Characterization of *Helicobacter pylori* adhesin thiol peroxidase (HP0390) purified from *Escherichia coli*

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The antioxidant protein, adhesin thiol peroxidase (*Hp*Tpx or HP0390), plays an important role in enabling *Helicobacter pylori* to survive gastric oxidative stress. The bacterium colonizes the host stomach and produces gastric cancer. However, little information is available about the biochemical characteristics of *Hp*Tpx. We expressed recombinant *Hp*Tpx in *Escherichia coli*, purified to homogeneity, and characterized it. The results showed that *Hp*Tpx existed in a monomeric hydrodynamic form and the enzyme fully retained its peroxidase and antioxidant activities. The catalytic reaction of the enzyme was similar to an atypical 2-cysteine peroxiredoxin (Prx). The conformation of the enzyme was observed in the presence and absence of dithiothreitol (DTT); similar to other known thiol peroxidases, conformational change was observed in *Hp*Tpx by the addition of DTT.

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1. Introduction

The human gastric pathogen *H. pylori* is considered to play an important role in the pathogenesis of gastritis, peptic ulcer and gastric cancer (Correa 1988). *H. pylori* induces a strong inflammatory response on colonizing the host. This response stimulates the production of reactive oxygen species (ROS) by gastric cells (Bagchi *et al.* 1996) and phagocytes (Ramarao *et al.* 2000). Increased levels of ROS have been observed in the gastric mucosa of *H. pylori*-infected patients (Wang *et al.* 2006). To survive in this difficult environment, *H. pylori* produces a series of antioxidant enzymes, including superoxide dismutase (SOD), catalase and peroxiredoxin that can remove the ROS (Wang *et al.* 2006).

Peroxiredoxins, reduced peroxide levels produced in the cell, are the most abundant of these antioxidant enzyme in *H. pylori*. Generally, member of the Prx family are divided into three groups according to their enzymatic mechanism: 1-cys Prx, typical 2-cys Prx and atypical 2-cys Prx (Rhee *et al.* 2005). Mammalian Prx is known to have dual antioxidant and signal transduction functions. Prx removes H_2O_2 inside the cell, but if the concentration of H_2O_2 is high, the peroxidatic cystein of the Prx is converted to sulphinic acid leading to inactivation of the Prx, thus allowing H_2O_2 to participate in signal transduction (Wood *et al*. 2003). Prx inactivation has also been observed in plants (Kitajima 2008) and yeast (Park *et al.* 2000; Koo *et al.* 2002). In addition, bacterial Prx is inactivated in high concentrations of H_2O_2, but is less sensitive than mammalian Prx (Parsonage *et al.* 2008).

Thiol peroxidase (Tpx) is one of the subfamilies of Prx proteins and has a similar biochemical character. The crystal structure of *E. coli* Tpx (*Ec*Tpx) reveals an

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Abbreviations used: CD, circular dichroism; Cp, peroxidatic cysteine; Cr, resolving cysteine; DTT, dithiothreitol; FADD-DED, death effector domain of Fas-associated death domain; *Hp*Tpx, *Helicobacter pylori* adhesin thiol peroxidase; IPTG, isopropyl thiogalactoside; LB, Luria broth; MALDI-TOF MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; MCO, metal-catalysed oxidation; NADPH, nicotinamide adenine dinucleotide phosphate; HSQC, heteronuclear single quantum coherence; NMR, nuclear magnetic resonance; OD, optical density; PCR, polymerase chain reaction; Prx, peroxiredoxin; ROS, reactive oxygen species; SDS-PAGE, sodium dodecyl sulphate-polyacrylamide gel electrophoresis; SEC, size-exclusion chromatography; SOD, superoxide dismutase; Tpx, thiol peroxidase; Trx, thioredoxin; Trx-R, thioredoxin reductase

intramolecular disulphide bridge in the oxidized state (Choi *et al.* 2003). The substrate specificity of *Ec*Tpx favours alkyl hydroperoxide over H_2O_2. During the catalytic reaction, the peroxidatic cysteine is converted to sulphenic acid and becomes overoxidized by organic hydroperoxide, but not by H_2O_2 (Baker and Poole 2003). *M. tuberculosis* Tpx (*Mtb*Tpx) reduces H_2O_2, t-butyl hydroperoxide and cumene hydroperoxide, and is inhibited by sulphydryl reagents (Rho *et al.* 2006). Both these Tpxs form homodimers in solution. Recently, both the oxidized and reduced forms of the *Bacillus subtilis* Tpx structure were solved (Lu *et al*. 2008), and it was shown to undergo significant catalysis-coupled conformational changes in the active regions around the peroxidatic (Cp) and resolving (Cr) cysteines.

H. pylori adhesin thiol peroxidase belongs to the Prx family and is known to act as an H_2O_2 scavenger in the presence of the thioredoxin (Trx) system (Wan *et al*. 1997; Wang *et al*. 2005). *Hp*Tpx mutants have been found to be more sensitive to killing by peroxide and superoxide than wild-type organisms. *Hp*Tpx-null microorganisms exhibit a reduced ability to colonize the stomachs of host mice (Comtois *et al*. 2003; Wang *et al.* 2005), implying that *Hp*Tpx plays a critical role in combating oxidative stress. *Hp*Tpx was previously identified as a 1-cys Prx, even though it has two cysteine residues (Wang *et al.* 2006). Because cysteine residues are critical for catalytic activity, the classification of this protein remains unclear.

The pathogenesis of *H. pylori* in the human stomach and duodenum is closely related to the resistance of *H. pylori* to the ROS produced by the host. This heightened resistance may reflect a more robust antioxidant system. We report the *in vitro* characterization of recombinant *Hp*Tpx, which has not been well studied but is an important *H. pylori* antioxidant protein. In this study, we examined the enzymatic activities of recombinant *Hp*Tpx. We also investigated a conformational change in the different redox states of *Hp*Tpx by DTT. By studying site-directed mutagenesis, we were able reclassify *Hp*Tpx, as its classification was previously unclear.

2. Materials and methods

2.1 *Expression and purification of wild-type and mutant* Hp*Tpx*

The full-length DNA coding sequence of wild-type *Hp*Tpx (Gene bank accession no. AE000511) was cloned into pET15b using the NdeI and BamHI restriction sites. Mutants of *Hp*Tpx [C60S] and [C94S] were cloned into the pET15b vector by overlapping polymerase chain reaction (PCR) with T7 promoter and terminator primers, and site-specific mutant primers (C60S forward: 5′-tcggttt**c**tttgctccaa-3′; C60S reverse: 5′-ttggagcaaa**g**aaaccga–3′; C94S forward: 5′–gcaaattt**c**cggcgctga–3′; C94S reverse: 5′–tcagcgccg**g**aaatttgc–3′; bold characters indicate the mutated position). The recombinant plasmid, *Hp*Tpx-pET15b was expressed in the *E. coli* strain BL21 (DE3) (Novagen, USA). The inoculum was grown at 37°C in Luria broth (LB) medium, to an optical density $(OD)_{600}$ of 0.6–0.8 and induced by 0.5 mM isopropyl thiogalactoside (IPTG) for 4 h at 37°C. The cell paste was resuspended in a lysis buffer (50 mM sodium phosphate, 300 mM sodium chloride, pH 8.0, 1 mM phenylmethanesulphonyl fluoride) and stored at –80°C for subsequent purification. Frozen cells were lysed by sonication and the lysate was centrifuged at 23 000 × *g* at 4°C to separate the supernatant and pellet. The proteins were purified by an Ni-NTA agarose column (QIAGEN, USA) according to the manufacturer's instructions. Partially purified *Hp*Tpx was pooled and N-terminal His_6 was then cleaved with 1 unit thrombin/mg protein by incubating for 16 h at 4°C; the cleavage was analysed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). The protein was further purified by DEAE sepharose chromatography in 50 mM sodium phosphate buffer, pH 7.0. The purified protein was detected mainly in the flow-through and its purity was analysed by SDS-PAGE. The protein sample was then concentrated and stored at –80°C for further experiments.

2.2 *Circular dichroism analysis*

Far-UV circular dichroism (CD) spectroscopy was used to generate a wavelength scan and analyse the thermal unfolding transition of *Hp*Tpx by using a JASCO J-715 spectropolarimeter (JASCO, Japan). The purified protein (0.2 mg/ml) in 10 mM sodium phosphate buffer, pH 7.0, in the absence or presence of DTT (1 mM), was used for recording wavelength spectra from 260 nm to 195 nm at 20°C using a 1 nm nominal bandwidth with six accumulations. A temperature scan from 5°C to 100°C was performed at a rate of 1°C/min at 222 nm.

2.3 *Size-exclusion chromatography*

The oligomerization state of *Hp*Tpx was verified by size-exclusion chromatography (SEC) using an AKTA Basic UPC–FPLC system (Amersham Biosciences, USA) with a Superdex 200 10/300 GL column using running buffer (50 mM sodium phosphate, 100 mM sodium chloride, pH 7.0). The eluted protein was monitored by UV detection at a wavelength of 280 nm; the size of the eluted protein was determined by comparison with standard marker proteins. The reduced form of the protein was isolated by incubating the purified protein for 1 h with 10 mM DTT before being loaded into the column.

2.4 *Nuclear magnetic resonance spectroscopy*

All nuclear magnetic resonance (NMR) measurements were carried out at 25°C with a Variant 900 MHz NMR machine. N^{15} labelled *Hp*Tpx (0.5 mM) was prepared in 50 mM HEPES buffer, pH 6.5, with or without the addition of 10 mM DTT.

2.5 *MALDI-TOF MS analysis*

*Hp*Tpx protein (5 mg/ml) was prepared in 50 mM HEPES buffer, pH 7.0 in the presence or absence of 10 mM DTT for molecular mass analysis by matrix-assisted laser desorption/ ionization time-of-flight mass spectrometry (MALDI-TOF MS).

2.6 *Peroxidase assay*

Trx-dependent peroxidase activity of the purified *Hp*Tpx protein was determined based on the oxidation of nicotinamide adenine dinucleotide phosphate, reduced form (NADPH). It was indirectly monitored as a decrease in absorbance at 340 nm, essentially as described previously (Rho *et al.* 2006). The reaction mixture contained 0.1 mM NADPH, 12.5 μg/ml *E. coli* thioredoxin reductase (Trx-R), 12.5 μg/ml *E. coli* thioredoxin (Trx), 50 μg/ml purified *Hp*Tpx in 50 mM HEPES–NaOH (pH 7.0), and 100 μM H_2O_2 substrate. The reaction was initiated by the addition of NADPH, and a decrease in the absorbance at 340 nm was measured using a Biochrom (Libra S22) spectrophotometer. In control experiments, Trx-R, Trx or *Hp*Tpx was omitted from the reaction mixtures.

2.7 *DNA-based antioxidant activity assay*

DNA protection assays were performed as described previously (Rho *et al.* 2006) with minor modifications. Briefly, reaction mixtures containing 5 mM DTT, 15 μM $FeCl_3$, 200 ng/μl pUC19 DNA plasmid, and different concentrations of *Hp*Tpx were incubated for 30 min at 37ºC. The reaction was stopped by the addition of 50 mM EDTA, and DNA nicking was analysed by separating the mixture on 0.8% agarose gels. In various control experiments, DTT, $FeCl_3$ or *Hp*Tpx was omitted, or 50 mM EDTA was added before incubation.

2.8 *Intracellular antioxidant activity assay*

E. coli was used to test the intracellular antioxidant activity of *Hp*Tpx. The *E.coli* strain BL21 (DE3) was co-transformed with plasmids bearing a mouse death effector domain of Fas-associated death domain (FADD-DED) gene (Lee *et al.* 2000) and an *Hp*Tpx expression plasmid. For this, cells bearing *Hp*Tpx were made competent using a standard $CaCl_2$ protocol, and then transformed with mouse FADD-DED. After transformation, cells expressing both genes were then grown in LB medium to an OD_{600} of 0.4. The cells were spread onto an agar plate containing the appropriate antibiotic and IPTG, and incubated overnight at 37ºC. The number of colonies, reflecting surviving cells, was determined for *E. coli* transformed with FADD-DED only (control) and FADD-DED plus *Hp*Tpx.

3. Results and discussion

3.1 *Purification of recombinant* Hp*Tpx protein*

Recombinant His_6-*Hp*Tpx protein was overexpressed in *E. coli* and purified to homogeneity from lysates using nickel affinity chromatography followed by thrombin digestion to remove the N-terminal His_6. The protein was further purified by DEAE anion-exchange chromatography. A high-purity protein was achieved after two-step purification, yielding 30–40 mg protein per litre of culture broth with approximately 99% purity. The integrity of the purified protein was confirmed by N-terminal sequencing. The purified protein migrated with a molecular mass of about 18 kDa under denaturing conditions (figure 1A). Two functionally important mutants of *Hp*Tpx, [C60S] and [C94S], were also expressed and purified to homogeneity by the same procedure used to isolate the wild-type protein. The results showed that mutant [C60S] and [C94S] proteins appeared as a mixture of stable dimers and monomers under non-reducing conditions, and were completely transformed into monomers after reduction with DTT (figure 1B).

3.2 *Activity assay of* Hp*Tpx*

The known Tpx proteins had peroxidase as well as antioxidant activity (Rho *et al*. 2006; Fourquet *et al*. 2008; Lu *et al*. 2008). We also examined the activities of *Hp*Tpx in an *in vitro* system to characterize the protein. The peroxidase activity of *Hp*Tpx was analysed using the Trx and Trx-R systems, in which oxidation of NADPH was recorded as a decrease in absorbance at 340 nm (Rho *et al.* 2006). In this assay, the use of *E. coli* Trx and Trx-R rather than *H. pylori* Trx and Trx-R did not significantly affect the results, as described by Baker *et al.* (2001), but was convenient because of their commercial availability. The *in vivo* study showed that *Hp*Tpx is an H_2O_2 scavenger enzyme (Wan *et al.* 1997; Wang *et al.* 2005); therefore, H_2O_2 was used as a substrate for *Hp*Tpx in this experiment. The

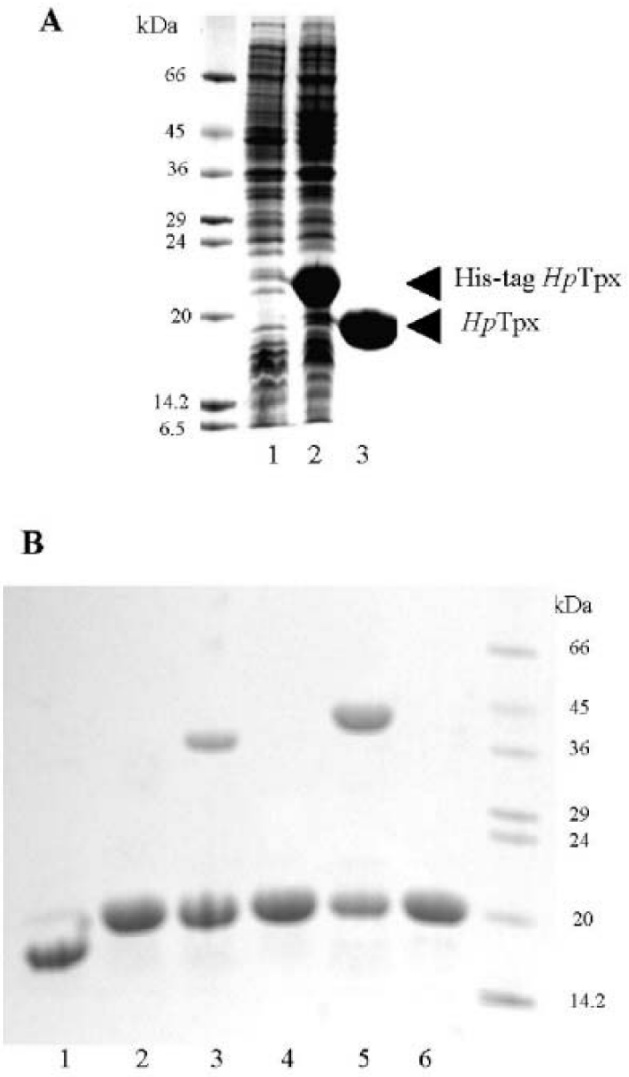

Figure 1. **(A)** Expression and purification of *Helicobacter pylori* adhesin thiol peroxidase (*Hp*Tpx) protein. Lane 1: the uninduced *E. coli* cell; lane 2: induced *E.coli* cell with 0.5 mM IPTG; lane 3: completely purified and His_6 removed *Hp*Tpx. **(B)** Non-reducing SDS-PAGE with wild-type and mutant *Hp*Tpx. Lanes 1, 3, 5: wild-type, C60S and C94S mutants, respectively, in the absence of dithiothreitol (DTT); lanes 2, 4, 6: wild-type, C60S and C94S mutants, respectively, in the presence of 10 mM DTT.

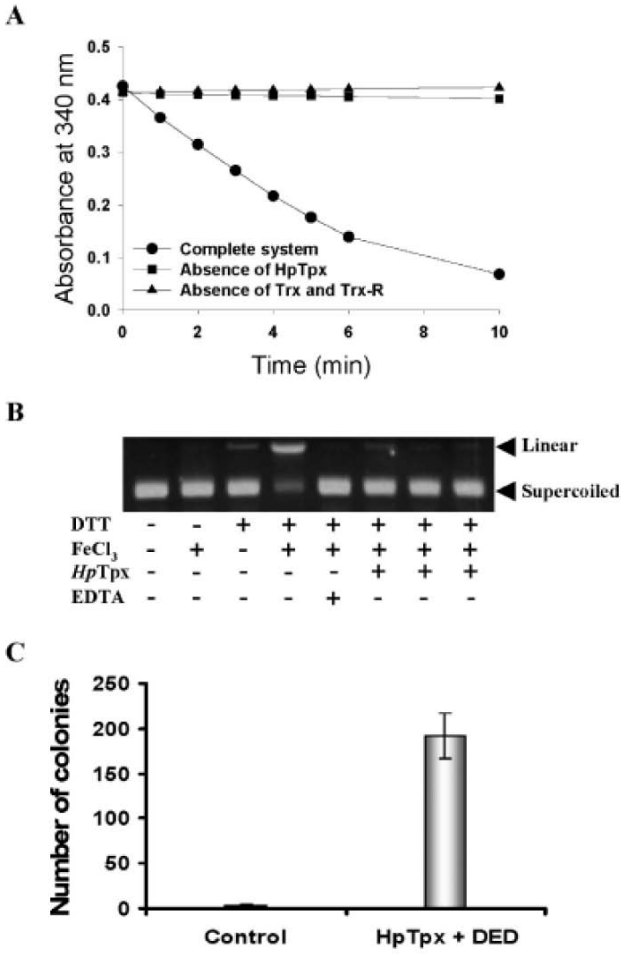

Figure 2. Activity assays of *Helicobacter pylori* adhesin thiol peroxidase (*Hp*Tpx). **(A)** NADPH-linked peroxidase activity of *Hp*Tpx showing it has activity in the presence of both thioredoxin (Trx) and thioredoxin reductase (Trx-R). Data represent averages of triplicate experiments. **(B)** DNA-based antioxidant activity of *Hp*Tpx. DNA is damaged in the presence of both $FeCl_3$ and dithiothreitol (DTT), which can be prevented by adding *Hp*Tpx or EDTA before the reaction starts. The last three lanes presented different concentrations of *Hp*Tpx (from left to right: 25 μM, 50 μM, and 100 μM, respectively). **(C)** Intracellular antioxidant activity of *Hp*Tpx. The co-transformation of *Hp*Tpx and mouse FADD-DED plasmids into *E. coli* BL21 (DE3) helped in cell survival. However, the transformation of only FADD-DED plasmid, labelled as control, results in the cell death. Data represent the averages of triplicate experiments.

results illustrate that recombinant *Hp*Tpx had a relatively high peroxidase activity (figure 2A). Oxidation of NADPH was not observed in the absence of either *Hp*Tpx or Trx and Trx-R, indicating that *Hp*Tpx is a thioredoxin-dependent peroxidase (figure 2A).

The DNA-based antioxidant activity of *Hp*Tpx was also tested by assessing its ability to protect DNA from oxidative damage. pUC19 is a small, supercoiled DNA plasmid that can be attacked by hydroxyl radicals produced by a metal-catalysed oxidation (MCO) system. Nicked plasmids produced as a result of oxidant activity adopt an open-circular form that can be distinguished from the supercoiled one by agarose gel electrophoresis (Rho *et al.* 2006). Our data showed that in the presence of both DTT and $FeCl_3$, pUC19 DNA was transformed entirely into the nicked form, which was further damaged to yield linear DNA molecules. On the contrary, no DNA damage was observed when either DTT or $FeCl_3$ was omitted from the reaction (figure 2B).

Additionally, by including *Hp*Tpx in the reaction mixture, DNA damage was either completely or partially prevented, depending on the concentration of *Hp*Tpx. *Hp*Tpx at a concentration of 50 and 100 μM completely prevented DNA damage – the plasmids remained in the supercoiled form – suggesting that *Hp*Tpx protected the DNA from ROS-induced damage caused by the MCO system (the last two lanes of figure 2B), while 25 μM *Hp*Tpx (figure 2B, the third lane from the right) did not completely protect the DNA. DNA damage was prevented by adding EDTA prior to initiating the reaction (figure 2B, fourth lane from right).

Expression of the FADD-DED in *E. coli* causes cell death due to elevated cellular levels of ROS (Lee *et al.* 2000). To confirm the antioxidant activity of *Hp*Tpx, which was observed in the cell-free MCO system in a more physiological setting, the ability of *Hp*Tpx to reduce the levels of DED-generated intracellular ROS was tested. In these experiments, cell survival was studied in *E. coli* BL21 (DE3) cells transformed with DED plasmid alone, or co-transformed with *Hp*Tpx and DED plasmids. In *E. coli* expressed with only DED, almost all of the cells died. In cells co-expressed with *Hp*Tpx and DED, *Hp*Tpx protected *E. coli* cells against the death effect of DED (figure 2C). These findings are consistent with the observations of DNA-based antioxidant activity. This study revealed that all known biochemical functions of recombinant *Hp*Tpx are retained in an *in vitro* system and the results are in agreement with the predicted characteristics of Tpx proteins.

3.3 *Conformational change of recombinant* Hp*Tpx protein*

A conformational change in the oxidized and reduced forms of Tpx proteins is a well-known property (Lu *et al.* 2008). Hence, we also studied the conformational change of recombinant *Hp*Tpx protein, especially since it is related to the activity of Tpx. Suppose the presence of DTT changes the protein to a reduced form, which is otherwise in an oxidized form. We observed the effect of DTT on the conformational change of *Hp*Tpx by non-reducing SDS-PAGE. A clear difference in the mobility of the protein was observed in the presence and absence of DTT (the reduced and oxidized states of protein) (figure 1B). Lanes 1 and 2 in figure 1B correspond to the wild-type *Hp*Tpx in the absence (oxidized form) and presence (reduced form) of DTT. The mobility of the protein was slower with the addition of DTT. Furthermore, *Hp*Tpx migrated as a single band corresponding to the monomeric size of the protein. The Cysteine-to-Serine mutations of *Hp*Tpx showed dimer formation in the absence of DTT (figure 1B, lanes 3 and 5) and were completely transformed into monomers after reduction with DTT (figure 1B, lanes 4 and 6). This reveals that the two cysteine residues in the wild-type *Hp*Tpx formed an intramolecular disulphide bridge in the oxidized form, i.e. in the absence of DTT. The experiments were performed under aerobic conditions, in which protein tends to remain in an oxidized form. Since *Hp*Tpx exists in a monomeric form and contains only two cysteine residues, these cysteines form an intramolecular disulphide bridge to achieve a stable conformation in the oxidized condition. DTT disrupts this disulphide bridge and converts it into a thiol group, causing a structural perturbation, which leads to slower movement of the protein on the gel. When cysteine residues are mutated individually, the remaining cysteine also tends to get oxidized, which results in dimer formation, i.e. intermolecular disulphide bridge formation (figure 1B, lanes 3 and 5). The addition of DTT disrupts this disulphide bridge; therefore, protein appears as a monomer at the same position as the wild-type protein (figure 1B, lanes 4 and 6).

This fact was further confirmed by size-exclusion chromatography; the native monomeric molecular weight of *Hp*Tpx was observed to be about 18 kDa. On treating the protein with DTT, the peak appeared earlier than in the absence of DTT (figure 3A). This property was also verified by MALDI-TOF MS analysis (figure 3B), by which the molecular mass of *Hp*Tpx is 18 587.12 Da and 18 639.74 Da in the absence and presence of DTT, respectively, with a difference in molecular mass of about 52.6 Da implying further oxidation of cysteine.

The effect of DTT was also studied by CD spectroscopy. A shift in the mean residual ellipticity in the presence of DTT indicated the stable nature of the protein in a reduced form (figure 3C, left panel). The thermal unfolding transition temperature of *Hp*Tpx at 222 nm was 65°C, which increased to 66.6°C with the addition of DTT (figure 3C, right panel). This suggests that the reduced form of the protein may have a different conformation compared with the oxidized form and DTT may be important in stabilizing the conformation of protein *in vitro*. The ^{15}N-heteronuclear single quantum coherence (HSQC) NMR spectra of *Hp*Tpx also revealed the clear effects of DTT on spectral resolution. It was observed that a comparatively lesser number of peaks, i.e. a lesser resolved spectrum appeared in the absence of DTT (figure 3D, left panel) compared with a higher number of peaks, i.e. more resolved spectrum in the presence of DTT (figure 3D, left panel). We speculate that at the higher concentrations used for the NMR experiment, the protein may have invisibly aggregated in the absence of DTT. The addition of DTT to the NMR sample improved the solubility of the protein and the spectrum. Overall, these results indicate that the hydrodynamic properties of *Hp*Tpx change drastically between the oxidized and reduced forms. These results are consistent with a previous report on the conformational change of thiol peroxidase protein (Lu *et al.* 2008).

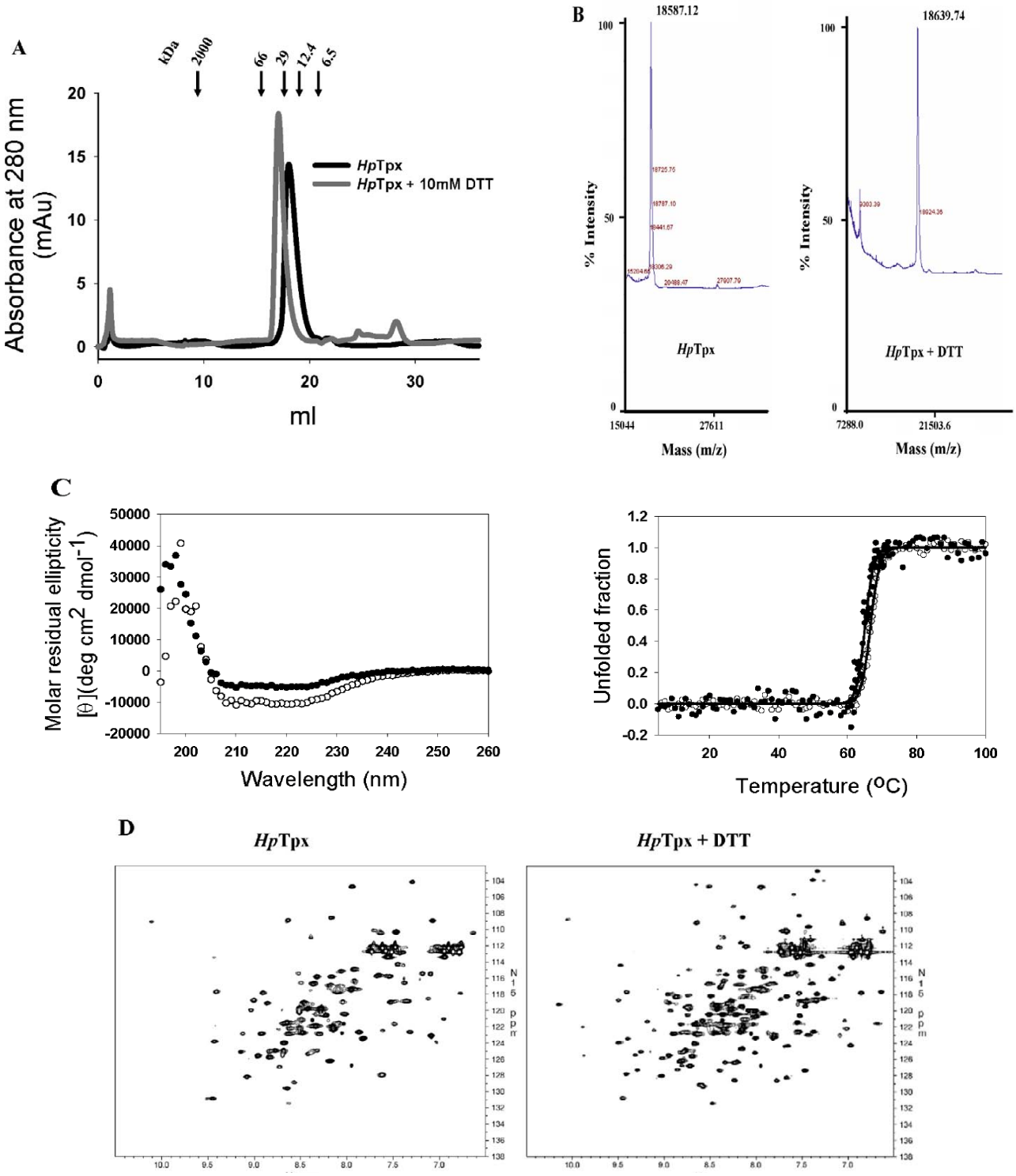

Figure 3. **C**onformational change of *Helicobacter pylori* adhesin thiol peroxidase (*Hp*Tpx). **(A)** Gel-filtration chromatogram of *Hp*Tpx shows a difference in the elution profile of *Hp*Tpx in the presence or absence of dithiothreitol (DTT). **(B)** Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) analysis of *Hp*Tpx in the presence or absence of DTT. The axes and molecular mass were re-drawn for convenience of observation. **(C)** Circular dichroism spectra of *Hp*Tpx. The secondary structure of *Hp*Tpx was analysed at 20ºC at pH 7 (left panel). Thermal unfolding of *Hp*Tpx measured at pH 7 at 222 nm (right panel). **(D)** Heteronuclear single quantum coherence spectra of N^{15} labelled *Hp*Tpx with and without DTT. Left panel, *Hp*Tpx without DTT; right panel, *Hp*Tpx with DTT.

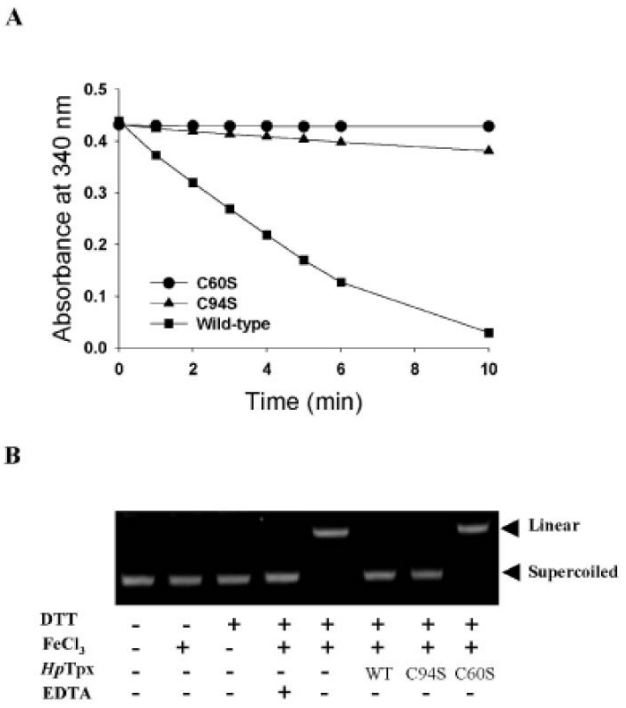

Figure 4. Point mutation-based determination of the peroxidatic and resolving cysteines of *Helicobacter pylori* adhesin thiol peroxidase (*Hp*Tpx). **(A)** Effect of mutations on reduced nicotinamide adenine dinucleotide phosphate (NADPH)-linked peroxidase activity. Data represent averages of duplicate or triplicate experiments. **(B)** Effect of mutations on antioxidant activity, measured by the DNA protection assay.

3.4 *Determination of peroxidatic cysteine*

*Hp*Tpx contains two cysteine residues at positions 60 and 94. In order to determine the importance of these cysteines in the activity of *Hp*Tpx, we individually mutated both cysteine residues to serine by site-directed PCR, and then purified the mutant proteins. The experiment was performed exactly as described by Rho *et al.* (2006). A comparison of the peroxidase activity of wild-type *Hp*Tpx with that of the mutant proteins revealed that mutants lost the ability to oxidize NADPH; thus, both cysteine residues are important in maintaining peroxidase activity (figure 4A). The importance of both cysteines was further investigated in catalysing antioxidant activity using the pUC19 plasmid DNA protection assay. In this assay, both wild-type *Hp*Tpx and *Hp*Tpx [C94S] showed almost complete protective activity. However, the C60S mutant could not protect supercoiled DNA from damage (figure 4B), implying that Cys^{60} is the more important of the two cysteines. These results indicate that the Cys^{60} of *Hp*Tpx is the peroxidatic cysteine, and Cys^{94} is the resolving cysteine. Therefore, unlike 1-cys Prx (Wang *et al.* 2006), *Hp*Tpx can be classified as an atypical 2-cys Prx. Although Trujilo *et al.* (2006) reported that mycobacterial thioredoxin peroxidase can act as a 1-cys Prx, the classification of *Hp*Tpx into an atypical 2-cys Prx group is more accurate.

4. Conclusions

*Hp*Tpx was purified as a monomer retained Trx-dependent peroxidase activity as well as antioxidant activity. We also found that the transition between the oxidized and reduced forms of *Hp*Tpx, induced by DTT, was accompanied by a conformational change. Furthermore, the addition of DTT stabilized the conformation of the protein. Similar to the known Tpxs, *Hp*Tpx should be classified as an atypical 2-cys Prx.

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