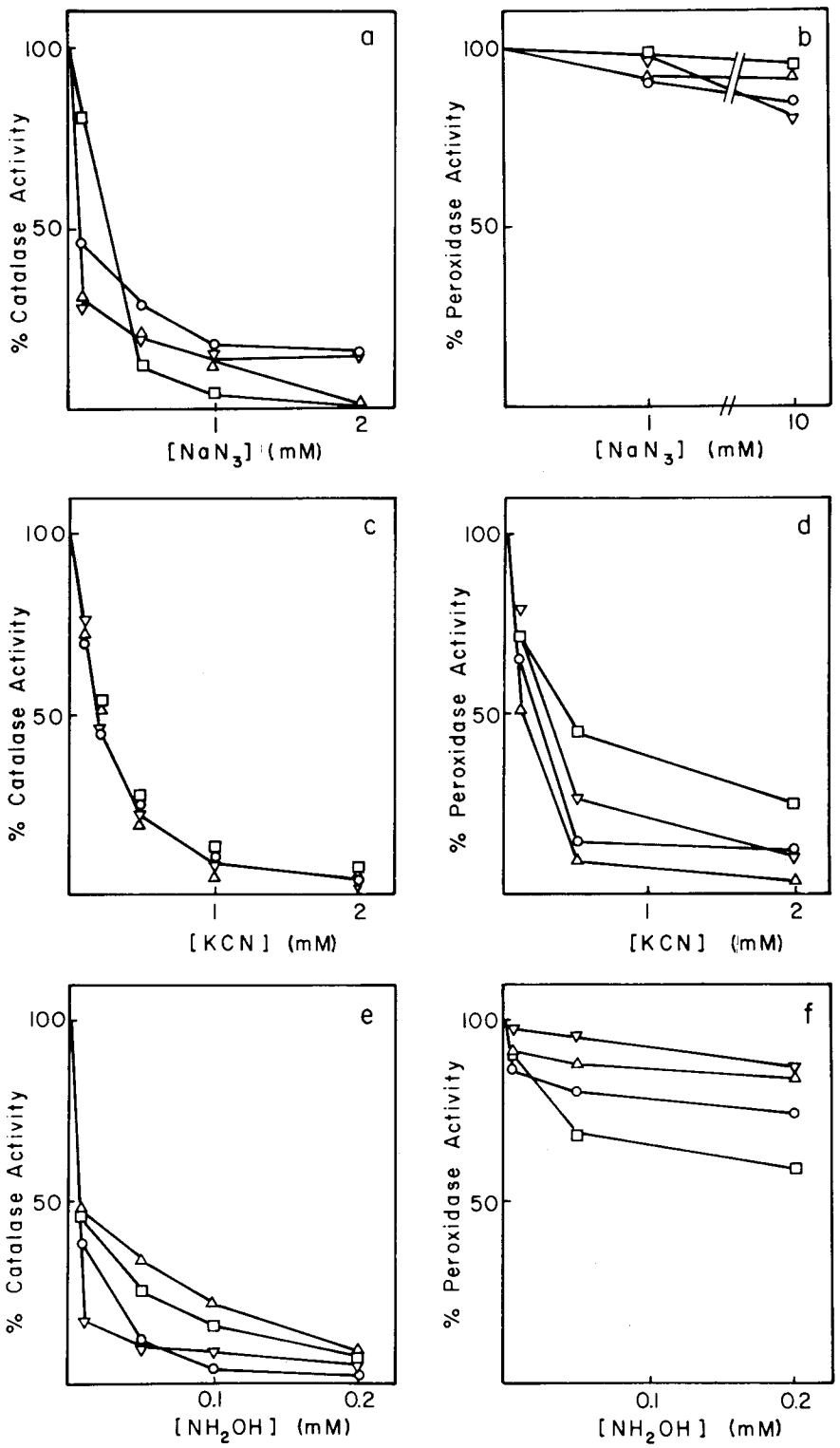


FIG. 4. Effect of various reagents on the catalase (*a*, *c*, *e*) and peroxidase (*b*, *d*, *f*) activities of the mutant KatG proteins. The effects of sodium azide (*a*, *b*), potassium cyanide (*c*, *d*), and hydroxylamine (*e*, *f*) were studied on wild-type KatG (○), KatG14 (□), KatG15 (△), and KatG16 (▽). For comparison, all activities were normalized.

accessed by hydrogen peroxide (Fita and Rossman 1985). It has been suggested that HPI and other similar catalase-peroxidases also may have the heme bound closer to the surface to fulfill the peroxidase role. This in turn may result in the heme being bound less tightly, allowing its loss during purification so that there would be less than the one heme per subunit normally found in most other catalases (Hochman and Shamesh 1987). The authors who originally purified HPI did not think that the heme was lost during isolation (Claiborne and Fridovich 1979), but in the case of the mutant enzymes such a loss might occur because of weaker binding.

The differential effect of sulfhydryl reagents on the catalase and peroxidase activities has never been extensively

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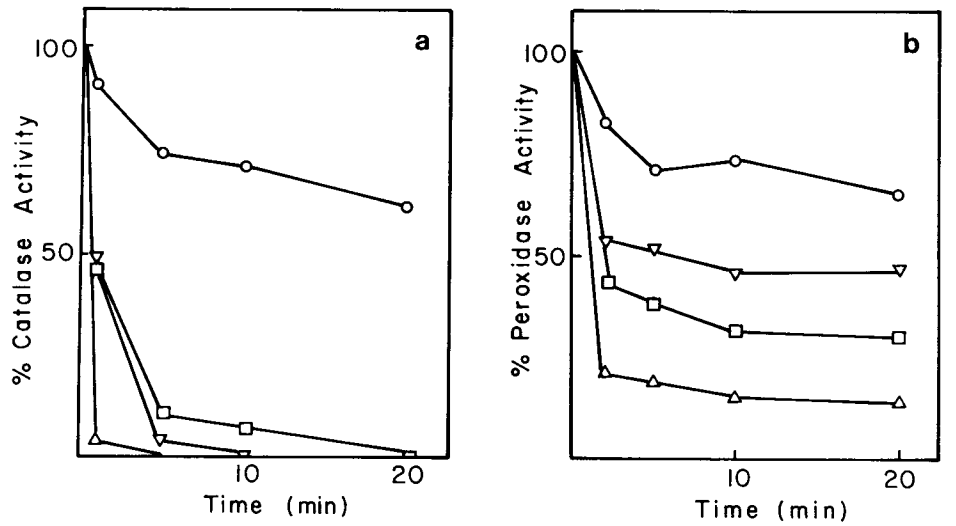

FIG. 5. Effect of 1 mM β-mercaptoethanol on the catalase (*a*) and peroxidase (*b*) activities of the mutant KatG proteins. Wild-type KatG (○), KatG14 (□), KatG15 (△), and KatG16 (▽), were studied. For comparison, all activities were normalized.

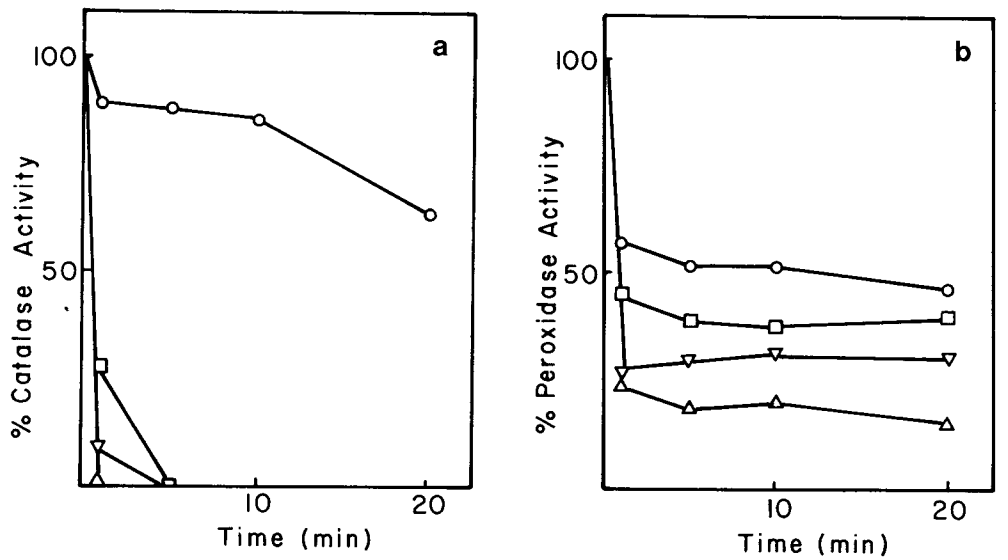

FIG. 6. Effect of incubation at 50°C on the catalase (*a*) and peroxidase (*b*) activities of the mutant KatG proteins. Wild-type katG (○), KatG14 (□), KatG15 (△), and KatG16 (▽) were incubated at 50°C for the times indicated before determination of the remaining activity. For comparison, all activities were normalized.

investigated. The single cysteine of HPI is in a location where its oxidation state does not appreciably affect the catalase activity but does greatly influence the peroxidase activity. The conformational changes resulting from the amino acid substitutions in all three mutants resulted in a change in location of the cysteine side chain, such that its reduction interfered with the catalase activity. The change in location of the sulfhydryl group had little effect on its interference with the peroxidase activity. A more detailed study of the role of the sulfhydryl group may lead to a better understanding of the differences between the peroxidase and catalase mechanisms.

Only one of the mutants, KatG14 (Phe139), exhibited a change in kinetic parameters that would suggest a change in reaction mechanisms. That was a significant change in the concentration of H_2O_2 required to attain 50% of apparent maximal catalase activity to 20.2 from 4.4 mM. This change could be explained by a lower rate of conversion of compound I (enzyme oxidized by H_2O_2) to compound II (Hochman and Shamesh 1987) for the Phe139-containing mutant. As a consequence there would be less compound II present which reacts only slowly with H_2O_2 and more compound I which reacts more rapidly with the second molecule of H_2O_2 to produce molecular oxygen.

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