The Antioxidant Protein Alkylhydroperoxide Reductase of Helicobacter pylori Switches from a Peroxide Reductase to a Molecular Chaperone Function

Abstract / Helicobacter pylori, an oxygen-sensitive microaerophilic bacterium, contains many antioxidant proteins, among which alkylhydroperoxide reductase (AhpC) is the most abundant. The function of AhpC is to protect H. pylori from a hyperoxidative environment by reduction of toxic organic hydroperoxides. We have found that the sequence of AhpC from H. pylori is more homologous to mammalian peroxiredoxins than to eubacterial AhpC. We have also found that the protein structure of AhpC could shift from low-molecular-weight oligomers with peroxide-reductase activity to high-molecular-weight (HMW) complexes with molecular-chaperone function under oxidative stresses. Time-course study by following the quaternary structural change of AhpC in vivo revealed that this enzyme changes from low-molecular-weight oligomers under normal microaerobic conditions or short-term oxidative shock to high-molecular-weight complexes after severe long-term oxidative stress. This study revealed that AhpC of H. pylori acts as a peroxide reductase in reducing organic hydroperoxides and as a molecular chaperone for prevention of protein misfolding under oxidative stress.

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Chronic infection of Helicobacter pylori (H. pylori), a Gram-negative bacteria, could lead to various gastrointestinal diseases including chronic gastritis, gastric and duodenal ulceration, and gastric cancer¹⁻³. After successful isolation and culture of Helicobacter pylori from patients with gastritis about 20 years ago by Warren and Marshall⁴, the complete genome sequences of various H. pylori strains have been identified. However, the specific functions of most gene products remain to be defined. Previous investigations in this field mainly focused on the post-infection pathogenic events and more recently on the alteration of gene expression and protein modification under environmental stresses.

To address the influence of oxidative stress on H. pylori, we have compared proliferation, urease activity and the protein expression profile of H. pylori incubated under normal microaerophilic (5% O₂) and aerobic stress (20% O₂) conditions⁵. Oxidative-stress cells displayed coccoid morphology and time-dependent decrease in proliferation. The urease activity was completely abrogated after 32 hours. We have further compared the protein expression profiles of H. pylori under normal growth and oxidative-stress conditions by a global proteomic analysis, which includes high-resolution two-dimensional gel electrophoresis (2-DE) followed by matrix-assisted laser desorption/ionization — time of flight (MALDI-TOF) mass spectrometry and bioinformatic databases search/ peptide-mass comparison. The results revealed that more than ten proteins were differentially expressed under oxidative stress. Most notably, the protein expression level of UreE (urease accessory protein E, an essential metallochaperone for urease activity) and alkylhydroperoxide reductase (AhpC) with antioxidant potential are greatly down-regulated under stress conditions. We have further cloned and expressed the AhpC gene of H. pylori from patients with duodenal ulcers and...
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Fig 1  Sequence alignment and homology comparison of *H. pylori* AhpC with homologous AhpC and Prxs from other species. (A) Alignment of the amino acid sequences of AhpC and Prxs (2-Cys Prx) from several representative prokaryotes and eukaryotes. Identical and consensus amino acids in all sequences were shown in black boxes (shaded). (B) Pair-wise comparison of protein sequence identity and divergence and (C) construction of phylogenetic tree based on sequence divergence between the AhpC sequences from clinical HP isolates (HC28 and HD30) and AhpC homologues from other species.

Fig 2  Structural and molecular-size changes of purified AhpC under oxidative stress (Left) One-dimensional *in vitro* native-PAGE (4-12% gradient gel) analysis after pre-treatment with 10 mM H$_2$O$_2$ for 12 h. All the different forms of AhpC isolated from normal or stressed conditions showed a 26 kDa monomer band on SDS-PAGE. (Right) Oxidative stress-dependent switching of AhpC structures *in vivo*. Crude proteins extracted from two clinical HP isolates were separated on 4-12% native-PAGE and subjected to immunoblotting with an anti-HP-AhpC antibody. The proteins formed oligomers under short-term (8 h) stress; all AhpC converted to HMW complexes with molecular chaperone activity after long-term stress (16 h).

gastric cancer, and studied the structural and functional alterations of AhpC caused by oxidative stresses.

In order to combat the reactive oxygen species released from the host immune system and surviving in the gastric mucosa, pathogenic *H. pylori* is equipped with a number of detoxifying proteins. Among these, the most abundant
Figure 3: Electron micrographs (EM) showing structures of purified AhpC proteins under (Left) normal condition or (Right) after treatment with 10 mM H₂O₂. The scale bar represents 100 nm.

Figure 4: The dual-function activity of *H. pylori* AhpC. (Left) Peroxide reductase activity assay of AhpC in the absence or the presence of Trx system with or without 10 mM H₂O₂ as measured by the decrease of NADPH absorbance at 340 nm. (Right) Chaperone activity assay of AhpC after treatment with H₂O₂ for 1 or 12 h as measured by the turbidity change at 360 nm by using 10 mM DTT-induced insulin aggregation analysis at 25 °C.

One is alkylhydroperoxide reductase (AhpC). Several functional studies of *H. pylori* AhpC have shown that the AhpC-deleted mutants increase sensitivity to oxygen and overexpression of NapA, and accelerate inactivation of catalase.

As a member of 2-Cys peroxiredoxins, AhpC has been shown to be Trx-dependent. Although the structure of bacterial AhpC was shown to be similar to eukaryotic Prxs, the co-factors for AhpC and Prxs were different. The peroxide reductase activity of AhpC from *Salmonella typhimurium* was shown to be flavin-dependent, while the AhpC in *Mycobacterium tuberculosis* was lipoamide and AhpD-dependent. All these data indicated that the bacterial AhpC proteins were more heterogeneous in functional characteristics than eukaryotic Prxs.

We have previously demonstrated that the enzyme activity of AhpC in *H. pylori* of clinical isolates was suppressed under long-term oxidative stress(5). When comparing the sequences of AhpC and Prxs from different species, including bacteria, yeasts and mammals, we found that the AhpC of *H. pylori* is more homologous to human...
Prx than to eubacterial AhpC. These results suggest that a long-term infection of \textit{H. pylori} may facilitate the recombination of its AhpC gene with human Prx genes to form a human-like AhpC.

Since the AhpC of \textit{H. pylori} is closer to eukaryotic Prx than other prokaryotic AhpC phylogenetically, it was found that \textit{H. pylori} AhpC also acts as a molecular chaperone as does yeast Prx. When \textit{H. pylori} encounters intracellular ROS attack, the AhpC will react to ameliorate peroxides and oxygen radicals generated under short-term oxidative stress. However, some AhpC will be converted to HMW chaperones to prevent the misfolding or unfolding of proteins under long-term stress conditions. If the oxidative stress is too severe because of the presence of excessive damaged proteins (perhaps including some essential transcription factors for AhpC transcription), all expressed AhpC may be switched to molecular chaperones for salvage of unfolded proteins. It has also been found that chaperone activities as exhibited by AhpC of different \textit{H. pylori} strains isolated from gastric cancer and duodenal ulcer patients were greatly different. Thus the study of AhpC from clinical isolates of patients with different pathological manifestations may form a basis for the prognosis and diagnosis of varied gastric-duodenal diseases associated with \textit{H. pylori} infection.