Helicobacter pylori defense against oxidative attack

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Stent A, Every AL, Sutton P. Helicobacter pylori defense against oxidative attack. Am J Physiol Gastrointest Liver Physiol 302: G579–G587, 2012. First published December 22, 2011; doi:10.1152/ajpgi.00495.2011.—Helicobacter pylori is a microaerophilic, gram-negative pathogen of the human stomach. Despite the chronic active gastritis that develops following colonization, H. pylori is able to persist unharmed in the stomach for decades. Much of the damage caused by gastric inflammation results from the accumulation of reactive oxygen/nitrogen species within the stomach environment, which can induce oxidative damage in a wide range of biological molecules. Without appropriate defenses, this oxidative damage would be able to rapidly kill nearby H. pylori, but the organism employs a range of measures, including antioxidant enzymes, biological repair systems, and inhibitors of oxidant generation, to counter the attack. Despite the variety of measures employed to defend against oxidative injury, these processes are intimately interdependent, and any deficiency within the antioxidant system is generally sufficient to cause substantial impairment of H. pylori viability and persistence. This review provides an overview of the development of oxidative stress during H. pylori gastritis and examines the methods the organism uses to survive the resultant damage.

reactive oxygen species; gastric inflammation; human stomach

SINCE ITS DESCRIPTION in 1983 by Warren and Marshall (104), the bacterium Helicobacter pylori has come to be recognized as a pathogen of great importance in human medicine. It is now understood that inflammation resulting from H. pylori infection is the leading cause of gastric adenocarcinoma (96), gastric mucosal-associated lymphoid tissue lymphoma (106), and peptic ulcer disease (63). The development of these associated diseases is a direct result of the ability of H. pylori to chronically colonize the extreme, hostile environment of the gastric mucosa, with infection typically commencing during childhood and lasting for the life of the host.

H. pylori have developed a range of defensive strategies to facilitate long-term survival within the stomach environment, despite the generation of a ubiquitous inflammatory response by the host. Many of these, such as the production of large quantities of urease to buffer the low pH resulting from stomach acid, have been well studied. H. pylori also possess high motility and chemotactic ability, which allow it to penetrate the mucus layer to the relatively protected area adjacent to the epithelial surface.

Perhaps less generally appreciated, however, is the fact that H. pylori is continuously exposed to attack from reactive oxygen species (ROS) or reactive nitrogen species (RNS) generated during inflammation (8). These molecules are capable of initiating harmful oxidative or nitrosative reactions in biological systems, and much of the damage caused by gastritis results from the actions of these molecules at the site of inflammation (5, 56). As a microaerophile, H. pylori should ostensibly be susceptible to this attack. However, the organism possesses oxidative defense mechanisms (summarized in Fig. 1 and discussed in detail below), enabling it to withstand the harmful effects of ROS/RNS and facilitating its ability to chronically infect the host.

Sources of ROS in H. pylori Infection

H. pylori are exposed to ROS generated by numerous cell types. Gastric epithelial cells, for example, have been shown to produce ROS following exposure to H. pylori, resulting in a substantial increase in superoxide secretion by these cells (5, 6). This ROS production appears to result from activation of the enzyme mitogen oxidase 1 (57) and is particularly enhanced by exposure to H. pylori strains expressing the CagA cytotoxin, a virulence factor correlated with more severe gastritis (71). Recent reports also suggest that H. pylori stimulates H2O2 production by gastric epithelial cells, via CagA-mediated induction of the ROS-generating enzyme spermine oxidase (17). This process results in demonstrable DNA damage and apoptosis of epithelial cells, although the effects, if any, on H. pylori viability remain uncertain. Regardless, it provides an additional source of ROS to which H. pylori could potentially be exposed.

The phagocytic immune cells, neutrophils and macrophages, are important effectors of innate immunity and can also produce large quantities of ROS via the membrane-associated enzyme NADPH oxidase (13). Neutrophils are known to pass through the tight junctions between epithelial cells and adhere to the luminal surface of the gastrointestinal epithelium (25), as reported in the stomachs of H. pylori-infected humans (59). Such transepithelial neutrophils would be in close proximity with H. pylori, the vast majority of which are located within the...
Antioxidant Defenses of H. pylori

Prevention of oxidant generation. At the vanguard of its antioxidant defenses, H. pylori are able to minimize the generation of peroxynitrite from superoxide. Through secretion of arginase and induction of arginase II production by macrophages, H. pylori are able to deplete arginine within phagocytes (41, 42). Arginine is required for translation of inducible nitric oxide synthase, the enzyme responsible for nitric oxide generation (30). As nitric oxide reacts with superoxide to form the highly toxic RNS peroxynitrite, blockade of nitric oxide synthesis prevents the generation of this damaging oxidant.

While this helps minimize exposure to oxidative damage, other potentially toxic ROS remain in the environment. Patients with H. pylori infection have increased concentrations of ROS within the gastric mucosa as measured by chemiluminescence (28), confirming that substantial quantities persist at the site of infection. The presence of these ROS demonstrates the requirement for a second line of defense against oxidative damage. This role is filled by a wide range of neutralizing antioxidant enzymes.

H. pylori antioxidant enzymes. SUPEROXIDE DISMUTASE. One of the most important antioxidant enzymes for neutralization of exogenous ROS is superoxide dismutase (SOD). This enzyme is nearly ubiquitous throughout nature, being produced by eukaryotes and prokaryotes, including many anaerobic bacteria (14). SOD is responsible for catalyzing the dismutation of the superoxide radical into H₂O₂ and oxygen via the reaction

$$2\text{O}_2^- + 2\text{H}^+ \rightarrow \text{H}_2\text{O}_2 + \text{O}_2$$

This decay proceeds spontaneously, but the reaction follows second-order kinetics (i.e., the rate of the reaction is concentration-dependent) and dramatically slows as superoxide concentration decreases. SOD accelerates the reaction by an estimated factor of $10^{10}$ (40) and, under physiological conditions, also reduces the influence of concentration and pH on dismutation (61). By strongly catalyzing this pathway, the enzyme plays a key role in preventing the development of more toxic molecules, such as peroxynitrite.

SOD can be divided into several families, distinguished by their metal cofactor. SOD cofactored to copper and zinc is designated CuZn-SOD, and this family occurs primarily in eukaryotes. In contrast, SOD produced by prokaryotic cells can also damage lipids and amino acids and trigger peroxidation cascades, destroying plasma membrane integrity and inactivating enzymes (107).
generally uses a manganese (Mn-SOD) or iron (Fe-SOD) catalytic cofactor (43). Mn-SOD is also found within the mitochondria of eukaryotes (74), and Fe-SOD is present within the plastids of plants (105).

In contrast to many other bacteria that express multiple SODs of different families, *H. pylori* is somewhat unusual, in that it produces a single Fe-SOD, designated Hp-SOD. The reason for the absence of other forms is unclear. The development of multiple forms of SOD would be assumed to provide an evolutionary advantage, as different forms are inhibited by different compounds, and alternate forms may be useful, depending on the availability of individual metal cofactors. One explanation may be that iron availability, although low, is relatively constant in the stomach, because of iron-binding properties of gastric mucus (53). *H. pylori* is also well equipped with iron-scavenging mechanisms, which allow it to maintain a relatively constant supply (94). It may be that the availability of cofactors other than iron in the stomach may be sufficiently erratic to select against their use in Hp-SOD.

Three different isoforms of Hp-SOD, differentiated only by minor amino acid substitutions, have been characterized (11). All these isoforms possess the same activity, suggesting that the active site is not altered. The enzyme occurs as a tightly bound dimer comprising two identical 24-kDa subunits (90). Apart from other bacteria within the order Campylobacterales, which share close SOD homology (32), basic local alignment search tool (BLAST) analysis reveals Hp-SOD to have the closest protein sequence homology with facultative intracellular bacteria such as *Francisella spp.* (1). However, all iron-containing SODs have a conserved structure, particularly within the active site (32, 52). There is little homology with human SOD forms, with protein sequence analysis revealing homology of 11%, 30%, and 3% with human SOD1, SOD2, and SOD3, respectively (36).

Similar to other Fe-SOD, the active site of Hp-SOD is trigronal pyramidal, with the catalytic iron molecule held in place by three basally arranged amino acid residues (His73, His160, and Asp156) combined with another axial histidine (His26) (32). These amino acid ligands from nonadjacent regions of the protein pinch the iron molecule and affix it in place, forming the active site. The active site is protected within a deep pocket close to the dimeric interface, surrounded by a hydrophobic shell of amino acids (52). The route of access is undetermined, although it has been speculated to occur along the dimeric interface axis parallel with the NH2-terminal helix A (91).

As well as being located within the bacterial cytosol, where it is presumably required for neutralization of superoxide by-product produced through metabolic processes, Hp-SOD has been found to be surface-bound or secreted (4, 90). Our own immunogold electron-microscopic analysis found that cell surface Hp-SOD is heavily expressed on the flagella of *H. pylori* and that Hp-SOD is also present in secreted outer membrane vesicles (Fig. 2) (68). While the role of these outer membrane vesicles in the pathophysiology of *H. pylori* is incompletely understood, they are thought to represent extracellular release of bioactive compounds for interbacterial and host interactions and are known to be capable of binding to host epithelial cells (77).

Although Hp-SOD is clearly present on the external surface, no membrane transport mechanism has been discovered, and it remains unclear how the enzyme reaches the surface. It is possible that Hp-SOD may be actively translocated to the surface by a yet to be identified mechanism, but the enzyme lacks a recognized signal peptide sequence (90), making this less likely. Alternatively, Hp-SOD may merely adhere to the cell surface following cellular release, by exocytosis in outer membrane vesicles or autolytic release from adjacent bacteria, in a process sometimes referred to as “altruistic autolysis” (80). This hypothetical mechanism, originally suggested to explain urease release, proposes that a proportion of *H. pylori* within a colony undergoes spontaneous autolysis to release enzymes that aid the remaining bacteria in colonization (29). This second mechanism would fit with the presence of a 20-residue extended COOH-terminal tail on Hp-SOD, which Esposito et al. (32) postulated may be involved in adsorption to the bacterial outer membrane.

SOD gene expression appears to be dependent on stage of colonization, with evidence that transcription is suppressed upon bacterial attachment to gastric epithelial cell monolayers (60). Hp-SOD is also regulated depending on environmental conditions, although the only known regulation pathway is mediated by the ferric uptake regulator (Fur) polypeptide, which can suppress Hp-SOD production in iron-restricted environments (31). Fur regulation appears to be dispensable, however, as *H. pylori* resistance to metronidazole in some cases arose due to mutations in Fur that reduced the regulator’s binding affinity (95). This resulted in deregulation of Hp-SOD production, which subsequently attenuated the superoxide-mediated toxic effects of metronidazole. Interestingly, these
resistant strains displayed dose-dependent upregulation of Hp-SOD in the presence of metronidazole, suggesting that additional oxidative stress-responsive regulation pathways may exist.

_H. pylori_ are heavily reliant on the antioxidant properties of Hp-SOD for survival in vivo. Enzyme-deficient mutants exhibit a severe impairment in their ability to colonize mouse stomachs (85). In addition, Hp-SOD-deficient _H. pylori_ strains are hypersensitive to superoxide and H₂O₂ in vitro. The increased sensitivity to H₂O₂ is interesting, as H₂O₂ is not a substrate for Hp-SOD. It is likely that the protective effect of Hp-SOD in this instance reflects the enzyme’s ability to eliminate superoxide before it can reduce ferric iron to the ferrous form. Reduced iron is required for generation of hydroxyl radicals via the Fenton reaction (44); thus the action of Hp-SOD can limit this reaction, thereby preventing oxidative damage even when superoxide is not directly involved. This fits with the known regulation of Hp-SOD, whereby its production is derepressed in environments that contain abundant iron (31), as a possible means of preventing iron reduction.

**Catalase**. Working in close concert with SOD is the antioxidant enzyme catalase. Similar to SOD, catalase occurs in all aerobic organisms and is also occasionally found in some anaerobic organisms (14). The two enzymes have a complementary relationship: catalase promotes the decomposition of H₂O₂, the product of SOD activity, into water and oxygen via the pathway

$$2\text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2$$

Thus catalase provides a final step in neutralizing ROS before they can damage cellular components.

Catalases are exceptionally stable enzymes, with studies on *Escherichia coli* catalase HPII demonstrating substantial resistance to thermal denaturation and enzymatic digestion (19, 92). It has also been observed, however, somewhat counterintuitively, that catalase is susceptible to oxidative damage. Exposure to hypochlorous acid causes oxidation of methionine residues within the enzyme, resulting in enzyme oligomerization and loss of function (67). *H. pylori* counters this through the actions of two enzymes, methionine sulfoxide reductase (MsRA) and GroEL heat shock protein, which appear to work synergistically to repair catalase (67). While MsRA is known to reduce the oxidized methionine residues, GroEL also seems to be required for catalase to regain functionality, presumably by correctly refolding the enzyme through its chaperoning ability. It is unlikely that catalase is the only target of MsRA, however, as MsRA-deficient mutants were more susceptible to neutrophilic killing than catalase-deficient mutants, suggesting that MsRA has additional functions unrelated to catalase repair (67).

As with SOD, while many other bacteria possess multiple catalases, _H. pylori_ produce only a single form of catalase, encoded by the gene _katA_. _H. pylori_ catalase (KatA) is a heme-containing, monofunctional enzyme. This contrasts with other catalases, which may have secondary peroxidative functions [such as the capability to catalyze oxidation of ethanol (93) or formaldehyde (97) in the presence of H₂O₂] or may contain other, manganese-based active sites (20). _H. pylori_ catalase is further subclassified as a clade 3 catalase (so-called “small subunit catalase”), characterized by its tetrameric form comprising four identical ~50-kDa subunits (48). Although classified as a clade 3 catalase, KatA is unusual, in that it does not appear to bind NADPH, a property of all other known clade 3 catalases that is theorized to increase stability of the enzyme (65). BLAST protein sequence analysis reveals that, outside the genus *Helicobacter*, KatA is most homologous with the catalases of bacteria from the order Burkholderiales, which includes pathogens such as *Bordetella pertussis* (64.9% homology) (1). Homology with human catalase is 53% on the basis of BLAST protein sequence alignment.

The active site of KatA is centered on a heme ligand deep within the enzyme, which H₂O₂ is presumed to access via a long channel from the surface (55). This channel varies in structure between catalases; in KatA, the channel opening is marginally smaller (by ~0.5–1 Å) than that of most other known catalases, but beyond this point the channel opens into a wider chamber (65). Amino acid substitution studies have shown that this channel opening is sensitive to size changes, with a substantial reduction in enzyme activity observed after structural alteration of the channel dimensions (18).

Regulation of KatA production appears to be similar to regulation of Hp-SOD production, in that while the enzyme is constitutively expressed, the rate of production is partially upregulated by Fur in response to environmental iron concentrations (46). The function of KatA appears to be associated with a downstream gene, _kapA_, which encodes the KatA-associated protein (KapA) (46). There is evidence that KapA may be involved in translocation of the catalase enzyme into the periplasmic space, as _H. pylori_ KapA mutants had reduced catalase activity in the periplasmic fraction (45). In addition to its occurrence in the cytoplasm and periplasmic space, catalase has been characterized as a secreted protein (4, 89) and an outer membrane protein (4, 7, 86), supporting a role for the enzyme in defense against exogenous ROS. _H. pylori_ catalase is also present within outer membrane vesicles (77).

Catalase is obviously an enzyme of key importance to _H. pylori_, as it accounts for ~1% of total bacterial protein (10a). Its role in oxidative stress resistance has been clearly demonstrated with catalase imparting resistance to extracellular ROS generated by phagocytes (81). As for Hp-SOD, the importance of KatA has been clearly demonstrated by KatA-deficient _H. pylori_ mutants, which are defective in their capacity for long-term colonization of mice and also more sensitive to oxidative stress (47). The same phenotype was displayed by KapA-deficient mutants (47), confirming the essential interdependent nature of these enzymes.

**Thiol Peroxidase and Other Peroxiredoxins.** Peroxiredoxins (Prxs) are another family of antioxidant enzymes important for defense against exogenous ROS. _H. pylori_ produce three enzymes from this family: thiol peroxidase (Hp-Tpx), alkyl hydroperoxide reductase (AhpC), and bacterioferritin comigratory protein (Bep).

Prxs can catalyze the reduction and detoxification of a wide range of hydroperoxides (83). Thus, while Prxs can perform a role similar to catalase in degrading H₂O₂, they often possess much broader target specificity. Their peroxidatic function can be generalized as

$$\text{ROOR} + 2\text{H}^+ + 2e^- \rightarrow \text{ROH} + \text{R OH}$$

where R and R’ are organic moieties. This reaction is particularly important for prevention of lipid peroxidation cascades initiated by ROS, thereby maintaining cell membrane integrity. The structure of the binding site of _E. coli_ Tpx appears to be...
optimized for recognition of fatty acid hydroperoxides (22), suggesting that this is a major function of the enzyme. The three Prxs of \textit{H. pylori} have different substrate preferences: AhpC and Hp-Tpx exhibit broad protective activity against a range of hydroperoxides (9, 26), and Bcp has a distinct substrate preference for linoleic acid (103). AhpC from \textit{H. pylori} and other bacterial species also possess activity against peroxynitrite, which has not been observed for the other enzymes (15).

The enzymes are distinguished by the number of conserved cysteine residues within the structure, with AhpC classified as a “typical” 2-Cys Prx (78), Hp-Tpx as an “atypical” 2-Cys Prx (70), and Bcp as a 1-Cys Prx (103). Typical and atypical 2-Cys Prxs differ in the conformation of the disulfide bond that forms upon oxidation of the cysteine residues: the disulfide bond of typical 2-Cys Prxs is intermolecular, leading to dimer formation, whereas the bond in atypical 2-Cys Prx is intramolecular, resulting in monomer formation (83). BLAST protein sequence analysis reveals that homology with human Prxs is low, with the closest homolog of Hp-Tpx, Prx 3, having only 29% homology, while AhpC has 48% homology, with human Prx 2 (1).

Under stress, such as in oxidizing environments, typical 2-Cys Prxs, including \textit{H. pylori} AhpC, undergo oligomerization to form high-molecular-weight complexes (24, 54). These multimeric forms possess distinct chaperoning activity, protecting proteins from inactivation through unfolding or aggregation. This indicates that AhpC may have a role in protecting intracellular proteins from oxidative damage (24). Chaperoning functionality has not been described for atypical 2-Cys and 1-Cys Prxs. However, it has recently been suggested that atypical 2-Cys Prxs may also exist in multimeric forms (10, 84). Nguyen et al. (70) found Hp-Tpx to be a purely monomeric, 18-kDa protein. However, nuclear magnetic resonance analysis of the enzyme detected peaks of higher molecular weight (70) that may represent multimeric forms. This suggests that chaperoning ability in this class of Prx cannot be discounted completely, although because of the low level of oligomerization, its role is likely to be minor.

In a physiological setting, \textit{H. pylori} Prx enzymes require the thioredoxin system for regeneration of the oxidized form following antioxidant activity (26). In this system, thioredoxin (Trx1) acts as an electron donor to the Prx, and Trx1 is subsequently replenished by the enzyme thioredoxin reductase (TrxR), with NADPH used as a substrate (9). Without this system, the enzymes are unable to regain function and remain inactivated.

Hp-Tpx and AhpC appear to have important physiological roles in stomach colonization, as mutants are highly susceptible to oxidative damage and are severely impaired in their ability to colonize mice (75). In contrast, Bcp appears to be less important for survival, as mutants deficient in this enzyme exhibit only mild oxidative sensitivity and impairment of colonization (26, 103). The precise relationship between the functions of the three Prxs remains unclear. However, there is some suggestion that Hp-Tpx may be more important for protection from external ROS, rather than regulation of normal cellular redox equilibrium, as Hp-Tpx mutants grow at near-normal rate under standard microaerophilic conditions but exhibit increased sensitivity to exogenous oxidative stress induced by superoxide, H$_2$O$_2$, and organic hydroperoxides (26, 75). Conversely, AhpC has been primarily associated with neutralization of endogenous hydroperoxides generated through normal metabolism in \textit{E. coli} (87). Although this has not been demonstrated specifically for \textit{H. pylori}, expression of AhpC is greatly reduced under oxidative stress conditions (23), suggesting that this enzyme may not have a major role in protection against oxidative attack.

The assertion that Hp-Tpx is involved in defense against exogenous oxidative attack is strengthened by the fact that Hp-Tpx has been reported as a potential membrane-associated protein (4, 108) and has been detected in \textit{H. pylori} outer membrane vesicles (68). Although other studies have failed to detect Hp-Tpx extracellularly (89), this may reflect differences in growth conditions, as Tpx has been shown to have variable extracellular expression in other bacterial pathogens, depending on media composition and atmospheric oxygen concentration (38). While this condition-dependent expression variability has not been specifically assessed in \textit{H. pylori}, mouse adaptation was associated with altered membrane expression of Hp-Tpx (108), suggesting that environmental changes can influence extracellular expression. Other reports indicate that Tpx is expressed extracellularly by \textit{Mycobacterium tuberculosis} (84), \textit{Actinobacillus actinomycetemcomitans} (38), and \textit{Bacillus anthracis} (2) and periplasmically in \textit{E. coli} (16), despite the apparent absence of a signal sequence. Interestingly, Tpx also appears to play a role in protecting \textit{Enterococcus faecalis} against phagocytosis (62). If analogous to \textit{H. pylori}, this may explain, in part, \textit{H. pylori}’s resistance to phagocytic digestion.

Proteins with potential antioxidant roles. Neutrophil-activating protein A. Neutrophil-activating protein A (NapA) is a dodecameric bacterioferritin comprising 12 identical 15-kDa subunits (34). As its name suggests, the primary role of NapA was originally thought to be neutrophil stimulation, by promoting neutrophil adhesion to endothelial cells and stimulating NADPH oxidase activity (35). However, investigations in human patients found no correlation between neutrophil activation and \textit{H. pylori} NapA expression, suggesting that this function may not be important clinically (64). Subsequently, the NapA protein was implicated in oxidative stress responses, as NapA-deficient \textit{H. pylori} are more susceptible to oxidative damage in vitro (27). Deficiency of a range of antioxidant enzymes, including Hp-SOD, KatA, Hp-Tpx, and AhpC, results in a compensatory increase in NapA production (76), suggesting that it may function as a secondary antioxidant that becomes important when other defenses are overwhelmed. This is supported by the fact that NapA mutant strains of \textit{H. pylori} are able to colonize mice normally (99), indicating that the enzyme is not required for survival and that some functional redundancy may be present. Although the precise mechanism of its antioxidant activity is unknown, it is possible that the iron-sequestering properties of NapA may help prevent secondary ROS formation from superoxide (99). When iron-loaded, NapA can also bind to DNA with great affinity and, therefore, may play a role in protecting DNA from oxidative damage. Indeed, NapA-deficient mutants possess significantly more fragmented DNA after aerobic incubation than the wild-type strain (99).

\textbf{NADPH quinone reductase.} Quinones are potentially toxic aromatic metabolites of the bacterial respiratory chain. They are capable of spontaneous redox cycling with their partially reduced semiquinone, resulting in the generation of superoxide.
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NADPH quinone reductase (MdaB) bypasses this superoxide generation by fully reducing quinones to quinol via a two-electron reduction (101).

Investigation of the role of MdaB in oxidative stress protection was initiated after it was observed that expression of the enzyme is upregulated in AhpC:NapA double mutants (101). MdaB-deficient mutant bacteria were unable to colonize mouse stomachs and were more sensitive to oxidative stress (101). However, as quinones are not known to be generated as a component of host defense, the primary role of this enzyme is likely to be in detoxifying metabolites of the respiratory chain, an assertion borne out by the fact that MdaB has not been reported to be secreted or surface-associated.

DNA REPAIR ENZYMES. The methyl-directed mismatch repair system is a highly conserved mechanism for DNA repair. The role of two of these enzymes, MutS and MutY, in oxidative stress has been investigated in H. pylori. Both enzymes appear to be important for repair of base mismatches resulting from oxidation of guanine to 8-oxoguanine (33, 98). Knockout of either gene individually resulted in an increase in DNA base pair mismatch and mutagenesis under oxidative stress. In addition, these mutant strains were impaired in their ability to colonize mice.

Endonuclease III (EndoIII) is another DNA repair enzyme reported to be important for oxidative stress resistance (73). EndoIII is able to excise potentially mutagenic oxidized pyrimidine bases from DNA strands. While displaying normal growth in vitro, mutants deficient in EndoIII exhibit reduced colonization of mice and increased sensitivity to superoxide and H2O2 (73), suggesting that EndoIII is specifically protective against exogenous oxidative damage.

H. pylori lacks homologs of many DNA repair enzymes found in other bacterial species, including the E. coli enzymes EndoVIII, MutM, MutH, MutL, Fpg, and AlkA (94). This restricted range of enzymes suggests that H. pylori may be more sensitive to DNA damage than other bacteria and may account for the high mutation rate in H. pylori (100).

HIGH TEMPERATURE REQUIREMENT A AND OTHER PROTEASES. H. pylori high temperature requirement A (HtrA) is a secreted serine protease that has been shown to cause disruption of the gastric epithelial barrier through cleavage of the E-cadherin intercellular adhesion protein (49). Unusually, however, expression of HtrA by H. pylori is upregulated under conditions of oxidative stress (50, 102), suggesting a potential additional role in combating oxidative damage. In other bacteria, deficiency of HtrA has been found to be associated with an increase in sensitivity to oxidative stress (21, 88), although this has not been investigated for H. pylori. HtrA has been found to be membrane-associated (4) and present within outer membrane vesicles (77), indicating that the enzyme has a strong presence at the exterior of the bacterium, which may facilitate a role in defense against oxidative stress.

However, another protease of H. pylori, ATP-dependent caseinolytic protease (Clp), has been shown to be important for resistance to exogenous oxidative stress. H. pylori Clp forms a hybrid complex consisting of a chaperoning subunit (ClpA or ClpX) combined with the proteolytic ClpP subunit (66). Although each of the subunits possess some enzymatic activity individually, when combined, the chaperoning subunit is able to efficiently coordinate presentation of protein substrate cleavage sites to the proteolytic subunit (82). Double mutation of the ClpA/ClpP subunits resulted in increased sensitivity to oxidative attack as well as increased susceptibility to killing by macrophages (66). The growth of these mutants under normal cultivation conditions was unaffected, however, suggesting that the enzyme may have a specific role in combating oxidative attack. Mutants deficient in Clp were also defective in colonizing mice. Although the protective mechanism of Clp is uncertain, it may stem from the ability of this complex to chaperone or cleave oxidized proteins.

Conclusion

H. pylori thrive in a ROS-rich environment. While the organism can inhibit the production of toxic oxidants such as peroxynitrite, it primarily relies on its antioxidant system to prevent cellular damage. However, for an organism heavily dependent on antioxidant defenses for survival, the system is surprisingly underdeveloped. H. pylori produces only singular isoforms of several antioxidant enzymes compared with multiple forms in other bacteria and lacks other enzymes entirely. The relative paucity of oxidative defense pathways means that there is very little redundancy within the system, demonstrated by reduced viability in nearly all the enzyme-deficient mutants. Simplification of the system in H. pylori may reflect specific adaptation to the stomach environment and loss of pathways that are not essential for colonization. However, because H. pylori lacks the ability to compensate for impairment of the oxidative defense system, these pathways represent an attractive target for therapeutic exploitation.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

A.S., A.L.E., and P.S. prepared the figures; A.S. drafted the manuscript; A.S., A.L.E., and P.S. edited and revised the manuscript; A.S., A.L.E., and P.S. approved the final version of the manuscript.

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Helicobacter pylori (H. pylori) is a common human pathogen that colonizes the stomach and is associated with chronic gastritis, peptic ulcer disease, and an increased risk of gastric adenocarcinoma. One of the mechanisms by which H. pylori withstands the highly oxidative stomach environment is through the production and utilization of catalase, a key enzyme in the detoxification of hydrogen peroxide. The iron-binding properties of gastric juice are crucial for the survival of H. pylori under oxidative stress induced by hydrogen peroxide. The interaction of Helicobacter pylori with host macrophages and mouse tissue is another significant factor in this process. The study of H. pylori defense mechanisms against oxidative attack is critical for understanding the pathogenesis of gastritis and related diseases.
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