Vol. 154, No. 1, 1988 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
July 15, 1988 Pages 392-397

COMPARTMENTALIZATION OF CATALASES IN ESCHERICHIA COLI

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Received May 9, 1988

Escherichia coli possesses three catalase genes: *katG* codes for protein HPI and *katE* codes for HPII; *katF* is also needed for HPII but may be a positive regulatory gene for *katE*. We have assayed for HPI and HPII in the outer cell membrane, the periplasmic space, the inner membrane, and in the cytoplasm of *E. coli*. Following synthesis of catalase in the cytoplasm, the active *katG* gene product (HPI) was found in the periplasmic and in the cytoplasmic membrane fractions. HPII remained in the cytoplasm.

Escherichia coli katG codes for catalase HPI, *katE* codes for protein HPII, and *katF* may act as a positive regulator gene for *katE* (Loewen, personal communication). HPI is a tetramer (Mol. Wt. 337,000) containing two molecules of protoheme IX per tetramer (1) and can be observed electrophoretically on a 9.5% polyacrylamide gel (PAGE) (2); under some conditions, it appears on gels as an electrophoretic variant pair (Fig. 1), HPI-A and HPI-B (3). The synthesis of both variants is under the control of the *katG* gene (4). HPI is bifunctional; in addition to dismutation of H_2O_2 to O_2 and H_2O, HPI also exhibits broad spectrum peroxidase activity. HPI is inducible, but HPII is constitutive (5). HPII, the product of *katE* gene (6, 7) is also a tetramer of identical subunits containing two molecules of protoheme IX per tetramer. HPII possesses only hydroperoxidase activity and thus differs from HPI (8). No serological cross-reaction occurs between the two (9).

Since it is obvious that the two *E. coli* catalases do not have identical functions, we examined the possibility that they may act at different cellular sites. It has been reported that a heme protein possessing catalase activity is associated with the *E. coli* cytoplasmic membrane (10). It accounts for less than 5% of the total heme; it has not been specifically identified as HPI, although it was found to have broad spectrum peroxide activity. There is other evidence that HPI biological activity may occur at the membrane level. When *E. coli* cells (wildtype) are treated with low, non-lethal doses of H_2O_2, they immediately lose the capacity to incorporate amino acids (11). This change in permeability lasts for about 30 minutes and is one of the characteristics of the amino acid uptake delay phenomenon (12). If the cells receive a challenge dose of H_2O_2 30 min. after the inducing dose, the amino acid uptake delay is over-ridden; i.e., the uptake of amino acids is delayed for only 1 to 2 min. This suggests that the over-ride is a result of the induction and synthesis of critical proteins. A *katG* mutant deficient in HPI activity, exhibits no over-ride of the amino acid uptake delay; i.e., a second H_2O_2 dose is

0006-291X/88 $1.50

accompanied by a second, equally long, amino acid uptake delay (11). These observations suggest that induced catalase HPI is active at a cell permeability site and is important in recovery from uptake delay. However, no reports have been found of the site of action of HPI nor of a specific physiological role. We now report that the product of *katG*, HPI, was found to be associated with the inner membrane on the periplasmic side, but that HPII remained in the cytoplasm. Thus, there may be functional differences between the two catalases.

MATERIALS AND METHODS

Bacterial Strains: *E. coli* strains are listed in Table 1. These include wildtype, mutant and plasmid strains in which only HPI or HPII would be present, or over-produced.

Since a mutation of either *katE* or *katF* resulted in no synthesis of active HPI, and since plasmid strains of each of the two both resulted in excessive synthesis of HPII, only figures utilizing *katF*:: TN10 and the $katF^+$ plasmid strain are presented.

Cell Fractionation:

Whole Cell Extracts: Cells were grown at 37° C to late exponential phase in LB medium. Cultures (20 ml) were harvested by centrifugation at 4° C, pellets were rinsed in O.1M Tris HCl (pH 8.0), resuspended in 0.5 ml of buffer, and ultrasonically disrupted with five 20 sec. pulses at 0° C. Unlysed cells were removed by centrifugation at 2,000 X g for 10 min. Cellular membranes were obtained by centrifugation at 180,000 X g at 4° C for 2 hours.

Spheroplast Preparation: Spheroplasts were obtained by resuspending the pellet of 20 ml of centrifuged cells in 1 ml of 0.1M Tris HCl (pH 8.0), 10 μl of 2mg/ml lysozyme, and 20 μl of 0.1M EDTA pH 8 (13). Cells were incubated for 10 min at 23° C then centrifuged at 8,000 X g for 10 min. The supernatant, which contained "leaked" periplasmic proteins and portions of the outer membrane (14), was saved for further analysis. The pellet was suspended in 0.5 ml of 50mM Tris HCl (pH 8) and disrupted ultrasonically. Cell membrane and debris were removed as described above.

Cellular Membrane Preparation: The membrane fraction (obtained as described above), was resuspended in Tris (pH 7.8), 1% SDS. The membranes were heated to 99° C for 10 minutes to solublize membrane polypeptides.

Glucose-Pulse: We shifted an exponentially growing culture in 1% casamino acids (CAA) M-9 containing no glucose to a 1% CAA/M9 medium supplemented with 1% glucose medium for 1 hour at 37° C (15). Cells (20 ml) were harvested and fractionated as described above.

Non-Denaturing Gel: Total protein (25 ul) in 10% glycerol was applied to a nondenaturing polyacrylamide mini gel. The 9.5% gel continuous buffer system, was electrophoresed for 1.5 hours at 200 V. Catalase activity was assayed *in situ* by a method

TABLE 1

Strain, main genotype and source (reference)	
AB1157 (wildtype)	Our Laboratory
UM202 *katG*::Tn10	Loewen
UM122 *katF*::Tn10	Loewen
UM228 pBT22 $katG^+$ plasmid	Loewen
UM258 pMM $katF^+$ plasmid	Loewen
UM120 *katE*::Tn10	Loewen
pAM $katE^+$ plasmid	Loewen

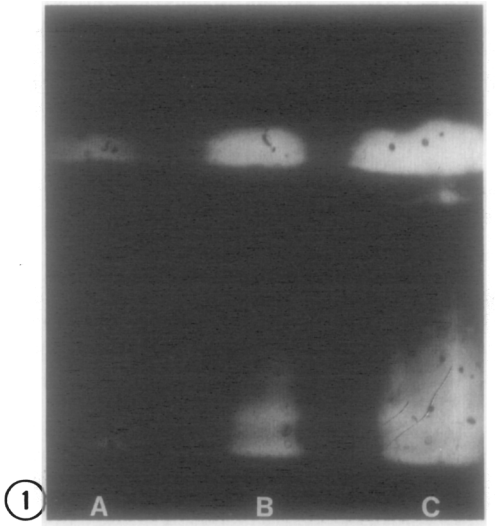
previously described (16). The modified procedure was as follows: we soaked the gel in .03% H_2O_2 for 15 min., rinsed with H_2O, and stained with a 50:50 mixture of 2% $K_6(FeCN)_6$ and 2% $FeCl_3$.

RESULTS

When wildtype cells were grown in LB and PAGE assayed for catalase, the two types, HPI and HPII, were readily observed (Fig. 1). After cells were fractionated, the spheroplast fraction was collected; it lacked the proteins of the periplasmic space and portions of the outer membrane. The periplasmic fraction consisted of outer cell membranes, periplasmic contents and various proteins normally bound to the cytoplasmic membrane, but which may have become unbound in the fractionation process.

As may be seen from Fig. 2, when wildtype cells were broken open and fractionated, the spheroplast fraction (lane A) contained a large quantity of HPII (upper band), and a very small quantity of HPI (lower band). In contrast, the periplasmic material (lane B), contained a large quantity of HPI and a very small amount of HPII. When the entire membrane (outer and inner) fraction was collected (lane C), only HPI activity was detected.

The above results were confirmed when mutants of *katE* or *katF* (lacking HPII) or *katG* (lacking HPI) were used, as illustrated in Figs. 3 and 4. For the *katF* mutant (Fig. 3), HPI activity was abundant in the whole cell preparation (lane A), greatly reduced in the



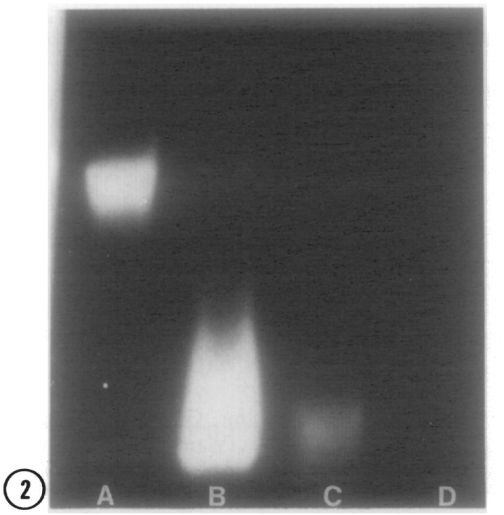
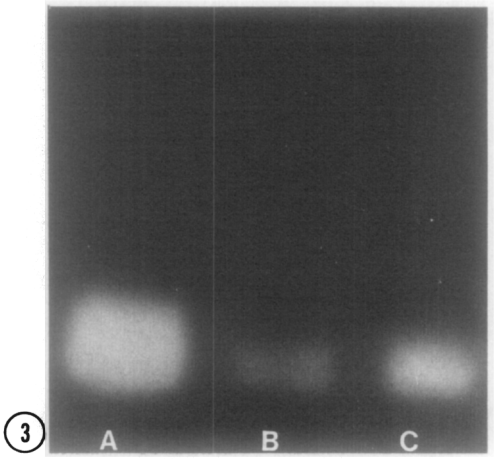


FIGURE 1: Strain AB1157 (wildtype). Three different concentrations of protein (Lanes A, B and C, = 300, 600, 900 μg) respectively were loaded on a 9.5% non-denaturing polyacrylamide gel. The top band is HPII, the product of *katE*, the lower bands are the electrophoretic variant pair HPI-A and HPI-B, the products of *katG*. Bubbles of oxygen often appear when H_2O_2 is added to gel in staining process. These appear in photographs as black dots.

FIGURE 2: Strain AB1157 (wildtype). Spheroplast fraction (lane A) contained a large quantity of HPII and a decreased amount of HPI. In contrast, the periplasmic fraction (lane B) contained an abundance of HPI. Only HPI was present in the whole cell membrane fraction (lane C) which consisted of inner and outer membranes. The inner membrane (lane D) was devoid of either catalase activity.



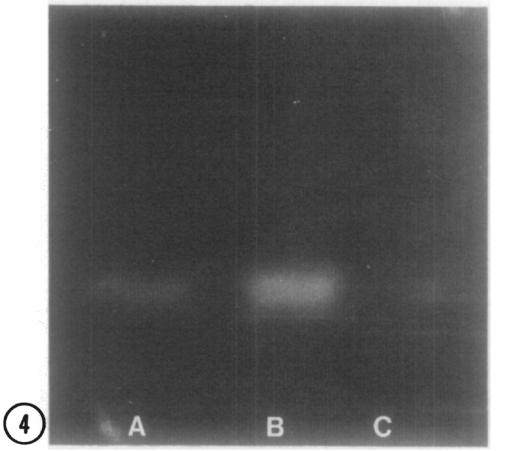


FIGURE 3: Strain UM122 *katF*::Tn10. Only HPI activity was present in the whole cell extract (lane A). It was reduced in the spheroplast fraction (lane B), and present in large quantities in the periplasmic fraction (lane C).

FIGURE 4: Strain UM202 *katG*::Tn10. Whole cell extract (lane A) and the spheroplast fraction (lane B) contained exclusively HPII. No catalase activity was detected in the periplasmic fraction.

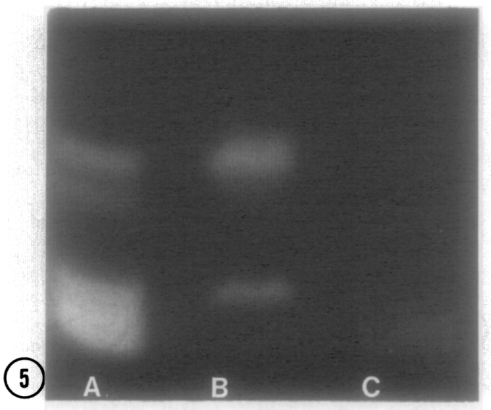
spheroplasmic fraction (lane B), but a large quantity was seen in the periplasmic fraction (lane C). For the *katG* mutant (Fig. 4), HPII was found in the whole cell and spheroplast fractions, (lanes A and B), but no catalase activity was detected in the periplasmic fraction (lane C).

When plasmid strains were tested, a similar pattern was seen. For the *katG* plasmid strain (Fig. 5), there was an abundance of HPI and the normal amount of HPII in the whole cell (lane A). In the spheroplast fraction (lane B), HPI was greatly reduced, but was present in the periplasm (lane C). In the case of the *katF* plasmid-carrying strain (Fig. 6), first it should be noted that HPII was greatly overproduced (lane A). It was present in the spheroplast (lane B), but absent in the periplasmic space.

All of these observations verify the compartmentalization of catalase activity, with HPII active in the cytoplasm, but HPI active in the periplasmic fraction.

We additionally examined the membrane fraction of wildtype AB1157. The whole cell membrane fractions, consisting of inner and outer membranes, contained only HPI (Fig. 2) (lane C). The spheroplast membrane fraction, principally the inner membrane (lane D), was devoid of either catalase activity.

To determine if newly synthesized molecules in transit to other cellular sites might obscure subcellular localization, cells were grown in 1% glucose for 1 hr. prior to collecting cells for assay. Since glucose retards catalase synthesis (13, 15), shifting cells to medium with 1% glucose reduces the amount of newly synthesized catalase and allow endogenous catalase molecules to localize to sites of activity. Cells exposed to glucose showed identical subcellular distribution of endogenous catalase as those cells not exposed to glucose. Thus, transient catalase was not a significant factor in observing localization.



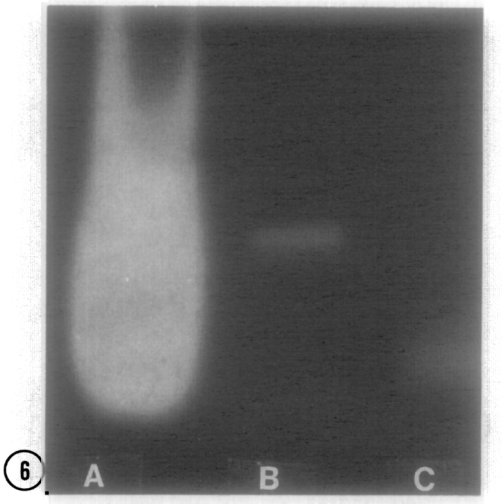


FIGURE 5: UM228 *recA* PBT22 *katG*$^{+}$. Abundance of HPI and normal amounts of HPII in the whole cell (lane A); however HPI was reduced in the spheroplast fraction (lane B). Only HPI was present in the periplasmic fraction (lane C).

FIGURE 6: UM258 pMM *katF*$^{+}$. Overproduction of HPII (whole cell extract) (lane A). The spheroplast fraction (lane B) contained HPII with a trace amount of HPI while the periplasmic fraction (lane C) contained only HPI.

Results of experiments utilizing *katF* mutant and plasmid strains are presented in figures; however, assays utilizing *katE* mutant and plasmid strains were also performed, but since the results were the same as that observed for *katF*, separate figures are not presented.

DISCUSSION

These results indicate that the two catalases may have functional differences associated with their locality in the cell. HPI may be involved in very specific decomposition of peroxides at the membrane level, while HPII may decompose H_2O_2 that arises within the cytoplasm.

Trace amounts of HPII found in the periplasm might indicate slight hypotonicity of the spheroplast conversion solutions resulting in lysing of some cells and therefore contamination of the sample with the cytoplasmic contents. Also, trace amounts of HPI found in the spheroplast fraction may indicate that catalase either was not totally dissassociated from the cytoplasmic membrane or had reassociated. Catalase HPI was found in the whole cell (inner and outer) membrane fraction but could not be detected in the spheroplast (inner) membrane fraction. Since the inner membrane was obtained from the spheroplast fraction, various membrane-bound proteins could have dissociated into the periplasm, particularly if they were located on the periplasmic side of the inner membrane. Thus, HPI on the periplasmic side probably disassociated during sample preparation.

Since we did not observe HPII in the periplasmic space, we concluded that *E. coli* mutants that lack HPI do not compensate for this lack by redistributing HPII to the periplasmic side of the inner membrane.

ACKNOWLEDGEMENT

This investigation was supported in part by National Science Foundation (DMS-85027-08) and by University of Missouri Institutional Biomedical Research Support Grant RFR 07053 (N. I. H.) A. H. was a recipient of an undergraduate summer award under the NSF-REU Program.

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