This paper describes the purification of thioredoxin reductase (TR) and the characterization, purification, and cloning of thioredoxin (Trx) from *Helicobacter pylori*. Purification, amino acid sequence analysis, and molecular cloning of the gene encoding thioredoxin revealed that it is a 12-kDa protein which possesses the conserved redox active motif CGPC. The gene encoding Trx was amplified by polymerase chain reaction and inserted into a pET expression vector and used to transduce *Escherichia coli*. Trx was overexpressed by induction with isopropyl-1-thio-β-D-galactopyranoside as a decahistidine fusion protein and was recovered from the cytoplasm as a soluble and active protein. The redox activity of this protein was characterized using several mammalian proteins of different architecture but all containing disulfide bonds. *H. pylori* thioredoxin efficiently reduced insulin, human immunoglobulins (IgG/IgA/IgG4/IgA/sIgA), and soluble mucin. Subcellular fractionation analysis of *H. pylori* revealed that thioredoxin was associated largely with the cytoplasm and inner membrane fractions of the cell in addition to being recovered in the phosphate-buffered saline-soluble fraction of freshly harvested cells. *H. pylori* TR was purified to homogeneity by chromatography on DEAE-52, Cibacron blue 3GA, and 2′,5′-ADP-agarose. Gel filtration revealed that the native TR had a molecular mass of 70 kDa which represented a homodimer composed of two 35-kDa subunits, as determined by SDS-polyacrylamide gel electrophoresis. *H. pylori* TR (NADPH-dependent) efficiently catalyzed the reduction of 5,5'-dithiobis(nitrobenzoic acid) in the presence of either native or recombinant *H. pylori* Trx. *H. pylori* Trx behaved also as a stress response element as broghn vaccinated bacteria secreted Trx in response to chemical, biological, and environmental stresses. These observations suggest that Trx may conceivably assist *H. pylori* in the process of colonization by inducing focal disruption of the oligomeric structure of mucin while rendering host antibody inactive through catalytic reduction.

Redox control of a broad range of biochemical and immunological processes is now well documented to exercise a pivotal role in the cellular activity of both eukaryotes and prokaryotes. To date the redox properties of *Helicobacter pylori* have received little attention. Accumulating evidence indicates that the redox status of cells controls various cellular functions including cellular activation and proliferation in addition to growth inhibition and apoptosis (for reviews, see Refs. 1–5). Cellular redox status is maintained by intracellular redox-regulating molecules, including thioredoxin (Trx), glutaredoxin, and protein disulfide isomerase, which catalyze the formation and reduction of disulfide bonds in proteins.

The redox protein Trx and the associated enzyme thioredoxin reductase (TR) constitute a thiol-dependent reduction-oxidation system that can catalyze the reduction of certain proteins by NADPH, usually with high selectivity (1). In anaerobic bacteria, the generation of low redox potential reductants, such as Trx, can be used to assist electron flow to specific substrates. The capacity with which a given Trx can participate in this process is governed largely by the redox potential of the molecule. This in turn is directly associated with the nature of the amino acid residues flanked by 2 redox active vicinal cysteines in the active site. These cysteine residues participate in various redox reactions resulting in the catalysis of dithiol-disulfide exchange reactions. This CXXC motif has been termed elegantly as a molecular rheostat (6), whereby the resulting family of thiol-disulfide oxidoreductases vary greatly in their redox potential and therefore their ability to assist electron flow. Thioredoxin reductase catalyzes the reduction of oxidized thioredoxin (Trx-S2) by NADPH, and reduced thioredoxin (Trx-(SH)2) is the disulfide reductase. Typically, enzymes of this family contain 2 identical protein subunits, each subunit containing one redox-active disulfide, 1 mol of FAD per subunit, and conserved FAD and NADPH binding motifs. The flavin moiety mediates the transfer of reducing equivalents from NADPH to the disulfide bond of thioredoxin.

We sought to identify and characterize processes in the gastric pathogen *H. pylori* which were susceptible to redox regulation, with an overall view to exploring the effects of the bacterium’s redox environment on pathogenic mechanisms in addition to studying the effects of host factors on the redox activity of the bacterium. Initially we focused on members of the thioredoxin superfamily and specifically the Trx system. Here we describe the purification, overexpression, and characterization of the Trx system from *H. pylori*.

**EXPERIMENTAL PROCEDURES**

*Materials—2′,5′-ADP-agarose, Cibacron blue 3GA, imidazolactic acid-Sepharose 6B, ρ-aminobenzamidine-agarose, bovine insulin, bovine submaxillary gland mucin, DTT (1,4-dithio-DL-threitol) and *Escherichia coli* thioredoxin, and anti-E. coli thioredoxin were obtained from Sigma,

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* The abbreviations used are: Trx, thioredoxin; TR, thioredoxin reductase; 2′,5′-ADP-agarose, adenosine 2′,5′-bisphosphate linked to agarose through a 6-aminohexyl group; Trx-S2 and Trx-(SH)2, oxidized and reduced thioredoxin, respectively; DTT, 1,4-dithio-DL-threitol; *Escherichia coli* thioredoxin, and anti-E. coli thioredoxin were obtained from Sigma,
Poole, Dorset, United Kingdom. Sephacryl S-300 was obtained from Amersham Pharmacia Biotech. Isopropyl-β-D-thiogalactoside, NADPH, NADP+, and NADH were obtained from Roche Molecular Biochemicals, Bell Lane, Lewes, East Sussex, U.K. DEAE-52 was purchased from Whatman ( Maidstone, U.K.). Factor Xa was purchased from New England Biolabs, Beverly, Mass., U.S. Polyethylene glycol (PEG) 6000 and IgA were obtained from Calbiochem, Beeston, Nottingham, U.K. Anti-IgG subclass antibodies (anti-IgG1, IgG2, IgG3, IgG4) were purchased from The Binding Site Ltd., Birmingham, U.K. Anti-IgA and anti-secretory component antibodies were from Dako Ltd. All buffer reagents for SDS-PAGE were prepared in deionized water (Elga Prima osmosis, conductivity 1.2 μS/cm). All other chemicals were of analytical reagent grade.

Western Blotting and SDS-PAGE—Discontinuous SDS-PAGE was performed essentially as described previously (7). Proteins from SDS-PAGE gels were electroblotted (0.9 mA/cm² for 1 h) to polyvinyldene difluoride membrane (Gelman) using a semi-dry blotting apparatus (Amersham Pharmacia Biotech), essentially as described by Towbin et al. (8). Immunooblots were processed and developed by enhanced chemiluminescence as described previously (9). For NH$_2$-terminal sequencing the protein was electroblotted to ProBlot and stained briefly with freshly prepared Amido Black. Two-dimensional SDS-PAGE was performed essentially as described (54). Briefly, pellets of cells (~100 μg) of protein) from broth cultures of H. pylori were resuspended in isolation buffer (IEP) overnight at 4 °C and subjected to microcentrifugation for 16 h at 3000 V and for an additional 1 h 15 min at 800 V. Following IEP, the gels were equilibrated in SDS-PAGE sample buffer for 30 min prior to electrophoresis in the second dimension on 15% acrylamide gels. Broad range carrier ampholytes (pH 3–10) were used in the IEF gels.

Protein Measurements—Protein was measured by the method of Markwell et al. (10) with bovine serum albumin as the protein standard.

Bacterial Strain and Growth Conditions—The reference strain of H. pylori used in this study (NCTC 11638, VacA+ and CagA+) was obtained from the National Collection of Type Cultures, Public Health Laboratory, London, U.K. All components for H. pylori culture media were obtained from Oxoid, Unipath Ltd., Basingstoke, Hampshire, U.K. H. pylori was grown under microaerobic conditions (Oxoid Campylobacter system, 5% O$_2$, 10% CO$_2$) for 4 days on 7% lysed horse blood Columbia agar at 37 °C. Cells were harvested into ice-cold phosphate-buffered saline (pH 7.5). The cells were washed twice by centrifugation to remove intact cells and cellular debris (12,000 g, 10 min, 4 °C) and the resulting supernatant was applied to a DEAE-cellulose column (3.5 ml). Activity was calculated as micromole of NADPH oxidized/min in accordance with the relationship ΔA$_{412}$/13.6 × 2. Thioredoxin reductase activity was assayed also using a minor modification of the insulin reduction assay (11). The reaction mixture consisted of 0.1 m potassium phosphate buffer (pH 7.0) containing EDTA (1 mM), insulin (0.1 mg/ml), NADPH (0.2 mM), and H. pylori histidine-tagged Trx (2 μM) in a final volume of 1 ml. The reaction was initiated by the addition the enzyme to the mixture at 25 °C and the oxidation of NADPH was monitored at 340 nm. The amount of NADPH oxidized was determined from the relationship ΔA$_{256}$/0.2.

Purification of Native H. pylori Trx—Thioredoxin was purified by a combination of ion exchange chromatography on DEAE cellulose and gel filtration on Sephadex G-50. Thioredoxin containing Trx were identified using the spectrophotometric insulin reduction assay (11).

Fluorescence Spectroscopy—Fluorescence emission spectra of Trx of a Jasco FP 750 spectrophotometer by excitation at 280 nm and emission was recorded from 290 to 390 nm. All measurements were performed in Tris-HCl (50 mM, pH 7.3) containing EDTA (1 mM) and Trx (15 μg/ml) at 25 °C. To prevent oxidation of Trx, all solutions were sparged with N$_2$. Complete reduction of Trx was achieved by the addition of DTT (1 mM).

Expression and Purification of Recombinant H. pylori Trx—Transformants of E. coli BL21(DE3)pLysS with plasmid pET-16b (Novagen) containing the Trx gene (HP 824) were grown at 37 °C in LB broth supplemented with ampicillin (100 μg/ml) and chloramphenicol (30 μg/ml). H. pylori Trx was expressed as an NH$_2$-terminal decahistidine fusion protein in E. coli. The gene coding for Trx was amplified by polymerase chain reaction using Expand® (Roche Molecular Biochemicals), using the amplification conditions recommended by the manufacturer. Under these conditions a single product was obtained and this was cloned into the expression plasmid via the BamHI and NdeI restriction sites. The following primers were used: forward primer, 5'-CGCGATATGAGTCACTATATTGAATTAAC-3', reverse primer 5'-GGGGTACCCTGGTTAGATTTGTTGTGCG-3'. Overexpression of the fusion protein was induced by adding 1 mM isopropyl-β-D-thiogalactoside at exponential phase and the incubation continued for 3 h at 37 °C. The induced cells were harvested by centrifugation (10,000 × g, 10 min, 4 °C), washed once with 50 mM Tris-HCl (pH 7.5), and subjected to sonication (3 × 1 min). The soluble fusion protein was purified to homogeneity by metal chelate chromatography on a Ni$^{2+}$ column (3 ml) according to the manufacturer’s instructions. The protein was eluted with 0.4 M imidazole in 20 mM Tris-HCl (pH 7.5) containing 0.5 mM NaCl. Typically, 2–3 mg of homogenous Trx/100 ml of culture was obtained by this procedure. Both the histidine-tagged fusion protein and the recombinant Trx obtained after cleavage of the histidine tail by Factor Xa were indistinguishable in their spectroscopic properties and redox behavior.

Antiserum—Hyperimmune bovine anti-H. pylori antiserum was raised in cows immunized with a sonicate prepared from agar grown H. pylori. The IgG fraction was obtained by purification on Protein G (Amersham Pharmacia Biotech) using standard procedures. Anti-H. pylori thiorerodoxin antiserum was obtained from rabbits hyperimmunized with purified recombinant H. pylori thiorerodoxin using standard procedures. Anti-E. coli thiorerodoxin antiserum was obtained commercially (Sigma).

Reduction of Immunoglobulins and Mucin by the Trx System—Proteins (soluble porcine submaxillary gland mucin and immunoglobulins) to be reduced by the H. pylori Trx system were suspended in 50 mM Tris-HCl (pH 7.5) containing EDTA (1 mM). The reaction was performed at room temperature. At the indicated period of time, the reduction mixture was subjected to alkylation by the addition of 4-fold molar excess iodoacetamide (in N$_2$-sparged 0.2 M Tris-HCl (pH 8.8) over sulfhydryls to the sample. The mixture was left to incubate for 15 min under N$_2$ in the dark after which time an equal volume of double strength nonreducing sample buffer was added and the mixture boiled for 5 min prior to SDS-PAGE on 5–20% acrylamide gradient gels. The

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RESULTS

Purification, Subcellular Distribution, and Properties of Native Trx from H. pylori—Thioredoxin from H. pylori was purified to homogeneity in a two-step procedure which involved ion exchange on DEAE-cellulose followed by gel filtration over Sephadex G-50. An SDS-PAGE analysis of a sample taken from each step in the purification procedure is shown in Fig. 1 (panel A). Detection of Trx during its purification was based on its ability to reduce insulin disulfides, as described under “Exper-imental Procedures.” Thioredoxin from H. pylori exists as a monomer with an apparent molecular mass of 12 kDa as determined by SDS-PAGE under reducing and non-reducing conditions. The N-terminal 20 amino acid residues for Trx were found to be MSHYIELTEEXFESTIKGV, where X represents an unidentified residue. A search of the protein data bases confirmed that we had purified Trx, entry HP 0824 in the H. pylori genome data base (12). Subcellular distribution studies demonstrated that Trx from H. pylori is largely a cytoplasmic protein (Fig. 1, panel C) with a small proportion of the total protein associated with the inner membrane fraction of the cell (not shown). Interestingly, Trx was detected in the soluble extracellular fraction which was obtained by briefly suspending the bacteria in PBS to remove loosely cell-associated material (Fig. 1, panel C, lane 4).

Overexpression of Trx in E. coli—Routinely, 2–3 mg of recombinant Trx was obtained per 100 ml of broth culture and the protein was expressed exclusively as a soluble cytoplasmic protein in E. coli. Removal of the His-tag from the purified material was achieved by digestion with Factor Xa (25 μg/mg protein) for 16 h at room temperature. To terminate the reaction the Factor Xa was removed from the mixture by passage through a small (1 ml) benzamidine-agarose column followed by purification of the recombinant protein (minus His-tag) on a Ni²⁺ column. Removal of the histidine tag resulted in a 2-kDa decrease in the molecular mass of the protein.

Spectral Properties—The fluorescence emission spectra of the native and recombinant Trxs were determined for both the reduced and oxidized forms of the protein. The spectral characteristics of the reduced forms of the native and recombinant protein (+His-tag) were identical as were those of their oxidized forms (not shown). The reduction of the recombinant H. pylori Trx by DTT (1 mM) resulted in a 1.8-fold increase in the tryptophan fluorescence intensity at 343 nm following excitation at 280 nm (Fig. 2). It has been demonstrated previously that thioredoxin from E. coli shows a larger increase in fluorescence intensity (3.5-fold) upon reduction of the active site cysteines due to the quenching effect of the active site disulfide...
on the fluorescence of an adjacent tryptophan (13).

Reduction of Insulin—The ability of \( H. \) pylori Trx to catalyze the reduction of insulin by DTT was determined. Insulin reduction can be measured spectrophotometrically as an increase in turbidity due to precipitation of the free insulin B chain (88). We have compared the rates of porcine insulin reduction by DTT in the presence of \( H. \) pylori and \( E. \) coli Trxs. Both Trxs show activity as disulfide reductases with insulin as substrate (Fig. 3, panel A). Interestingly, Trx from \( H. \) pylori was approximately four times as efficient at reducing insulin than equivalent amounts of \( E. \) coli Trx when incubated with DTT (Fig. 3).

In addition, the initial rate of insulin reduction was greater with \( H. \) pylori Trx compared with Trx from \( E. \) coli. These observed redox activities (Fig. 3) and the differential spectral properties of reduced and oxidized Trx (Fig. 2) enabled us to examine the oxidation of fully reduced Trx by insulin and to assay the process continuously using a fluorimeter. Fluorescence spectroscopy of reduced thioredoxin re-oxidation by insulin demonstrated that the process occurred rapidly upon addition of an equal amount of insulin (Fig. 3, panel B) and that the thioredoxin was oxidized within 2 min of the initiation of the reaction. Taken together, these data indicate that we had a functional recombinant molecule which behaved in a manner expected for a functional thioredoxin reductase.

Reduction of Human Polyclonal IgG by the Trx System of \( H. \) pylori—In order to study the patterns of reduction of human IgGs, samples were reduced and alkylated over various periods of time and analyzed by both SDS-PAGE and Western blotting. Fig. 5 (panel A) shows the results obtained when human polyclonal IgG was reduced by the Trx system of \( H. \) pylori. Overall the SDS-PAGE analysis demonstrated that IgG was reduced almost to completion with time. In addition to the intact immunoglobulin (\( H\)-L, \( M_r: 146,000–170,000 \)) and the major fragments of heavy (\( H, M_r: 51,000–60,000 \)) and light (\( L, M_r: 22,000–25,000 \)) chains, the small number of minor bands, which appear transiently, presumably represent the formation of mixed disulfides (e.g. \( H\)-L, \( M_r: 124,000–145,000 \); \( H\)-L, 102,000–120,000; HL, 73,000–85,000) and these are identified in the margins of the figures, where appropriate. It is clear that human IgG is an efficient substrate for this system. IgG was completely reduced after a 3-h incubation (at room temperature) using equimolar amounts of Trx and Ig in the presence of TR and NADPH. Similarly, an identical cleavage pattern of IgG was observed when the reaction was monitored by Western blotting and probed with anti-human IgG and anti-IgG antibodies.
ECL, respectively (not shown). The reduction of Ig was dependent on reduced thioredoxin as neither oxidized thioredoxin nor thioredoxin reductase alone was able to reduce IgG (not shown). Furthermore, analysis of the subclass specificity of the reduction process revealed that thioredoxin could efficiently reduce IgG1, IgG2, IgG3, and IgG4 as shown by Western blotting with antisera raised against purified IgG subclasses (Fig. 5, panels B-E). The Western blotting data demonstrated that the interchain disulfide bonds of different subclasses of IgG were reduced in a time-dependent manner by the Trx system but with significant kinetic differences. IgG1 was reduced rapidly to H and L chains (by 10–20 min). Transient appearance of mixed disulfides (H2L, H2L, and HL) was seen also and these are indicated in Fig. 5 (panel B). Clearly the 4 interchain disulfides of IgG1 are readily reduced, whereas the remaining subclasses were less susceptible to reduction in the following preferential order; IgG3, IgG4, IgG2. Almost complete reduction of IgG3 (Fig. 5D) and IgG4 (Fig. 5E) was seen by 40–60 and 60–90 min, respectively. IgG2 (Fig. 5C) was the subclass most resistant to reduction although reduced H chains were apparent after only 5 min of incubation. In addition, fewer intermediate mixed disulfides were seen with IgG2 as substrate compared with the other subclasses. The kinetic differences observed in cleavage patterns are most likely due to structural differences in the Igs where steric constraints will effect both the accessibility and interaction of Trx with sulfhydryls in addition to altering the susceptibility of these thiols to reduction. Finally, it appears from our data that H. pylori Trx is unable to reduce the intra-chain disulfide bonds of the heavy (H) and light (L) chains. Only when the alkylated Trx-reduced H and L chains were subjected to complete reduction by 2-mercaptoethanol or DTT were the fully reduced H and L chains observed (data not shown). Cleland (14) has shown that DTT, because of its low redox potential, is capable of maintaining monothiols completely in the reduced state and of reducing disulfides quantitatively.

**Reduction of IgA**—The reduction of IgA1 and the secretory component (SC) of sIgA was performed and monitored exactly as described for IgG. The cleavage patterns of IgA1 and the secretory component are shown in Fig. 6. Monomeric nonsecretory IgA1 was readily reduced by the Trx system as can be seen from the appearance of the expected cleavage fragments with time (Fig. 6, panel A). The anti-IgA antibody only weakly recognized the L chain of IgA and this was only seen upon overexposure of the blot (not shown). The Trx-mediated reduction of dimeric sIgA proceeded more slowly than reduction of IgG, monomeric non-secretory IgA or polymeric IgA (not shown) presumably due to steric hindrance imparted by the SC and J-chain. Similarly, reduction of the SC of dimeric sIgA was observed using anti-SC antiserum (Fig. 6, panel B). The fragment appearing at 183 kDa most likely represents SC bound to monomeric sIgA (H2L2) as indicated in the figure. Free SC (68 kDa) is released from 5 min of incubation. From our data it appears

**Fig. 4.** Amino acid sequence alignment of thioredoxin and thioredoxin reductase. Multiple alignment of Trx (HP 824) from H. pylori and other species is shown in panel A. Panel B shows the paired alignment of the two species of Trx from H. pylori (HP 824, lower; HP 1458, upper). Panel C shows the homology between the two species of TR from H. pylori (HP 824, top; HP 1164, lower). The vertical bars indicate conservative substitutions/regions of strong similarity as opposed to identity and the dots indicate identical residues.
that removal of the secretory component may represent a rate-limiting step in the reduction of sIgA, as a considerable amount of the molecule remained bound to the dimeric sIgA even after 80 min of incubation. Presumably, however, the rate of reduction would be much greater if the reaction was performed at 37 °C rather than room temperature (21 °C) as described.

Reduction of Mucin—Upon reduction of soluble porcine submaxillary gland mucin by the Trx system the apparent molecular weight of the mucin was increased as demonstrated by analytical SDS-PAGE (Fig. 7A). This observed anomalously increase in the molecular mass of mucin upon Trx-mediated reduction (Fig. 7A, lane 2) is interpreted as the presence of residual intact intrachain disulfides which give the partially linearized mucin an electrophoretic mobility less than that of the nondenatured material (Fig. 7A, lane 1). Similarly, upon complete reduction of the mucin by 2-mercaptoethanol the electrophoretic mobility of the material is decreased yet further due to complete linearization of the mucin structure (Fig. 7A, lane 3). Fig. 7B shows the time course of mucin reduction by the Trx system and the associated gradual decrease in electrophoretic mobility. Similar anomalous electrophoretic mobilities have been found for the partially and fully reduced forms of IgG and L chains. This behavior is thought to be due to the loss of domain compactness induced by reduction (15, 39).

**Fig. 6.** Reduction of human IgA by the Trx system of *H. pylori.* Panels A and B show Western blots of the time course of Trx-mediated reduction of human IgA, and human sIgA, respectively. Reduction of IgA, and secretory component were monitored by developing the blots with peroxidase-conjugated anti-IgA (α-chains) and anti-secretory component antibody, respectively. The molecular weight markers used were identical to those described in the legend to Fig. 5.

**Fig. 7.** Reduction of mucin by the Trx system of *H. pylori.* Reduction of porcine submaxillary gland mucin by the Trx system of *H. pylori* was examined by SDS-PAGE. Panel A shows the results obtained after a 2-h incubation of mucin with the Trx system (lane 1, untreated mucin; lane 2, Trx-treated mucin; lane 3, 2-mercaptoethanol-treated mucin). Panel B shows the time course of mucin reduction by Trx (0.5–60 min). Also shown is the untreated starting material after the 60-min incubation (Con.) and the fully reduced protein (Red.). The molecular weight markers used are described in the legend to Fig. 1.

Importantly, it appears that the accumulation of thioredoxin in the medium is due to specific secretion rather than lysis of the bacteria as the extracellular levels of urease activity remained essentially constant throughout the duration of the experiment (Fig. 8B). In support of this view, Western blotting, developed with hyperimmune anti-*H. pylori* antiserum, demonstrated
that there was no apparent time-dependent accumulation of multiple \textit{H. pylori} antigens in the broth culture supernatant over the duration of the experiment (not shown). Furthermore, the viability of the bacteria was unaffected by the presence of anti-\textit{H. pylori} IgG as assessed by subculture of the organism after completion of the experiment. Moreover, a control experiment demonstrated that urease activity was unaffected by the polyclonal anti-\textit{H. pylori} IgG used, therefore the detectable urease activity in the broth supernatant represented active urease enzyme rather than residual activity as a consequence of antibody-mediated inhibition. In addition, low levels of thioredoxin reductase activity (0.3 ± 0.08 nmol/NADPH oxidized/min/50 μl of broth supernatant) were detected in the broth culture supernatant. Unlike thioredoxin, however, extracellular levels of thioredoxin reductase remained constant and did not increase with time upon exposure of the bacteria to antibody.

**DISCUSSION**

This paper describes the isolation and characterization of electrophoretically pure thioredoxin (HP 824) and thioredoxin reductase (HP 825) from the gastric pathogen \textit{H. pylori} and the cloning and overexpression of thioredoxin in \textit{E. coli}. This is the first reported characterization of a functional thioredoxin system in a gastric pathogen. The results show that the Trx system can specifically reduce interchain disulfides in insulin, mucin, IgG, and IgA. Of particular note is the finding that, in response to a variety of applied extracellular stresses, the expression of intracellular Trx is dramatically altered by \textit{H. pylori}, an observation which suggests that Trx behaves as a stress response element in this organism. Importantly, the accumulation of Trx in the medium of broth grown bacteria in response to physical, chemical, or biological insults suggests that this molecule has the ability to protect the bacterium by initiating the process of catalytic reduction of susceptible target proteins. This suggestion is supported by the detection of measurable amounts of thioredoxin reductase activity also in broth culture supernatants thus equipping the bacterium with a functional extracellular thioredoxin system.

Interestingly, while there was an increase in the amount of thioredoxin secreted in response to stress there was no detectable increase in the extracellular levels of TR over the time course of the experiments. The significance of the quantitative changes in secretion observed for TRx but not for TR remain to be established, however, the induction and secretion of Trx alone would increase the capacity of the bacterium to react to stress. Differential expression of Trx, TR, and other redox active proteins in response to various stressors has been observed in eukaryotic and mammalian cells (81–84) and, paradoxically, oxidative stress has been shown to result in the down-regulation of TR activity but to increase Trx activity in epithelial cells (85). Differential expression of Trx and TR by \textit{H. pylori} may occur. Clearly a portion of such an adaptive response would be regulated at the transcriptional level, however, the transcription factor(s) that regulates the expression of these promoters may show a differential response to various stimuli as has been documented for Trx expression in \textit{B. sub-
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With respect to H. pylori, however, the effector functions of IgA appear to be less well defined. Contrasting data from several groups have demonstrated the presence of widely varying IgA/sIgA titers in different populations of H. pylori infected subjects (e.g. Refs. 76 and 78). Indeed, it has been demonstrated that antibody-independent mechanisms of immunity can protect against Helicobacter infection (77, 79), whereas others hold the view that specific mucosal antibodies may prevent overgrowth of H. pylori thereby reducing the risk of gastric malignancies (80), observations which suggest that there is a tenuous relationship between specific mucosal antibody and protection from infection. The experimental approach adopted in many of these studies (ELISA) to determine antibody levels, however, would not discriminate between active and inactive antibody species. Therefore, it is not inconceivable that the fraction of mucosal antibody which comes into contact with H. pylori is partially or completely inactivated via complete or incomplete catalytic reduction and that this fraction represents only a small percentage of the total antibody present in the gastric lumen. We propose that gross modification to antibody structure would be an unlikely occurrence. Rather, catalytic reduction would only conceivably occur in the immediate microenvironment of the bacterium where it is required. Also, the thioredoxin system could only remain functional so long as the respective components of the system were in close proximity to one another.

H. pylori has been shown by several laboratories to adversely affect the physical and chemical properties of gastric mucins (26–29, 57, 58) and that eradication of the bacterium results in restoration of the viscoelastic and hydrophobic protective properties of mucus (50, 31, 56). Although still a controversial and disputed issue (see Refs. 59–61) the weight of experimental evidence greatly favors H. pylori-mediated alterations to mucin structure/function. Moreover, it has been well documented that H. pylori inhabits the adherent (water insoluble) mucus gel layer in addition to the more soluble viscus mucin of the lumen. So how does H. pylori gain access to the adherent mucus gel, a substance known to be impermeable to proteins with a molecular size greater than 17,000 Da (67)? As the motility of H. pylori is affected by the viscosity of the medium it inhabits (25) focal disruption of the mucus barrier would clearly facilitate rapid passage of the bacterium. Studies in vitro suggest that loss of gel structure may arise due to H. pylori-mediated proteolytic or phospholipase activity, alterations in local pH (62, 63), or reversible modifications to patterns of gastric mucin glycosylation (64). Although this contentious issue has yet to be resolved unequivocally, we propose an alternative mechanism. Conceivably, based on the data presented in this paper, H. pylori could gain access to the impermeable adherent mucous layer by inducing focal disruption to the barrier by catalytically reducing the disulfide bonds present in the cysteine-rich regions responsible for cross-linking mucin monomers (19, 65, 66). These cysteine-rich domains are susceptible to both proteolytic attack and thiol agents (67). In addition, thioredoxin would represent an ideal candidate molecule to fulfill this task given its small size (12 kDa) and reducing capacity. Clearly, reduction of mucin disulfides would thus facilitate the process of colonization as a direct consequence of the loss of gel-forming properties of polymeric mucin which provide protection for the exposed delicate surfaces of the gastric epithelia from microbial and physical insults (18–24).

Clearly, any reductive redox activity mediated by Trx in the vicinity of antibodies and/or mucin could result in complete or partial catalytic reduction of the disulfides present in these molecules. It is clear that human Igs and mucin are reduced readily by the Trx system of H. pylori. Interestingly, all four subclasses of IgG are reduced by the Trx system of H. pylori in contrast to the reported inability of the Trx system from E. coli to reduce human IgG2 (39). Of course, if such a reductive process were to occur in vivo it would require that the Trx system be capable of exerting its effects extracellularly. We have shown that Trx is present in the PBS-soluble extracellular (water soluble) fraction from freshly harvested H. pylori in addition to being secreted in an apparently specific manner when the bacteria are subjected to a variety of stresses and therefore reasonably speculate that Trx may be secreted from the cell or remain surface-associated, as required. The mechanism of Trx secretion from the bacterium is not clear at present. Although Trxs lacks a typical signal sequence there are a number of ways in which it could be released from the cell. Principal among these are specific secretion pathways (52, 73), a potential type IV secretory mechanism (74), spontaneous autolysis (53, 71), and the shedding of membrane vesicles (68–70, 73) some of which have been shown to account for the extracellular localization of several cytotoxins H. pylori proteins (e.g. Refs. 68 and 70). These in vitro observations of potential mechanisms for shedding/releasing H. pylori antigens are given credence by the localization in vivo of various H. pylori proteins, such as urease and HspB (53, 72) in the lamina propria of infected individuals. Moreover, it has been suggested that the extracellular release of H. pylori antigens may serve as a means of evading the host immune response and, with the presence of an extracellular functional thioredoxin system, a mechanism to incapacitate host antibody.

It is likely that the Trx system of H. pylori has an important function in this bacterium particularly in view of the fact that it possesses limited means for manipulating and maintaining a reducing intracellular environment with the exception of superoxide dismutase and catalase. H. pylori is a microaerophile and lives in an environment of low oxygen tension. Such an environment encourages optimal conditions for reductive reactions although it is likely that the bacterium has the ability to adapt to conditions of variable oxygen tension (16). Unlike many other prokaryotes, H. pylori does not appear to possess
the enzymes to generate glutathione nor does it possess other thiol reductants such as glutaredoxin. Accumulating evidence indicates that Trxs from several species have multifunctional roles, however, the precise functions of Trxs have yet to be established unequivocally. One difficulty in assigning specific in vivo functions to Trx will be compounded by its many proposed regulatory functions and the wide variety of substrates with which it interacts (e.g., Ref. 40). Despite the similarities of the conserved CXXC motif among Trxs from different species, the various members differ strongly in their redox potentials (−220 to −270 mV). Determination of the redox potential of *H. pylori* Trx will give an indication of the redox capacity of the molecule and enable us to investigate further its functions in vivo.

**REFERENCES**

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52. Chinese bacterium *H. pylori* is a complex organism that uses different species, the various members differ strongly in their redox potentials (−220 to −270 mV). Determination of the redox potential of *H. pylori* Trx will give an indication of the redox capacity of the molecule and enable us to investigate further its functions in vivo.