MINIREVIEW

Potential role of thiol:disulfide oxidoreductases in the pathogenesis of Helicobacter pylori

Nadeem O. Kaakoush, Zsuzsanna Kovach & George L. Mendz
School of Medical Sciences, The University of New South Wales, Sydney, Australia

Abstract

Helicobacter pylori infections are responsible for a sequence of molecular events which ultimately result in the development of gastric diseases. The pathogenesis of H. pylori has been studied extensively with strong focus on the identification of virulence factors. In contrast, the involvement of thiol:disulfide oxidoreductases in bacterial pathogenesis is less well understood. This paper provides a review of the current knowledge of H. pylori putative thiol:disulfide oxidoreductases, and their potential role in promoting virulence and colonization. Several bioinformatic analyses served to complete the information on these oxidoreductases of H. pylori.

Introduction

Helicobacter pylori-induced gastroduodenal pathogenesis depends on the persistence of the infection, the production of specific virulence factors that cause damage to gastric epithelial cells and disruption of the gastric mucosal barrier, and the inflammatory response of the host (Kusters et al., 2006). There have been many investigations of the factors involved in H. pylori gastric colonization and virulence. To date, there are only few studies on the contribution of H. pylori disulfide reductases to bacterial pathogenesis.

Host reactions to bacterial infections: oxidative and nitrative bursts

Among the factors influencing the fate of infecting bacteria, two important ones are their capacities to avoid inducing an oxidative metabolic burst (Beaman & Beaman, 1984) and to resist reactive products of oxygen metabolism.

In response to bacteria, several types of cells, including macrophages, neutrophils and epithelial cells, produce a metabolic burst increasing the concentrations of bactericidal reactive oxygen species (ROS) including oxygen ions (superoxide anion), free radicals (hydroxyl radicals) and highly reactive inorganic and organic peroxides. Similarly, reactive nitrogen species (RNS) are produced in response to infection (Nathan & Shiloh, 2000). Increased levels of these reactive species can produce significant damage to biomolecules and host tissues (Imlay, 2003), leading to oxidative and/or nitrative stress.

In addition to direct bactericidal effects, generation of ROS and RNS affects the adaptive immune system and leads to recruitment of immune cells and stimulation of inflammatory processes; secretion of chemotactic and growth factors, proteolytic enzymes, lipoxigenases and cyclooxygenases; inactivation of antiproteolytic enzymes; and activation of oncogenes and transcription factors.

Normally, host cells are able to defend themselves against ROS damage through the use of enzymes such as superoxide dismutases and catalases. Small antioxidant molecules such as ascorbic acid, glutathione, polyphenols and uric acid also play important roles in cell protection.

Host reactions to H. pylori infection

Colonization of the gastric mucosa by H. pylori first results in the induction of an inflammatory response, predominantly of the T-helper 1 (Th1) type. This local inflammatory response is characterized by an influx of neutrophils, mononuclear cells, natural killer cells and T cells, typically aimed at clearing the infection (Kusters et al., 2006). However, H. pylori is not
an intracellular pathogen, and thus the Th1 response results in epithelial cell damage rather than in the removal of H. pylori (Kusters et al., 2006). The continued presence of H. pylori thus causes a lifelong inflammatory response, responsible for the ongoing production of ROS that cause cellular damage.

The microaerophily of H. pylori

Microaerophilic bacteria, like Helicobacter, have oxygen-dependent growth but cannot grow under fully aerobic conditions at 21% O2 tension (Bury-Mone et al., 2006). Aerotolerance varies between Helicobacter species and strains (Franklin et al., 1996), and the optimal content of the atmosphere for growth in vitro is 5–8% partial O2 tension. Although H. pylori can be grown in atmospheres with different oxygen contents, there is evidence that significant changes occur in the physiology and pathogenicity of the bacterium which depend on the growth atmosphere (Cottet et al., 2002; Bury-Mone et al., 2006).

Microaerophiles are particularly vulnerable to the detrimental effects of ROS and oxidative stress in general. To colonize its specific niche H. pylori has developed an impressive array of antioxidant defenses, which allows its persistent colonization of the human gastric mucosa (Wang et al., 2006). Thus, the defenses of H. pylori against the host contribute to make the infection chronic, and to bring about long-term tissue damage through the oxidative stress response of the host. This relationship is central to the pathogenesis of H. pylori.

Antioxidant defenses and H. pylori pathogenesis

Helicobacter pylori has evolved a complex molecular machinery to reduce the efficiency of oxygen-dependent antimicrobial systems (Wang et al., 2006). Some of its protective mechanisms are common to aerobic bacteria, but H. pylori has devised also a battery of unique strategies which play a central role in its survival and colonization of the host.

The bacterium avoids damage from oxygen metabolism by-products or oxidative host responses by expressing ROS scavengers such as superoxide dismutase SodB (Bereswill et al., 2000; Nathan & Shiloh, 2000; Seyler et al., 2001), catalase KatA (Nicholls et al., 2001; Harris et al., 2002; Basu et al., 2004) and KatA-associated protein KapA (Harris et al., 2002). The activities of alkyl hydroperoxide reductase AhpC (Bryk et al., 2000; Baker et al., 2001; Wang et al., 2004), and thiol peroxidases Bcp and Tpx (Wang et al., 2005) afford H. pylori protection against organic peroxides. Also involved in resistance to oxygen stress are the NADPH quinone reductase MdbA (Olczak et al., 2002; Wang & Maier, 2004), and the iron-binding protein NapA (Evans et al., 1995; Cooksley et al., 2003; Olczak et al., 2005). Systems to repair oxidative DNA damage in H. pylori include endonuclease III Nth (O’Rourke et al., 2003), 8-oxoguanine binding protein MutS (Pinto et al., 2005) and endonuclease RuvC (Loughlin et al., 2003).

Helicobacter pylori has a very low number of regulators potentially acting on the expression of antioxidant proteins. The involvement of the ferric uptake regulator Fur in the expression of ferritin Pfr, KatA and SodB indicates a close connection between iron homeostasis and resistance to oxidative stress (Harris et al., 2002; Waidner et al., 2002; Ernst et al., 2005a,b). The posttranscriptional regulator CsrA is necessary for H. pylori full motility and survival to oxidative stress, probably by contributing to mRNA stabilization (Barnard et al., 2004). CsrA is involved in regulating Fur and the heat shock protein regulator HspR, suggesting a regulatory hierarchy in which posttranscriptional regulation plays a major role in stress responses in H. pylori (Barnard et al., 2004). Knockout mutants generated from many of these genes have all resulted in either a defective (< 5% of wild-type) or attenuated (significant less than wild-type) colonization (Wang et al., 2006).

Pathways to synthesize glutathione or other thiol redox compounds are not present in H. pylori. Instead the bacterium has a thioredoxin system comprising the thioredoxins TrxA1 and TrxA2, a thioredoxin reductase TrxB1 (Baker et al., 2001), and another protein annotated as a second thioredoxin reductase TrxB2 (Alm & Trust, 1999). The thioredoxin system mediates resistance to oxidative and nitrosative stress in H. pylori (Comtois et al., 2003). The system is active in reducing AhpC (Baker et al., 2001), Tpx and Bcp (Comtois et al., 2003), as well as the methionine sulfoxide reductase Msr (Alamuri & Maier, 2004). TrxA1 also acts as an arginase chaperone capable of renaturing the enzyme to a catalytically active state (McGee et al., 2006).

Mechanisms to repair oxidized proteins indirectly confer cell resistance against oxidative stress (Vogt, 1995). Together with cysteinyls and tryptophanyls, methionyls are some of the most easily oxidized amino acids (Vogt, 1995). Numerous proteins lose functional activity by oxidation of methionyls. The reduction of methionine sulfoxide to methionine is catalysed by the TrxA1-TrxB1-dependent methionine sulfoxide reductases MsrA and MsrB, which in H. pylori are fused into one polypeptide (Alm & Trust, 1999). Helicobacter pylori mutants with either an inactivated MsrB domain or both domains show compromised growth under oxidative stress conditions (Alamuri & Maier, 2006).

The pathogenicity of H. pylori urease is not confined to its roles in producing toxic ammonia from urea hydrolysis (Sommi et al., 1996), promoting colonization by buffering the microenvironment of the bacterium (Weeks et al., 2000), and contributing to generate RNS through the ammonia produced and the recruitment of neutrophils (Suzuki et al.,
Several studies have linked the disulfide reductases and the pathogenesis of Bacillus subtilis defenses, disulfide reductases are involved also in virulence. As with other antioxidant metal detoxication (Moore 1992). In macrophages, urease activates induced nitric oxide synthase (iNOS) and the resulting increase in nitric oxide (NO) production contributes to H. pylori pathogenesis (Gobert et al., 2002). To avoid being killed by NO, the bacterium produces arginase whose substrate arginine is also an iNOS substrate. Thus, NO synthesis is regulated by the competition for the common arginine substrate, and arginase provides a protective mechanism to H. pylori against oxidative and nitrosative stresses (Gobert et al., 2001).

**Involvement of disulfide reductases in pathogenesis**

Oxidoreductases, and specifically disulfide reductases, play a major role in the antioxidant defense mechanisms of bacteria. A central function of disulfide reduction is the maintenance of intracellular redox balance. Disulfide reductases have roles also in other cellular processes such as heavy metal detoxication (Moore et al., 1992; Hayashi et al., 2000; Zegers et al., 2001) and drug resistance (Mendz & Mgraud, 2002; Kaakoush & Mendz, 2005). As with other antioxidant defenses, disulfide reductases are involved also in virulence.

For example, StoA is a membrane-bound thiol:disulfide oxidoreductase important in spore cortex synthesis in Bacillus subtilis (Erlendsson et al., 2004). The cortex is composed of a thick peptidoglycan layer that helps to maintain the dehydrated state of the spore (Erlendsson et al., 2004). Peptide methionine sulfoxide reductase (Msr) activity is involved in the virulence of the pathogens Escherichia coli, Streptococcus pneumoniae, Erwinia chrysanthemi, Mycoplasma genitalium and Neisseria gonorrhoeae (Taha et al., 1991; Wizemann et al., 1996; El Hassouni et al., 1999; Dhadayuthapani et al., 2001). The PilB protein of Neisseria spp. participates in the virulence of bacteria of this genus. This protein has three subdomains which contain Msr activity, and performs a similar role to that of Msr in other bacteria (Olry et al., 2002). A Salmonella enterica serovar typhimurium trxA-negative mutant does not exhibit any growth defects or decreased tolerance to oxidative or nitric oxide stress in vitro, yet it has pronounced decreases in intracellular replication and virulence in mice (Bjur et al., 2006). The attenuation of virulence caused by a thioredoxin deficiency was restored by expression of wild-type thioredoxin in a complemented mutant (Bjur et al., 2006).

**Disulfide reductases and the pathogenesis of H. pylori**

Several studies have linked the H. pylori thioredoxin system and Msr with the colonization capability of the bacterium. It has been hypothesized that thioredoxin assists H. pylori in the process of colonization by inducing focal disruptions of the oligomeric structure of mucus while rendering host antibodies inactive through catalytic reduction (Windle et al., 2000). *Helicobacter pylori* Bcp is a thiol peroxidase that depends on the reducing activity of the thioredoxin system, and plays a significant role in efficient host colonization (Wang et al., 2005). Mouse colonization studies in H. pylori have shown that Msr is an important factor for effective longer-term colonization (Alamuri & Maier, 2004). Complementation of an msr knockout strain by chromosomal insertion of a functional gene restored its mouse colonization ability (Alamuri & Maier, 2004).

**Functions of thiol:disulfide oxidoreductases**

One way in which pathogenic bacteria interact with their hosts is by secreting proteins. In Gram-negative bacteria, several types of secretion pathways have been identified. In all of them, many of the proteins residing in or transiting through the periplasmic space form disulfide bonds after their translocation across the inner membrane, suggesting that disulfide bond formation is crucial for the protein-folding pathway of many cell envelope proteins (Miki et al., 2004). Through this function, the family of thiol:disulfide reductases Dsb contributes to the structural integrity of some enzymes, and modulates their activities (Fabianek et al., 2000). Dsb reductases have been implicated also in cytochrome c maturation (Fabianek et al., 2000).

Periplasmic oxidoreductases have activities different from those in the cytoplasm. The cytoplasmic proteins are generally reducing proteins, while the periplasmic proteins are oxidizing, reducing or isomerizing (Fabianek et al., 2000). The functions of the periplasmic enzymes are: the oxidative formation of disulfide bonds, which is often necessary for folding and stability of secretory proteins; the reduction of nonnative disulfides; or the isomerization of disulfide bonds in proteins, especially when wrong disulfide bonds are formed (Fabianek et al., 2000). The periplasmic enzyme DsbA and the integral membrane protein DsbB are involved in the oxidation pathway in the periplasm of many Gram-negative bacteria (Raina & Missiakas, 1997; Rietsch & Beckwith, 1998; Fabianek et al., 2000). The isomerization pathway includes the periplasmic protein DsbC, and the inner membrane protein DsbD, which receives electrons from thioredoxin and transports them across the inner membrane (Raina & Missiakas, 1997; Rietsch & Beckwith, 1998; Fabianek et al., 2000). The periplasmic enzyme DsbG has not been fully characterized, but evidence suggests that it is responsible for redox balance in the periplasm.

**Pathogenicity of thiol:disulfide oxidoreductases**

Thiol:disulfide oxidoreductases contribute to the pathogenicity of diverse organisms through their role as an essential catalyst promoting the correct folding of secreted or surface-presented factors such as toxins, adherence factors and components of type III secretory systems (Yu & Kroll, 2007).
1999). Examples of these virulence factors are the cholera toxin of *Vibrio cholerae* (Peek & Taylor, 1992; Yu et al., 1992), the heat-stable toxin of enterotoxigenic *E. coli* (Yamanaka et al., 1994; Okamoto et al., 1995), the molecular chaperone, PapD, of the P pili of uropathogenic *E. coli* (Jacob-Dubuisson et al., 1994), the bundle-forming pilus and intimin of enteropathogenic *E. coli* (Zhang & Donnenberg, 1996; Hicks et al., 1998), and the invasin of *Yersinia pseudotuberculosis* (Leong et al., 1999). DsbA is required also for the proper function of the Type III secretion pathway in *Yersinia pestis* (Jackson & Plano, 1999), *Shigella flexneri* (Watarai et al., 1995), *Salmonella enterica* serovar typhimurium (Miki et al., 2004) and *Pseudomonas aeruginosa* (Ha et al., 2003). In-activation of DsbA affects the intracellular survival and virulence of *Shigella flexneri* (Yu, 1998). This protein is involved also in the correct folding of the cellulase EGZ in the plant pathogen *Erwinia chrysanthemi* (Yu & Kroll, 1999). DsbA and DsbC are required for secretion of Pertussis toxin by *Bordetella pertussis* (Stenson & Weiss, 2002).

**Identification of *H. pylori* thiol:disulfide oxidoreductases**

Little is known about the enzymes catalysing disulfide bond formation in *Epsilonproteobacteria*. In *H. pylori*, the DsbB-like protein DsbI encoded by *hp0377* has been identified experimentally (Raczko et al., 2005), and a putative DsbC encoded by *hp0377* has been annotated in the genome of *H. pylori* strain 26695. In addition, the cytochrome c biogenesis protein encoded by *hp0265* has sequence similarity to thiol:disulfide oxidoreductases from related bacterial species.

Generally, thiol:disulfide oxidoreductases are characterized by two features. They share an active site containing two cysteines arranged in a CXXC motif; the cysteins are either in the reduced state forming two thiols or in the oxidized state forming an intramolecular disulfide bond (Fabianek et al., 2000). The second feature of most of these oxidoreductases is a common tertiary structure known as the thioredoxin-like fold, which is present despite very low primary sequence similarities (Fabianek et al., 2000). Bioinformatic identification of Dsb proteins in *H. pylori* using these two features was performed. Searches in the genome of *H. pylori* strain 26695 for proteins containing a CXXC motif identified a total of 149 proteins.

The thioredoxin-like fold common to enzymes that catalyse disulfide bond formation and isomerization is an example of an \( \alpha/\beta \) protein fold that has oxidoreductase activity. Its spatial topology consists of a four-stranded \( \beta \) sheet sandwiched between two \( \alpha \) helices (Copley et al., 2004). *Helicobacter pylori* proteins which contain this fold in their structure were identified using the database INTERPRO (Mulder et al., 2005). A total of eight proteins were found to contain the thioredoxin-like fold, four of which also contained the CXXC motif (Table 1). These four proteins were the two thioredoxins, the putative DsbC and a hypothetical protein encoded by *hp0377*. The DsbI protein encoded by *hp0377* had a CXXC motif in its sequence but did not contain a thioredoxin-like fold in its structure.

Sequence alignments of the three putative *H. pylori* Dsb proteins with the *E. coli* Dsb proteins were performed. An indication of the functional similarity within each pair was the alignment and conservation of the motifs (Fig. 1). The sixth residue before the N-terminal cysteinyl of the motif was also highly conserved among the three pairs of proteins (Fig. 1). The predicted coding region HP0231 aligned with *E. coli* DsbG; the *H. pylori* putative DsbC aligned with *E. coli* DsbC; and the first part of the *H. pylori* DsbI protein aligned with *E. coli* DsbB, as indicated by Raczko et al. (2005). These authors speculated that the second part of the *H. pylori* Dsb protein, a \( \beta \)-propeller domain, could act as a platform for recruiting a protein with an active missing second pair of cysteines (Raczko et al., 2005). In *H. pylori*, the functional replacement of DsbB with DsbI and the absence of DsbA provided evidence for a novel Dsb oxidizing system.

**Table 1. Helicobacter pylori strain 26695 proteins containing a thioredoxin-like fold as identified by INTERPRO**

<table>
<thead>
<tr>
<th>ORF</th>
<th>Protein</th>
<th>CXXC motif</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>hp0096</em></td>
<td>2-Hydroxyacid dehydrogenase</td>
<td>No</td>
</tr>
<tr>
<td><em>hp0136</em></td>
<td>Bacterioferritin comigratory protein</td>
<td>No</td>
</tr>
<tr>
<td><em>hp0231</em></td>
<td>Hypothetical protein</td>
<td>Yes</td>
</tr>
<tr>
<td><em>hp0377</em></td>
<td>Putative thiol:disulfide interchange protein</td>
<td>Yes</td>
</tr>
<tr>
<td><em>hp0390</em></td>
<td>Adhesin-thiol peroxidase</td>
<td>No</td>
</tr>
<tr>
<td><em>hp0824</em></td>
<td>Thioredoxin</td>
<td>Yes</td>
</tr>
<tr>
<td><em>hp1458</em></td>
<td>Thioredoxin</td>
<td>Yes</td>
</tr>
<tr>
<td><em>hp1563</em></td>
<td>Alkyl hydroperoxide reductase</td>
<td>No</td>
</tr>
</tbody>
</table>

The proteins were identified among those containing CXXC motifs in their sequences.

![Fig. 1. Sequence alignment of the motifs of the three putative Helicobacter pylori Dsb proteins with the motifs of the Escherichia coli Dsb proteins.](image-url)
Thiol:disulfide oxidoreductases and the pathogenesis of H. pylori

Four proteins of interest encoded by hp0231, hp0265, hp0377 and hp0595 were identified as putative Dsb proteins in H. pylori. Investigation of their potential involvement in virulence or colonization indicated that HP0231 and HP0595 are related to the colonization efficiency of H. pylori (Haas et al., 2002; Sabarth et al., 2002; Godlewksa et al., 2006), but to date no studies have linked HP0265 or HP0377 to the pathogenesis of the bacterium.

Proteins secreted by H. pylori may contribute to gastric inflammation and epithelial damage in the host. In vitro analysis identified HP0231 as a secreted protein enriched more than 10-fold compared with UreB, and it may be implicated in H. pylori-induced effects on the gastric epithelium (Kim et al., 2002). HP0231 was recognized by H. pylori-positive sera in a systematic, proteome-based approach used to detect candidate antigens of H. pylori for diagnosis, therapy and vaccine development, and to investigate potential associations between specific immune responses and manifestations of disease (Haas et al., 2002). A study of H. pylori antigens in specific-pathogen-free mice using multiparameter selection demonstrated that HP0231 conferred protective immunity in the mouse Helicobacter infection model with levels of protection generally considered the gold standard for Helicobacter immunization (Sabarth et al., 2002). These studies demonstrated that this protein is involved in the colonization by H. pylori.

The protein Dsb1 encoded by hp0595 is a novel component of the Dsb system in H. pylori important for disulfide bond formation in periplasmic proteins (Raczko et al., 2005). A dsbI-knockout mutant impaired in disulfide bond formation revealed a greatly reduced ability to colonize the gastric mucosa of mice, suggesting a role for DsbI in the pathogenesis of H. pylori (Godlewksa et al., 2006).

The importance of the Dsb system in the pathogenesis of bacteria has become clearer in the past decade. Among the large number of studies published on H. pylori pathogenesis, only a few have addressed the family of thiol:disulfide oxidoreductases. This review serves to highlight the importance of these enzymes and to identify avenues for future experimental investigations. Evidence supports the hypothesis that H. pylori has a novel Dsb system, and characterizing this system will be required to understand fully the bacterium’s periplasmic environment, and how it interacts with the host.

Acknowledgements

This work was made possible by the support of the Cancer Council of New South Wales, Australia.

References


