The Mechanism of *Mycobacterium tuberculosis* Alkylhydroperoxidase AhpD as Defined by Mutagenesis, Crystallography, and Kinetics*

Aleskey Koshkin, Christine M. Nunn, Snezana Djordjevic, and Paul R. Ortiz de Montellano

From the Department of Pharmaceutical Chemistry, University of California, San Francisco, California 94143-2280 and Department of Biochemistry and Molecular Biology, University College, Gower Street, London WC1E 6BT, United Kingdom

AhpD, a protein with two cysteine residues, is required for physiological reduction of the *Mycobacterium tuberculosis* alkylhydroperoxidase AhpC. AhpD also has an alkylhydroperoxidase activity of its own. The AhpC/AhpD system provides critical antioxidant protection, particularly in the absence of the catalase-peroxidase KatG, which is suppressed in most isoniazid-resistant strains. Based on the crystal structure, we proposed recently a catalytic mechanism for AhpD involving a proton relay in which the Glu118 carboxylate group, via His137 and a water molecule, deprotonates the catalytic residue Cys133 (Nunn, C. M., Djordjevic, S., Hillas, P. J., Nishida, C., and Ortiz de Montellano, P. R. (2002) *J. Biol. Chem.* 277, 20033–20040). A possible role for His132 in subsequent formation of the Cys133-Cys130 disulfide bond was also noted. To test this proposed mechanism, we have expressed the H137F, H137Q, H132F, H132Q, E118F, E118Q, C133S, and C130S mutants of AhpD, determined the crystal structures of the H137F and H132Q mutants, estimated the pKs values of the cysteine residues, and defined the kinetic properties of the mutant proteins. The collective results strongly support the proposed catalytic mechanism for AhpD.

* Mycobacterium tuberculosis* infects an estimated 8 million per year and kills 2–3 million people in the same time span (1). It is estimated that ~2 billion people have been exposed to this lethal pathogenic organism and thus are at risk of developing the active disease. The majority of infected individuals reside in the third world, but the rates of infection in other areas that are undergoing rapid social change, such as the Soviet Union, are increasing at an alarming rate (2). Furthermore, partially as a result of the symbiotic relationship between human immunodeficiency virus and tuberculosis, the incidence of multidrug-resistant tuberculosis is rapidly increasing (3). The resurgence of tuberculosis as a world-wide phenomenon, in conjunction with the recent determination of the *M. tuberculosis* genome, has fuelled a renewed search for agents that are active against drug-resistant strains, completely sterilize the infection, and/or shorten the duration of drug therapy and thus promote drug compliance.

Mutations in the *M. tuberculosis* catalase-peroxidase KatG result in resistance to the prodrug isoniazid, because KatG is required to oxidize this drug to its biologically active form (4–9). In a similar manner, mutations in the flavoprotein monooxygenase EtaA result in resistance to ethionamide, because it is also a prodrug that must be oxidized by EtaA to its active form (10, 11). Interestingly, even though the activating enzymes differ, the mutations of KatG and EtaA that cause resistance to isoniazid and ethionamide, respectively, result in elevated expression of AhpC (4, 12–15, 17). AhpC is thought to provide protection against the oxidative stress associated with both of these mutations, particularly against loss of the KatG peroxidase activity (12). AhpC is a member of the ubiquitous peroxiredoxin family. In the peroxiredoxins, a cysteine residue reacts with a peroxide or other oxidant to give a sulfenic acid (–SOH) intermediate (19, 20). This sulfenic acid is then converted to a disulfide bond by intramolecular or intermolecular reaction with a second sulfhydryl group. Finally, the disulfide bond is reduced to regenerate the cysteine thiol groups. The disulfide bond is reduced by different mechanisms in different organisms; in yeast, the reduction is mediated by thioredoxin and thioredoxin reductase (21) and in *Salmonella typhimurium* by a flavoprotein known as AphP (22). The *M. tuberculosis* thioredoxin and thioredoxin reductase, however, do not reduce the corresponding AhpC (23), and no homologue of AphP is detected in the genome of this organism by BLAST searches. However, a gene (Rv2429) coding for AhpD, a protein with no sequence identity to AhpC or AphP, is located immediately adjacent to the AhpC gene. Recent work has shown that AhpD functions as the reducing partner for AhpC. AhpD itself is reduced by a novel system consisting of dihydrolipoamide succinyltransferase (SucB), a lipoamide-containing protein, and dihydrolipoamide dehydrogenase (25). SucB can be replaced in this system by dihydrolipoamide itself. Interestingly, AhpD, in addition to being the reducing partner for AhpC, has independent alkylhydroperoxidase activity of its own when AhpF from *S. typhimurium* is used as a surrogate reducing partner (24).

The crystal structure of AhpD was independently determined by two laboratories (25, 26). AhpD is a homotrimeric protein in which the individual subunits consist of 177 amino acids with a molecular mass of 18,781 Da (25, 26). Two cysteines, Cys130 and Cys133, are present in the AhpD sequence (numbering based on inclusion of the initial methionine) in a Cys-His-Ser-Cys motif within a novel protein fold. Site-specific mutagenesis of the cysteines has shown that both cysteines, but particularly Cys133, are critical for the alkylhydroperoxidase activity supported by the *S. typhimurium* AhpF (24, 25).
Despite the absence of structural homology to other proteins, analysis of the detailed structure of AhpD identified several residues that could be involved in the catalytic activity of the enzyme (26) based on local structural analogy to the active site of a functional analog, thioredoxin. Specifically, the carboxylate anion of Glu118 is 2.6 Å from the nitrogen of His137 and is hydrogen-bonded to it (Fig. 1). His137 is hydrogen-bonded to a water molecule 2.5 Å away that, in turn, interacts with Cys133 at a distance of 3.3 Å. These hydrogen bonding interactions provide a reasonable mechanism for deprotonation of Cys133, the residue thought to react with the O–O bond of peroxides or the disulfide bond present in the oxidized form of AhpC. The location of His137 4.8 Å from Cys133 and 3.7 Å from Cys132 in the reduced enzyme suggested that it might also play a role in the catalytic mechanism. These mechanistic proposals have been tested in the present study by mutating the residues, determining the crystal structures of some of the mutants, and determining the effects of the mutations on the catalytic activities in both the AhpF and AhpDAhpCh/lopoamide/dihydrolipoamide dehydrogenase assay systems.

EXPERIMENTAL PROCEDURES

Materials—All chemical reagents were purchased from Sigma. Escherichia coli strain BL21(DE3) was from Novagen, and strain DH5α was from Invitrogen. Q-Sepharose Fast Flow was purchased from Amersham Biosciences, and polyethyleneimine was from Research Biochemicals International (Natick, MA). LB medium was obtained from Invitrogen. Chloramphenicol, NaCl, NaOH, SDS, and MOPS1 were from Fisher. Protein molecular weight standards were from Invitrogen. Purified proteins were purchased from Research Biochemicals International (Natick, MA). Lipoamide, NADH, 20 mM NADPH, 20 mM NADH, 200 μM TPP, and 100 mM lipoamide dehydrogenase was measured as described by Hill et al. (27) and a molar extinction coefficient of 15,720 M⁻¹ cm⁻¹. In contrast to AhpD a single native PAGE chromatographic protocol was insufficient to purify the Ahp E118Q and H137Q mutants. Further purification by gel filtration on Superdex 200 using 50 mM MOPS, pH 7.2, 100 mM KCl, 20% glycerol, 5 mM dithiothreitol, and 0.2 mM EDTA was therefore carried out with all proteins. The proteins were judged to be >95% pure by denaturing SDS-PAGE. The proteins were finally aliquoted into 25- or 100-μl volumes, frozen on dry ice, and stored at −80 °C until used.

AhpF-dependent Activity Assays—Rates of hydroperoxide reduction were determined anerobically in a coupled assay with AhpF, monitoring the decrease in absorbance at 340 nm because of NADH oxidation as reported previously (24). The assays typically contained 2 μM hydroperoxide substrate in 100 mM potassium Pi, pH 7.0, 1 mM EDTA, 0.25 mM NADPH, 20 μM AhpD or mutant, and 10 μM AhpF. Background NADH oxidation caused by AhpF was monitored, the hydroperoxide substrate was added, and the enzymatic rate was observed.

AhpD-dependent Peroxidase Activity AhpC Assays—The rate of NADH oxidation catalyzed by AhpC in the presence of AhpD, lipoamide, and bovine lipoamide dehydrogenase was measured as described by monitoring the change in absorbance at 340 nm (25). Typical conditions for the assays were as follows: 50 mM potassium Pi, pH 7.0, 1 mM EDTA, 200 μM NADH, 2.5 μM AhpC, 2.5 μM AhpD, and 0.2 units of bovine dihydrolipoamide dehydrogenase, and 50 μM lipoamide. This assay can be used to evaluate the activity of AhpD and AhpD mutants, because the AhpD-supported activity of AhpC depends directly on the activity of AhpD. Each steady-state kinetic assay, the substrate concentration was varied, and data were fit to the equation v = Vmax [S]/(Km + [S]).

Measurement of Thiol pKα Values by UV Absorption—The pH-dependent ionization of the cysteine thiols was monitored by the absorbance of the thiolate anion at 240 nm as described (28, 29). All of the measurements were carried out at 25 °C, with 10 μM AhpF or AhpD mutant in 1 mM each of acetate and phosphate buffer containing 0.2 mM KCl and 5 mM dithiothreitol. The solution was titrated with 0.2 M KOH.

Crystallization—Crystallization of the AhpD H137F and H132Q mutants was carried out by the hanging drop vapor diffusion method reported previously (26). Hanging drops were prepared by mixing equal volumes of protein solution (1 mg/ml in 25 mM sodium phosphate, 10 μM EDTA, containing 50 mM KCl, 10% glycerol, 0.1 mM EDTA, and 5.0 mM dithiothreitol) with a reservoir solution containing 100 mM sodium citrate buffer at pH 5.6 containing 200 mM ammonium acetate and 26% polyethylene glycol 4000. Crystals grew as rhombohedral prisms over a...
Mechanism of Mycobacterium tuberculosis AhpD

RESULTS

Catalytic Activities of AhpD Mutant Proteins—The recently determined crystal structure of AhpD suggests important roles for Glu118, Cys130, Cys133, His132, and His137 in the catalytic mechanism of AhpD (25, 26). To ascertain the roles of these residues, single mutants were constructed in which each of the cysteines was mutated to a serine, and each of the two histidines and the glutamic acid was mutated to either a phenylalanine or a glutamine. The mutant proteins were expressed and purified by minor modifications of the protocol reported earlier for purification of wild-type AhpD (24, 26). The two AhpD Cys→Ser mutants and the two His→Phe mutants were expressed in yields comparable with those of the wild-type protein (25 mg/L), but the AhpD H137Q, E118F, and E118Q mutants were expressed at significantly lower levels (45–1% of wild-type). The glutamine mutants do not appear to bind to Q-Sepharose as strongly as native AhpD or the other mutants and tend to unfold and degrade relatively easily. The glutamine substitution may destabilize the protein fold or may force the protein into a conformation that exposes proteolytically sensitive sites.

The catalytic activities of the mutants were evaluated in two assay systems. In the first system, the ability of the AhpD mutants to reduce cumene hydroperoxide in the presence of the S. typhimurium AhpF and NADH was evaluated (24). This assay system tests the ability of AhpD to directly reduce a hydroperoxide in a reaction supported by a surrogate electron donor partner. The AhpF-supported catalytic activities of wild-type AhpD and its mutants are shown in Fig. 2. As reported previously (24), the two cysteine mutations impair the alkyl peroxidase activity of AhpD, with mutation of Cys→Ser completely suppressing the activity and mutation of Cys→Glu decreasing it to less than 5% of the wild-type activity (Fig. 2). Likewise, the two His to Phe mutations decrease the catalytic activity, but mutation of His→Phe causes a greater decrease than mutation of His→Glu. Mutation of the two His residues to glutamines also decreases the level of activity but to a lower extent than mutation to phenylalanines. As found for the phenylalanine mutants, the H137Q mutation decreased the activity more severely than the H132Q mutation. Replacement of Glu by a
glutamine only modestly lowered the catalytic activity, whereas replacement by a phenylalanine decreased the activity to a much higher extent.

In the second assay system, reduction of cumene hydroperoxide was measured in the fully reconstituted system consisting of AhpC, the AhpD mutant, lipoamide, bovine dihydrolipoamide dehydrogenase, and NADH (25). The lipoamide-dihydrolipoamide dehydrogenase-AhpC assay (Fig. 3) gives higher absolute alkylhydroperoxidase activities than the AhpF-dependent assay (Fig. 2). Nevertheless, as shown in Fig. 3, the impairment of the catalytic activities caused by the mutations follows the same trends as seen for direct reduction of the peroxide by AhpD/AhpF. The ratios among the activities of the different mutants are similar to those found in the AhpF assay. Thus, the two cysteine mutants are completely inactive in this assay, in agreement with an earlier report (25), the His132 mutation causes a greater impairment than the His137 mutation, and the E118Q mutation has a relatively minor effect when compared with the E118F mutation.

**The pH Dependence of Wild-type and Mutant AhpD Proteins in the Lipoamide-Lipoamide Dehydrogenase-AhpC Assay** — The pH dependence of the activities of native AhpD and three of its mutants are shown in Fig. 4. If either AhpC or AhpD is assayed in the absence of the other, the level of activity is several times lower than when both enzymes are present. The pH profiles of AhpD and its mutants are bell shaped. The pH dependence of AhpD alone may be considered as the background pH-dependent activity, as the activity of AhpC alone does not appear to be sensitive to pH in the pH 5–10 range. The optimum activity of native AhpD occurs at pH 7.2. Below pH 7.0 and above pH 7.5, there is a gradual decrease in the activity. Substitution of either His132 or His137 by a Gln shifts the pH optimum to a value of 7.5. This shift to a higher pH optimum indicates that His137 and His132 help to determine the $pK_a$ values of critical catalytic residues, as implied by the postulated catalytic mechanism (26).

**The $pK_a$ Values of the Cysteine Residues in AhpD** — To evaluate the $pK_a$ of the two cysteine residues in AhpD the thiolate anion absorbance at 240 nm (29) was monitored between pH 6 and 9. The increase in the 240-nm absorption with pH indicated it was caused by deprotonation of a thiol with $pK_a = 7.2 \pm 0.1$ (Fig. 5). In an effort to identify the cysteine with the indicated $pK_a$ value, the thiol titration experiment was carried out with the C130S and C133S mutants of AhpD. Measurements of the increase in the absorbance at 240 nm indicated that Cys133 in the C130S mutant had a $pK_a$ of 6.9 (Fig. 5). In contrast, similar measurements with the C133S mutant indicated that the remaining cysteine, Cys130, had a $pK_a$ of 7.5. The $pK_a$ of...
Mechanism of Mycobacterium tuberculosis AhpD

The incubation mixture contained the following: 0.5 mM H$_2$O$_2$, 200 mM dihydrolipoamide dehydrogenase, and 50 mM lipoamide, the two cysteine mutations active Cys130 and Cys133 residues lie close to the substrate binding site (26).

DISCUSSION

The mechanism proposed for catalytic turnover of the M. tuberculosis AhpD, based on the structure of the wild-type protein, involves reaction of the Cys133 thiolate with the substrate. The reaction could involve attack at either the dioxygen bond of a peroxide or the disulfide bond of oxidized AhpC (26). This reaction was postulated to be facilitated by a decrease in the pK$_a$ of Cys133 because of a relay system in which the thiol proton is removed by a water molecule whose basicity is increased by hydrogen bonding to His137, which in turn is activated by hydrogen bonding to the carboxylate group of Asp118.

We have demonstrated here that the pK$_a$ of the Cys133 thiol group is 6.9, a value significantly lower than that of 7.5 for Cys130. At a pH of 7.2, the optimum for wild-type AhpD activity (Fig. 4), the major fraction (~66%) of the thiol group in Cys133 is protonated, whereas the majority of Cys130 would be in the deprotonated, thiolate, form. These results are consistent with the proposal that Cys133 is involved in a nucleophilic attack on the substrate (26).

Mutagenesis shows that a C133S mutation suppresses the AhpF-supported alkylperoxidase activity of AhpD, and a C130S mutation is almost as effective (Fig. 2) (24). When the activity of AhpD is assayed in a system also containing AhpC, lipoamide dehydrogenase, and lipoamide, the two cysteine mutations completely eliminate catalytic activity (Fig. 3). These results are consistent with the crystal structure of the oxidized form of

![Graph](image_url)
AhpD, which shows that Cys\textsuperscript{130} is linked to Cys\textsuperscript{133} via a disulfide bond (25).

To explore the role of His\textsuperscript{137} in activating Cys\textsuperscript{133} for catalytic attack on the substrate, we have mutated it to a phenylalanine and a glutamine. As shown in Figs. 2 and 3, mutation to a phenylalanine, which is not able to hydrogen bond to the proposed catalytic water molecule, severely depresses the activity of the enzyme in both the AhpF and AhpC/lipoamide dehydrogenase, lipoamide catalytic systems. Mutation to a glutamine,
a residue with a side chain that is still able to enter into hydrogen bonding interactions, depresses the activity relative to the wild-type but to a much lower extent than the H137F mutation. The crystal structure of the H137F mutant supports these results, as phenylalanine is unable to engage in a water-mediated interaction with Cys133. Although aromatic hydrogen bonding interactions are possible between phenyl aromatic rings and hydrogen bond donors (37) no such interactions are observed for H137F. If protonated, Glu118 could act as a hydrogen donor, in a hydrogen bond analogous to that of Glu118, His317 in the wild-type enzyme. However, the mean Phe(137)–Glu(118) separation for the H137F mutant is 3.8 Å, and the geometry is inconsistent with hydrogen bonding between these side chains. The separation between His137 and Cys133 in both the native and H132Q AhpD structures (26) is spatially similar to that observed between the active site residues in thioredoxin (Fig. 8) (38). In thioredoxin the catalytic mechanism proceeds via a mixed disulfide intermediate whose breakdown is enhanced by the involvement of the residue Asp30 that acts as a base catalyst toward residue Cys39. The separation between Asp30 OD1 and Cys39 SG in the thioredoxin crystal structure is 5.9 Å and too large for direct proton transfer. It is proposed that proton transfer between Asp30 and Cys39 is mediated by a water molecule resulting in the subsequent nucleophilic attack of Cys39 on the mixed disulfide bridge (39). The effect of replacing the Asp by Asn in the active site of thioredoxin (16, 18) was of comparable magnitude to the effect on the catalytic activity created by replacement of His317 by Gln providing further parallels in the mechanisms of the two enzymes.

Mutation of Glu118, the residue postulated to activate His317, to a glutamine decreases catalytic activity in both assay systems but only to a small degree, whereas mutation to a phenylalanine lowered the activity to a higher degree. It is clear from this that glutamic acid can be replaced by a glutamine in the catalytic mechanism. Efforts to make the E118F mutant were not successful because of the instability of the resulting protein.

A role was also postulated for His132 in the catalytic mechanism (26), in this case in helping to deprotonate Cys130 in the E118F mutant, the nitrogen atom of His132 lies at 5.9 Å from the Asp30 OD1 and Cys39 SG in the thioredoxin crystal structure is 5.9 Å and too large for direct proton transfer. It is proposed that proton transfer between Asp30 and Cys39 is mediated by a water molecule resulting in the subsequent nucleophilic attack of Cys39 on the mixed disulfide bridge (39). The effect of replacing the Asp by Asn in the active site of thioredoxin (16, 18) was of comparable magnitude to the effect on the catalytic activity created by replacement of His317 by Gln providing further parallels in the mechanisms of the two enzymes.

Mutation of Glu118, the residue postulated to activate His317, to a glutamine decreases catalytic activity in both assay systems but only to a small degree, whereas mutation to a phenylalanine lowered the activity to a higher degree. It is clear from this that glutamic acid can be replaced by a glutamine in the catalytic mechanism. Efforts to make the E118F mutant were not successful because of the instability of the resulting protein.

A role was also postulated for His132 in the catalytic mechanism (26), in this case in helping to deprotonate Cys130 in the E118F mutant, the nitrogen atom of His132 lies at 5.9 Å from the Asp30 OD1 and Cys39 SG in the thioredoxin crystal structure is 5.9 Å and too large for direct proton transfer. It is proposed that proton transfer between Asp30 and Cys39 is mediated by a water molecule resulting in the subsequent nucleophilic attack of Cys39 on the mixed disulfide bridge (39). The effect of replacing the Asp by Asn in the active site of thioredoxin (16, 18) was of comparable magnitude to the effect on the catalytic activity created by replacement of His317 by Gln providing further parallels in the mechanisms of the two enzymes.

Mutation of Glu118, the residue postulated to activate His317, to a glutamine decreases catalytic activity in both assay systems but only to a small degree, whereas mutation to a phenylalanine lowered the activity to a higher degree. It is clear from this that glutamic acid can be replaced by a glutamine in the catalytic mechanism. Efforts to make the E118F mutant were not successful because of the instability of the resulting protein.

A role was also postulated for His132 in the catalytic mechanism (26), in this case in helping to deprotonate Cys130 in the E118F mutant, the nitrogen atom of His132 lies at 5.9 Å from the Asp30 OD1 and Cys39 SG in the thioredoxin crystal structure is 5.9 Å and too large for direct proton transfer. It is proposed that proton transfer between Asp30 and Cys39 is mediated by a water molecule resulting in the subsequent nucleophilic attack of Cys39 on the mixed disulfide bridge (39). The effect of replacing the Asp by Asn in the active site of thioredoxin (16, 18) was of comparable magnitude to the effect on the catalytic activity created by replacement of His317 by Gln providing further parallels in the mechanisms of the two enzymes.

Mutation of Glu118, the residue postulated to activate His317, to a glutamine decreases catalytic activity in both assay systems but only to a small degree, whereas mutation to a phenylalanine lowered the activity to a higher degree. It is clear from this that glutamic acid can be replaced by a glutamine in the catalytic mechanism. Efforts to make the E118F mutant were not successful because of the instability of the resulting protein.

A role was also postulated for His132 in the catalytic mechanism (26), in this case in helping to deprotonate Cys130 in the E118F mutant, the nitrogen atom of His132 lies at 5.9 Å from the Asp30 OD1 and Cys39 SG in the thioredoxin crystal structure is 5.9 Å and too large for direct proton transfer. It is proposed that proton transfer between Asp30 and Cys39 is mediated by a water molecule resulting in the subsequent nucleophilic attack of Cys39 on the mixed disulfide bridge (39). The effect of replacing the Asp by Asn in the active site of thioredoxin (16, 18) was of comparable magnitude to the effect on the catalytic activity created by replacement of His317 by Gln providing further parallels in the mechanisms of the two enzymes.

Mutation of Glu118, the residue postulated to activate His317, to a glutamine decreases catalytic activity in both assay systems but only to a small degree, whereas mutation to a phenylalanine lowered the activity to a higher degree. It is clear from this that glutamic acid can be replaced by a glutamine in the catalytic mechanism. Efforts to make the E118F mutant were not successful because of the instability of the resulting protein.

A role was also postulated for His132 in the catalytic mechanism (26), in this case in helping to deprotonate Cys130 in the E118F mutant, the nitrogen atom of His132 lies at 5.9 Å from the Asp30 OD1 and Cys39 SG in the thioredoxin crystal structure is 5.9 Å and too large for direct proton transfer. It is proposed that proton transfer between Asp30 and Cys39 is mediated by a water molecule resulting in the subsequent nucleophilic attack of Cys39 on the mixed disulfide bridge (39). The effect of replacing the Asp by Asn in the active site of thioredoxin (16, 18) was of comparable magnitude to the effect on the catalytic activity created by replacement of His317 by Gln providing further parallels in the mechanisms of the two enzymes.

Mutation of Glu118, the residue postulated to activate His317, to a glutamine decreases catalytic activity in both assay systems but only to a small degree, whereas mutation to a phenylalanine lowered the activity to a higher degree. It is clear from this that glutamic acid can be replaced by a glutamine in the catalytic mechanism. Efforts to make the E118F mutant were not successful because of the instability of the resulting protein.

A role was also postulated for His132 in the catalytic mechanism (26), in this case in helping to deprotonate Cys130 in the E118F mutant, the nitrogen atom of His132 lies at 5.9 Å from the Asp30 OD1 and Cys39 SG in the thioredoxin crystal structure is 5.9 Å and too large for direct proton transfer. It is proposed that proton transfer between Asp30 and Cys39 is mediated by a water molecule resulting in the subsequent nucleophilic attack of Cys39 on the mixed disulfide bridge (39). The effect of replacing the Asp by Asn in the active site of thioredoxin (16, 18) was of comparable magnitude to the effect on the catalytic activity created by replacement of His317 by Gln providing further parallels in the mechanisms of the two enzymes.

Mutation of Glu118, the residue postulated to activate His317, to a glutamine decreases catalytic activity in both assay systems but only to a small degree, whereas mutation to a phenylalanine lowered the activity to a higher degree. It is clear from this that glutamic acid can be replaced by a glutamine in the catalytic mechanism. Efforts to make the E118F mutant were not successful because of the instability of the resulting protein.

A role was also postulated for His132 in the catalytic mechanism (26), in this case in helping to deprotonate Cys130 in the E118F mutant, the nitrogen atom of His132 lies at 5.9 Å from the Asp30 OD1 and Cys39 SG in the thioredoxin crystal structure is 5.9 Å and too large for direct proton transfer. It is proposed that proton transfer between Asp30 and Cys39 is mediated by a water molecule resulting in the subsequent nucleophilic attack of Cys39 on the mixed disulfide bridge (39). The effect of replacing the Asp by Asn in the active site of thioredoxin (16, 18) was of comparable magnitude to the effect on the catalytic activity created by replacement of His317 by Gln providing further parallels in the mechanisms of the two enzymes.
The Mechanism of *Mycobacterium tuberculosis* Alkylhydroperoxidase AhpD as Defined by Mutagenesis, Crystallography, and Kinetics
Aleksey Koshkin, Christine M. Nunn, Snezana Djordjevic and Paul R. Ortiz de Montellano

*J. Biol. Chem. 2003, 278:29502-29508.*
doi: 10.1074/jbc.M303747200 originally published online May 21, 2003

Access the most updated version of this article at doi: 10.1074/jbc.M303747200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 36 references, 14 of which can be accessed free at
http://www.jbc.org/content/278/32/29502.full.html#ref-list-1