ELSEVIER

Gene 179 (1996) 39–44

GENE
AN INTERNATIONAL JOURNAL ON GENES AND GENOMES

Probing the structure of catalase HPII of *Escherichia coli* – a review [1]

Peter Loewen *

Department of Microbiology, University of Manitoba, Winnipeg, Manitoba, R3T2N2, Canada

Abstract

Escherichia coli produces two catalases or hydroperoxidases, HPI and HPII. HPI is a bifunctional catalase-peroxidase active as a tetramer of identical 80 049-Da subunits encoded by *katG*. The expression of *katG* is controlled at the basal level by σ^S (KatF), and its induction by H_2O_2 is regulated by OxyR. HPII is a monofunctional catalase active as a tetramer of identical 84 118-Da subunits encoded by *katE*. The induction of *katE* expression in the stationary phase is controlled by σ^S. The core of HPII is similar in sequence to other catalases including the conservation of several residues that have been implicated as playing a catalytic role, His^{128}, Asn^{201}, Ser^{167} and Tyr^{415}. These residues have served as targets for site-directed mutagenesis in a study that has demonstrated their role in the catalytic mechanism of HPII. In addition, the two Cys residues in HPII have been targeted in a similar study revealing that they do not have a catalytic role, but that Cys^{438} is blocked by a novel modification. Despite many structural similarities to bovine liver catalase, the heme component of HPII has proved to be quite different. The presence of a *cis* heme d was determined spectrally and chromatographically, and the inability of certain mutants to generate the modified heme revealed that it was HPII itself that was catalysing the oxidation of heme b to heme d. The recent solution of the crystal structure of HPII and mass spectrometry have revealed that the heme d bound to HPII is a spirolactone structure with a *cis* orientation of the oxygens on the proximal side of the heme. This has created the problem of explaining how the oxidation of the heme can occur on the opposite side of the heme ring, remote from the catalytic residues.

Keywords: Peroxidase; Heme; Site-directed mutagenesis; Hydroperoxidase; Catalysis

1. Background

The study of catalase genetics and physiology in *E. coli* evolved from an investigation of the mechanism of phage inactivation by ascorbate. Ascorbate is not transported into *E. coli* but does react with molecular oxygen in the medium to produce H_2O_2. This in turn can diffuse into the cell giving rise to hydroxyl radicals which can react with DNA to produce the single strand breaks responsible for phage inactivation (Richter and Loewen, 1982). Catalase (hydrogen peroxide:hydrogen peroxide oxidoreductase, EC 1.11.1.6) found in most aerobic organisms employs a two electron transfer mechanism in the dismutation of H_2O_2 to water and oxygen and is an obvious intracellular protectant against H_2O_2. Indeed we demonstrated that ascorbate caused an induction of catalase in *E. coli* (Richter and Loewen, 1981). As confirmation of the role of H_2O_2 in phage inactivation, catalase would be expected to modulate the effect and differences would be expected between catalase proficient and deficient mutants of the host. Unfortunately at that time, no catalase-deficient mutants of *E. coli* had ever been reported.

Despite the lack of genetic information, the biochemistry of catalases in *E. coli* had been extensively studied including the purification and characterization of two catalases, or hydroperoxidases, with very different properties. Hydroperoxidase I (HPI) was characterized as a bifunctional enzyme exhibiting catalatic and *o*-dianisidine peroxidatic activity in a tetrameric structure containing protoheme IX (Claiborne and Fridovich, 1979). Hydroperoxidase II was characterized as a monofunctional catalase containing a modified heme (Claiborne et al., 1979; Loewen and Switala, 1986) that gave the

* Corresponding author. Tel.: (1-204) 474-8334; Fax: (1-204) 275-7615; e-mail: Peter_Loewen@UManitoba.ca

[1] Presented at the Chulaborn Research Institute International Conference on 'Biotechnology Research and Applications for Sustainable Development (BRASD)', Central Plaza Hotel, Bangkok, Thailand, 7–10 August 1995.

Abbreviations: aa, amino acid(s); BLC, bovine liver catalase; bp, base pair(s); HP, hydroperoxidase; *katE*, gene encoding the HPII subunit; *katF*, gene encoding the sigma transcription factor σ^S; *katG*, gene encoding the HPI subunit; kb, kilobase(s) or 1000 bp; nt, nucleotide(s); OxyR, oxidative stress transcription regulator.

PII S0378-1119(96)00321-6

enzyme a characteristic green color. Any genetic studies of catalase would therefore be complicated by the presence of two unique enzymes.

2. Characterization of catalase-encoding genes in *E. coli*

A number of catalase mutants of the closely related *Salmonella typhimurium* had been reported (Levine, 1977) and four different loci identified. One locus, *katC*, was tentatively located on an F′ episome from *E. coli* resulting in it being included on the *E. coli* genetic map. None of these mutations were linked to an easily transducible marker which would have allowed their transfer into *E. coli* and a search for new mutants was initiated. Ultimately, a number of catalase-deficient mutants were isolated and used to identify three different loci that affected catalase levels. The first locus reported was *katE* (*katA* to *katD* had been reported in *S. typhimurium*) which mapped at 37.2 min and affected the production of HPII (Loewen, 1984). The second was *katF* which also affected HPII levels and which mapped at 59 min (Loewen and Triggs, 1984). Finally, *katG*, which affected HPI expression, was identified and mapped at 89.2 min (Loewen et al., 1985a, b).

As soon as the catalase-deficient mutants were isolated, work to clone the genes was initiated. The structural gene for HPI, *katG*, was first identified in the Clark and Carbon library (Loewen et al., 1983), subcloned for characterization (Triggs-Raine and Loewen, 1987), and sequenced (Triggs-Raine et al., 1988). The sequence of HPI inferred from the DNA sequence suggested that the protein was more closely related to plant peroxidases than to catalases. Subsequently, *katE* and *katF* were cloned (Mulvey et al., 1988) confirming that *katE* was the structural gene for HPII which had an inferred sequence (von Ossowski et al., 1991) very similar to the sequences of all other heme-containing catalases (von Ossowski et al., 1993). The sequence of *katF* (Mulvey and Loewen, 1989) revealed a protein that closely resembled σ transcription factors. This lead to the suggestion that KatF controlled expression of a regulon involved in stationary phase metabolism, including *katE*. Gene *katF* has since been renamed *rpoS* to make the genetic nomenclature consistent with that of other sigma factors in *E. coli* (Lange and Hengge-Aronis, 1991)

3. Catalase physiology

Early work had suggested differences in expression patterns between the two catalases in *E. coli* (Hassan and Fridovich, 1978) but the results were confused by the inability to clearly differentiate between the two catalase activities in cell extracts. Once available, mutants lacking one or the other of the catalases facilitated a study of in vivo variations in catalase levels in which there was a clear differentiation between the two enzymes (Loewen et al., 1985a, b). This study revealed that the synthesis of HPI was induced in response to H_2O_2 and the induction was ultimately shown to be modulated by OxyR (Morgan et al., 1986). Thus *katG* was part of the *oxyR* regulon in which OxyR controls expression of a number of genes in response to oxidative stress.

By contrast, HPII synthesis did not respond to H_2O_2 and remained low throughout early exponential phase, finally increasing six to ninefold as the cells made the transition to stationary phase (Loewen et al., 1985a, b). KatF was required for this induction and subsequent work by several groups has shown that a number of factors can influence *katF* expression (and therefore *katE* expression) including aromatic acids (Mulvey et al., 1990), weak acids generally (Schellhorn and Stones, 1992), and ppGpp (Gentry et al., 1993). The involvement of the latter nucleotide links the initial stages of the stationary phase response to the stringent response although the normal negative effect of ppGpp on RNA synthesis in the stringent response is reversed such that there is a positive correlation between ppGpp and the synthesis of KatF (or RpoS). Clarification of the mechanisms controlling KatF expression (and therefore HPII expression) has been complicated by the presence of multiple transcription start points (Takayanagi et al., 1994) upstream of *katF*, the absence of a clear mechanism for regulation of transcription initiation and the involvement of posttranscriptional controls (Loewen et al., 1993; McCann et al., 1993).

Induction of *katE* in stationary phase suggested a role in long term survival and this was tested by studying the survival of various catalase-deficient mutants during prolonged incubation in stationary phase, conditions that might be encountered by cells in the natural environment. Wild-type and *katG* mutants survived quite well whereas there was a striking decrease in survival in *katE*-containing mutants and an even greater drop in *katF*-containing mutants. HPII was important for long term survival under starvation conditions but the greater loss of viability in *katF*-containing mutants confirmed that KatF was controlling expression of a number of genes in addition to *katE* that had significant roles in survival (Mulvey et al., 1990). Most of these have yet to be characterized.

4. Structural relationships of HPII to other catalases

As already noted, the sequence of HPII derived from the DNA sequence of *katE* suggested a very close structural relationship with other catalases. At 753 aa and 81 kDa, the subunit of HPII was considerably larger than the subunits of normal catalases which classically

fell between 55 and 65 kDa. However, there was significant similarity between the core parts of the enzymes with the segments at the N and C-termini containing the extra sequence. This allowed the identification of a number of highly conserved aa among the catalases including several of which had already been identified in biochemical studies and in the X-ray structure of BLC as having potential roles in the catalytic mechanism (Murthy et al., 1981; Fita and Rossman, 1985). For example, His^{74} had been identified biochemically as being essential for catalytic activity in BLC and was subsequently found in the core of the active site situated immediately above the heme. Other residues implicated in the catalytic mechanism of BLC by their proximity to the active site included Asn^{147} and Ser^{113}. In addition Tyr^{357} was identified as the heme ligand on the proximal side. Following their identification, these key residues and the heme iron atom were combined in a mechanism for the reaction with H_2O_2 (Fita and Rossman, 1985). All of these residues were found to be conserved in HPII where the equivalents were His^{128}, Asn^{201}, Ser^{167} and Tyr^{415}.

We set out to confirm the catalytic importance of these residues by constructing a number of mutants with the residues changed by site-directed mutagenesis. Table 1 contains a list of the various mutants that were constructed and the specific activities of the mutants for which protein was actually isolated. The first point to note was that fewer than half of the mutants constructed actually accumulated protein. This was attributed to an inability of the mutant protein to fold correctly and thereby avoid proteolysis. Indeed most of the mutants produced protein only when grown at 28°C rather than 37°C suggesting more favorable folding conditions at the lower temperature either because of slower protein synthesis providing the chaperonins more time to interact with the protein or to slower proteolysis rates or a combination. Consequently, most mutant enzymes were isolated from cells grown at the lower temperature.

5. His^{128}

Early biochemical studies had implicated this residue (or rather its equivalent His^{74} in BLC) as an essential residue in catalysis (Chang and Schroeder, 1972) and our results confirm this. In BLC, the imidazole ring of His^{74} is situated immediately above ring III of the heme in a location where its participation as a catalytic factor is easily explained. Of the five His^{128} replacement mutants constructed, only two generated mutant enzyme, $His^{128} \rightarrow$Ala and $His^{128} \rightarrow$Asn, and both of these mutants were devoid of any detectable catalatic activity. These two mutants also had significantly different absorption spectra from the wild-type enzyme suggestive of a different heme being present. The significance of this will be addressed below.

6. Asn^{201}

In BLC, the equivalent Asn^{147} is located in the active site close to the heme and His^{74}. In the proposed mechanism, it plays a role in binding H_2O_2 causing the polarization of bonds that facilitates the reaction. The replacement mutants listed in Table 1 were constructed and all but one resulted in the production of mutant enzyme with varying levels of activity. Replacement of the Asn with either Ala or Asp resulted in an enzyme with 10% of non-mutant activity revealing that the Asn was not absolutely required for catalysis but that it was important in facilitating the reaction. Replacement with His or Gln resulted in an enzyme with less than 1% activity remaining revealing that the residue was located in a position where larger groups could sterically interfere with the reaction. Physical characterization of these mutants revealed that some exhibited different absorption spectra as compared to the wild-type enzyme suggesting the presence of different hemes, a fact which will be addressed below. The double mutant $Asn^{201} \rightarrow His/His^{128} \rightarrow Asn$ was constructed to see if simply having a His and an Asn in the active site, albeit in different positions, would be sufficient to reconstitute activity. Mutant protein accumulated but was found to be inactive indicating that the location of the residues was important in catalysis..

7. Tyr^{415} and Ser^{167}

The Tyr^{415}, equivalent of Tyr^{357} in BLC, is the proximal ligand to the heme iron and would be expected to be important, particularly for heme binding and folding. In peroxidases, His is commonly found as the distal ligand to the heme suggesting that a $Tyr^{415} \rightarrow$His mutant might be possible, but no protein accumulated. The equivalent to Ser^{167} of HPII is Ser^{113} in BLC where it is an integral part of a hydrogen bonded complex involving a water molecule and the propionate side chain on the heme. A number of mutants were constructed that replaced Ser^{167} but none resulted in the accumulation of mutant protein. Consequently, we could only conclude that these aa residues were essential for proper folding of the subunit and no other conclusions about the importance of the residues in catalysis could be drawn.

8. Cys^{438} and Cys^{669}

Catalases generally have a small number of Cys residues in comparison to their size and HPII is no exception having just two, Cys^{438} and Cys^{669}, among 753 aa residues, Both aa are found on the surface of the subunit well removed from the deeply buried active site although Cys^{438} is close to the entrance to the channel through which the H_2O_2 accesses the active site. It is also a residue that is conserved among the catalases. To investigate the role of these aa we began by changing them individually and in combination to Ala and Ser. Replacement of the conserved Cys^{438} with either Ala or Ser caused a small reduction in specific activity while replacement of Cys^{669} had no effect leading to the conclusion that neither Cys was required for catalysis. Reaction of HPII with mercaptoethanol caused an irreversible 50% inactivation that was independent of the Cys residues providing further evidence that the cysteines were not a direct factor in catalysis (Sevinc and Loewen, 1995).

In characterizing the Cys replacement mutants, it was observed that the sulfhydryl group of Cys^{438} was blocked and was not available for reaction with 5,5′-dithiobis-(2-nitrobenzoic acid) whereas Cys^{669} existed with a free sulfhydryl group. The blocking group was sensitive to alkali but did not react with methylamine, hydroxylamine or reducing agents, eliminating several possible modifications including disulfides, thiol esters, thiol carbamates and cysteic acid. The CNBr-cleaved peptide containing Cys^{438} was generated and subjected to mass spectrometry revealing that the modification had a mass of 43 ± 2. Having eliminated several possible structures, we are currently left with a hemithioacetal as the only structure consistent with the existing data, but further work is required to confirm this structure.

9. Heme structure

The original characterizations (Claiborne et al., 1979; Loewen and Switala, 1986) of HPII had noted that the enzyme exhibited an unusual absorption spectrum unlike that of normal catalases which contained heme b (protoheme IX). This was attributed to the presence of a modified heme which was ultimately characterized as cis heme d (Chiu et al., 1989) which could arise from heme b by a simple epoxide formation on ring III followed by hydrolysis to the *cis* diol (Timkovich and Bondoc, 1990) possibly involving a spirolactone intermediate (Fig. 1). This characterization did not determine the side of the heme on which the cis diol was situated nor the mechanism for modification. As noted above the spectra of some of the replacement mutants, notably both of the His^{128} mutant enzymes and the Asn^{201}→His mutant were different from wild-type HPII and more similar to

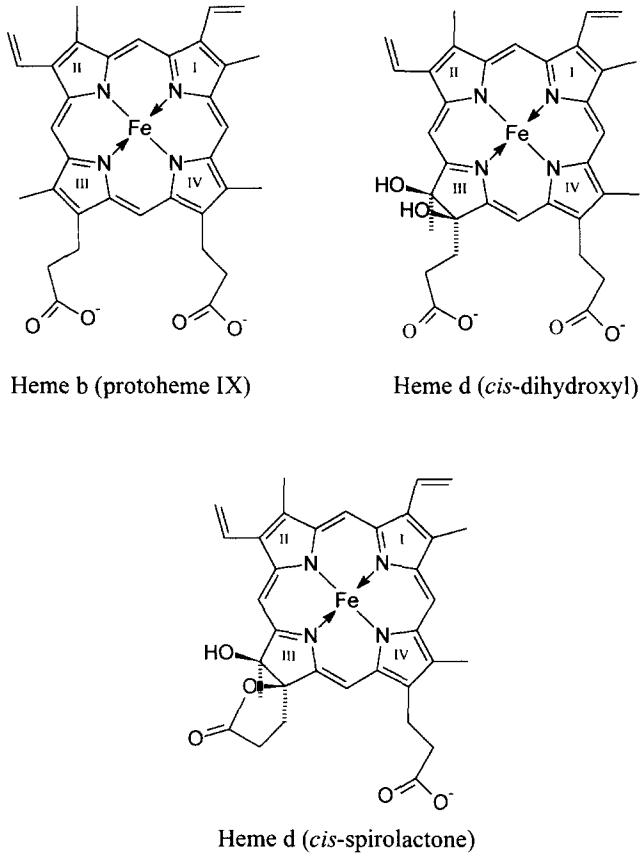

Fig. 1. Structures of heme b (protoheme IX), the *cis* dihydroxyl form of heme d and the spirolactome form of heme d with the oxygens in the *cis* conformation. The conformations presented here are as they exist in or are isolated from HPII or mutant HPII (Murshudov et al., 1996).

the spectra of heme b-containing proteins (Loewen et al., 1993). The presence of heme b was confirmed by spectral analysis and by HPLC elution of the isolated heme. It therefore appeared that these mutants were defective in either the binding or the generation of heme d.

Analysis of the heme content of wild-type HPII by HPLC revealed a small amount of heme b in addition to the predominant heme d suggesting that the enzyme might initially bind heme b which became the substrate for the cis hydroxylation by HPII in one of the early rounds of reaction with H_2O_2. To confirm this hypothesis, we isolated HPII from anaerobically grown cells and determined that most of the heme was heme b, but that upon treatment with H_2O_2 in the absence of any other protein, the heme was converted to heme d (Loewen et al., 1993).

Analysis of the heme content of the various mutants corroborated this conclusion. Both of the His^{128} replacement mutants contained only heme b, rather than heme d, suggesting that the absence of His^{128} was a block to the heme oxidation. Thus, the proposed role of His^{128} in binding H_2O_2 for the catalatic reaction may apply

equally to heme modification. Most of the Asn^{201} replacement mutants contained heme d indicating that this residue was not required for the modification. However, the Asn^{201}→His mutant contained only heme b and conversion to heme d was not possible suggesting that the larger His residue could sterically block the reaction.

HPII can therefore be considered a bifunctional enzyme. Its primary role is to remove H_2O_2, but it also has a heme oxidase activity. Whether the oxidized heme makes HPII more reactive has not been determined because the heme seems to be modified very rapidly in some of the first rounds of reaction with H_2O_2. To date a mutant that has full catalatic activity but which does not catalyse heme oxidation has not been isolated. It should be stressed here that the heme was extracted from the enzyme using acetone:HCl which could itself have caused modification of the heme such that the final product was not necessarily what existed within the enzyme complex. Other structures including an epoxide and a spirolactone could exist and be hydrolysed to the *cis* diol.

The recent solution of the crystal structure of HPII (Bravo et al., 1995) has finally provided us with a precise picture of the subunit such that we no longer have to base experiments on the structure of BLC. In fact there is considerable similarity between the enzymes with regards the locations of the key conserved residues which is not surprising given the high degree of identity between BLC and HPII in the core. For example, the key residues already studied, His^{128} and Asn^{201}, are in the active site pocket and close to the heme in positions analogous to their positions in BLC. However, HPII is larger at both the amino and carboxyl ends which adds considerable complexity to the structure of HPII.

Perhaps the most striking difference between the two

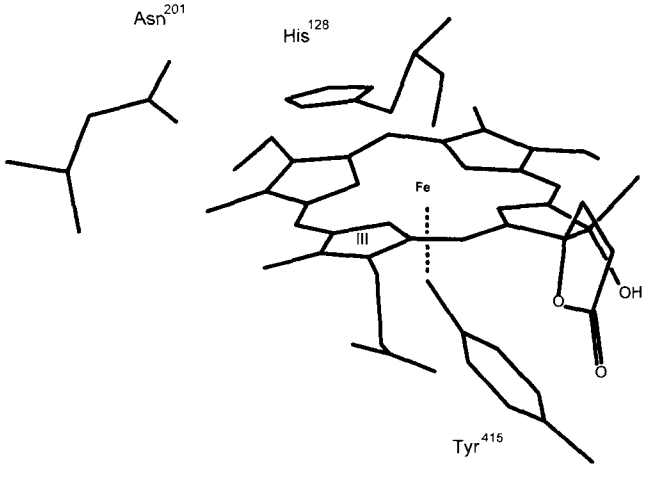

Fig. 2. Computer-generated diagram illustrating the steric relationships of heme d, the catalytic residues, His^{128} and Asn^{201} (Bravo et al., 1995). The oxygens of the *cis* spirolactone are on the proximal or Tyr^{415} side of the heme (Murshudov et al., 1996).

enzymes is the 'flipped' orientation of the heme in HPII compared to BLC resulting in the proximal side in BLC becoming the distal side in HPII. This results in the essential His^{128} and the adjacent Asn^{201} being situated over heme ring IV rather than ring III, but the proposed catalatic mechanism proposed for BLC can be adopted essentially unchanged for HPII. However, the 'flipped' heme results in the site of oxidation, ring III, being well removed from the catalytic residues His^{128} and Asn^{201} (Fig. 2). This has generated a fundamental question about the mechanism of catalysis of hydroxylation. How can residues that are distant from the reaction site participate in catalysis?

Another very surprising observation which has only just come to light is the actual identity of the heme. As noted above there has been a question about the identity of the bound heme as compared to the cis diol that is isolated. From the recently completed 2.2 Å structure of HPII (Murshudov et al., 1996), the heme has been identified as a spirolactone but with the unexpected orientation of the cis oxygens on the proximal side. Oxidation of the heme is therefore occurring on a portion of the heme that is not only remote from the catalytic residues His^{128} and Asn^{201}, but on the opposite face. This begs the question, what is the mechanism that gives rise to oxidation on the side opposite from the site of H_2O_2 approach, at a location that is buried and inaccessible? This is currently under investigation.

10. Conclusions

(1) *E. coli* produces two unique catalases that are expressed individually either in response to peroxide in the medium (HPI) or during the transition to stationary phase (HPII).
(2) HPI is a catalaseperoxidase with sequence identity to plant peroxidases.
(3) HPII is a monofunctional catalase with strong sequence identity to typical catalases.
(4) Residues including His^{128} and Asn^{201} have been confirmed as being essential for catalysis.
(5) HPII contains a spirolactone heme d in a 'flipped' conformation relative to the bovine catalase that is generated by the oxidation of heme b by HPII itself.
(6) The sulfhydryl group of Cys^{438} is blocked by a novel modification but neither Cys residue in HPII plays a role in catalysis.

Acknowledgement

This work was supported by grant OGP09600 and several postgraduate scholarships from the Natural Sciences and Engineering Research Council of Canada. The X-ray crystallography work was carried out in the

laboratory of I. Fita at the Universidad Politecnica de Cataluna , Barcelona, Spain.

References

Bravo, J., Verdaguer, N., Tormo, J., Betzel, C., Switala, J., Loewen, P.C. and Fita I.: Crystal structure of catalase HPII from *Escherichia coli*. Structure 3 (1995) 491–502.

Chang, J.Y. and Schroeder, W.A.: Reaction of 3-amino-1,2,4-triazole with bovine liver catalase and human erythrocyte catalase. Arch. Biochem. Biophys. 148 (1972) 505–508.

Chiu, J.T., Loewen, P.C., Switala, J., Gennis, R.B. and Timkovich, R.: Proposed structure for the prosthetic group of the catalase HPII from *Escherichia coli*. J. Am. Chem. Soc. 111 (1989) 7046–7050.

Claiborne, A. and Fridovich, I.: Purification of the *o*dianisidine peroxidase from *Escherichia coli* B. J. Biol. Chem. 254 (1979) 4245–4252.

Claiborne, A., Malinowski, D.P. and Fridovich, I.: Purification and characterization of hydroperoxidase II of *Escherichia coli* B. J. Biol. Chem. 254 (1979) 11664–11668.

Fita, I. and Rossmann, M.G.: The active center of catalase. J. Mol. Biol. 185 (1985) 21–37.

Gentry, D.A., Hernandez, V.J., Nguyen, L.H., Jensen, D.B. and Cashel, M.: Synthesis of the stationary-phase sigma factor, σ^S, is positively regulated by ppGpp. J. Bacteriol. 175 (1993) 7982–7989.

Hassan, H.M. and Fridovich, I.: Regulation of synthesis of catalase and peroxidase in *Escherichia coli*. J. Biol. Chem. 253 (1978) 6445–6450.

Lange, R. and Hengge-Aronis, R.: Growth phaseregulated expression of *bolA* and morphology of stationary-phase *Escherichia coli* cells are controlled by the novel sigma factor σ^S. J. Bacteriol. 173 (1991) 4474–4481.

Levine, S.A.: Isolation and characterization of catalase deficient mutants of *Salmonella typhimurium*. Mol. Gen. Genet. 150 (1977) 205–209.

Loewen, P.C.: Isolation of catalase-deficient *Escherichia coli* mutants and genetic mapping of *katE*, a locus that affects catalase activity. J. Bacteriol. 157 (1984) 622–626.

Loewen, P.C. and Switala, J.: Purification and characterization of catalase HPII from *Escherichia coli* K12. Biochem. Cell Biol. 64 (1986) 638–646.

Loewen, P.C. and Triggs, B.L.: Genetic mapping of *katF*, a locus that with *katE* affects the synthesis of a second catalase species in *Escherichia coli*. J. Bacteriol. 160 (1984) 668–675.

Loewen, P.C., Triggs, B.L., Klassen, G.R. and Weiner, J.H.: Identification and physical characterization of a ColE1 hybrid plasmid containing a catalase gene of *Escherichia coli*. Cell Biol. 61 (1983) 1315–1321.

Loewen, P.C., Switala, J. and Triggs-Raine, B.L.: Catalases HPI and HPII in *Escherichia coli* are induced independently. Arch. Biochem. Biophys. 243 (1985a) 144–149.

Loewen, P.C., Triggs-Raine, B.L., George, C.S. and Hrabarchuk, B.E.: Genetic mapping of *katG*, a locus that affects synthesis of the bifunctional catalase-peroxidase hydroperoxidase I in *Escherichia coli*. J. Bacteriol. 162 (1995b) 661–667.

Loewen, P.C., von Ossowski, I., Switala, J. and Mulvey, M.R.: KatF (σ^S) synthesis in *Escherichia coli* is subject to posttrascriptional regulation. J. Bacteriol. 175 (1993) 2150–2153.

McCann, M.P., Kidwell, J.P. and Matin, A.: The putative σ factor KatF is regulated posttranscriptionally during carbon starvation. J. Bacteriol. 175 (1993) 2143-2149.

Morgan, R.W., Christman, M.F., Jacobson, F.S., Storz, G. and Ames, B.N.: Hydrogen peroxide-inducible proteins in *Salmonella typhimurium* overlap with heat shock and other stress proteins. Proc. Natl. Acad. Sci. USA 83 (1986) 8059–8063.

Mulvey, M.R. and Loewen, P.C.: Nucleotide sequence of *katF* of *Escherichia coli* suggests KatF protein is a novel σ transcription factor. Nucleic Acids Res. 17 (1989) 9979–9991.

Mulvey, M.R., Sorby, P.A., TriggsRaine, B.L. and Loewen, P.C.: Cloning and physical characterization of *katE* and *katF* required for catalase HPII expression in *Escherichia coli*. Gene 73 (1988) 337–345.

Mulvey, M.R., Switala, J., Borys, A. and Loewen, P.C.: Regulation of transcription of *katE* and *katF* in *Escherichia coli*. J. Bacteriol. 172 (1990) 6713–6720.

Murshudov, G.N., Bravo, J., Grebenko, A.I., Barynin, V., Dauter, Z., Wilson, K.S., Ferran, J.M., Ferrer, J.C., Switala, J., Loewen, P.C., Fita, I. and Melik-Adamyan, W.: Structure of the heme d of *Penicillium vitale* and *Escherichia coli* catalases. J. Biol. Chem. 271 (1996) in press.

Murthy, M.R.N., Reid III, T.J., Sicignano, A., Tanaka, N. and Rossmann, M.G.: Structure of beef liver catalase. J. Mol. Biol. 152 (1981) 465–499.

Richter, H.E. and Loewen, P.C.: Induction of catalase in *Escherichia coli* by ascorbic acid involves hydrogen peroxide. Biochem. Biophys. Res. Commun. 100 (1981) 1039–1046.

Richter, H.E. and Loewen, P.C.: Rapid inactivation of bacteriophage T7 by ascorbic acid is repairable. Biochim. Biophys. Acta 697 (1982) 25–30.

Sevinc, M.S. and Loewen, P.C.: The cysteines of catalase HPII of *Escherichia coli*, including Cys438 which is blocked, do not have a catalytic role. Eur. J. Biochem. 230 (1995) 127–132.

Takayanagi, Y., Tanaka, K. and Takahashi, H.: Structure of the 5'upstream region and the regulation of the rpoS gene of Escherichia Coli. Molec. Gen. Genet. 243 (1994) 525–531.

Timkovich, R. and Bondoc, L.L.: Diversity in the structure of hemes. Adv. Biophys. Chem. 1 (1990) 203–247.

Triggs-Raine, B.L. and Loewen, P.C.: Physical characterization of katG, encoding catalase HPI of Escherichia coli. Gene 52 (1987) 121–128.

Triggs-Raine, B.L., Doble, B.W., Mulvey, M.R., Sorby, P.A. and Loewen, P.C.: Nucleotide sequence of katG, encoding catalase HPI of Escherichia Coli. J. Bacteriol. 170 (1988) 4415–4419.

von Ossowski, I., Mulvey, M.R., Leco, P.A., Borys, A. and Loewen, P.C.: Nucleotide sequence of Escherichicia coli katE, which encodes catalase HPII. J. Bacteriol. 173 (1991) 514–520.

von Ossowski, I., Hausner, G. and Loewen, P.C.: Molecular evolutionary analysis based on the amino acid sequence of catalase. J. Mol. Evol. 37 (1993) 71–76.