Thioredoxin Is an Arginase Chaperone and Guardian against Oxidative and Nitrosative Stresses*

The gastric human pathogen *Helicobacter pylori* faces formidable challenges in the stomach including reactive oxygen and nitrogen intermediates. Here we demonstrate that arginase activity, which inhibits host nitric oxide production, is post-translationally stimulated by *H. pylori* thioredoxin (Trx) 1 but not the homologous Trx2. Trx1 has chaperone activity that renatures urea- or heat-denatured arginase back to the catalytically active state. Most reactive oxygen and nitrogen intermediates inhibit arginase activity; this damage is reversed by Trx1, but not Trx2. Trx1 and arginase equip, and nitrogen intermediates inhibit arginase activity; this damage is reversed by Trx1, but not Trx2. Trx1 and arginase equip *H. pylori* with a “renox guardian” to overcome abundant nitrosative and oxidative stresses encountered during the persistence of the bacterium in the hostile gastric environment.

The gastric human pathogen *Helicobacter pylori* causes chronic gastritis and ulcers and has a strong link with gastric cancer. Despite enormous knowledge gleaned from two completely sequenced strains (1, 2), little is known about how this organism escapes the host innate and adaptive immune systems. The extensive inflammatory response observed in *H. pylori*-infected patients contributes to gastric damage; some of this damage is mediated by ROI/RNIs such as NO and hydrogen peroxide. Arginase (RocF), which hydrolyzes L-arginine to urea and nitrogen intermediates inhibits arginase activity; this damage is reversed by Trx1, but not Trx2. Trx1 and arginase equip *H. pylori* with a “renox guardian” to overcome abundant nitrosative and oxidative stresses encountered during the persistence of the bacterium in the hostile gastric environment.

EXPERIMENTAL PROCEDURES

Bacterial Strains, Growth Conditions, and Plasmids—Escherichia coli strains were grown at 37 °C in standard medium plus appropriate antibiotics (100 μg/ml ampicillin, 25 μg/ml kanamycin, 10 μg/ml tetracycline). *H. pylori* strains were cultured at 37 °C on Campylobacter agar containing 10% (v/v) defibrinated sheep blood in a microaerobic environment for 2 days in 5% O₂,10% CO₂ and 85% N₂ in humidified air. Kanamycin (5–10 μg/ml), chloramphenicol (20 μg/ml), or both antibiotics were added to the growth medium, as appropriate. Standard molecular biology procedures were used.

**Construction of *H. pylori* Mutants—**Construction of *H. pylori* rocF mutants of strains 26695 and SS1 (4) and 43504 (6) was described previously. The *rocF* mutant was constructed by transformation of wild type J75 with chromosomal DNA from the rocF mutant of strain 26695 and confirmed as described previously (4). The *ureArocF* double mutant was constructed by transforming the *rocF* mutant of strain 43504 with PHP902-ureA::cat (7) to yield an urease- and arginase-null strain that was resistant to both kanamycin and chloramphenicol. The *txxA1* mutant was constructed in strain 26695 by transformation with pSLC7 (8). The *txxA2* mutant has been described previously (8).

**Differential Ultracentrifugation—**French press (20,000 p.s.i.; two passages) lysates of the arginase/urease (rocF/ureA) double mutant of *H. pylori* strain 43504 were differentially centrifuged (TL-100 Beckman ultracentrifuge, TLA 100.3 rotor), and the membrane-enriched fraction (pellet of a 70,000 rpm centrifugation corresponding to 200,000 × g; 70K pellet) was retained. The corresponding supernatant (70K supe) was also retained. The fractions were assayed for arginase stimulatory factor (Asf) activity by addition to purified His₅-RocF.

**Preparation of Bacterial Extracts—**Ice bath-sonicated extracts were prepared as described previously (5). Soluble material from a low speed centrifugation (12,000 × g) was retained.

**Purification of RocF, Trx1, and Trx2—**His₅-RocF (5), Trx1 (9), and Trx2 (10) were purified as described. Previously, we were unable to establish dialysis conditions that still yielded catalytically active arginase (5). In this study, His₅-RocF was dialyzed in 1 liter of 10 mM glycine, 150 mM NaCl, pH 6.5, overnight at 4 °C. His₅-RocF was diluted 1:2 in 100 mM Tris pH 8.5, and the solution was dialyzed into 100 mM Tris pH 7.4, 150 mM NaCl, 1 mM MgCl₂, 1 mM DTT, and 10% glycerol.

**Blotting and Immunoblotting—**Aliquots (1 μg) of purified proteins were denatured in 2x SDS sample buffer with a long heat treatment (10 min at 100 °C), separated on a 4–20% gradient gel, and transferred onto a nitrocellulose membrane. Protein expression was detected using rabbit anti-His₅ antibodies (Novagen) and anti-rabbit horseradish peroxidase (HRP)-conjugated antibodies (Amersham). Protein expression was detected using a chemiluminescent reagent (Amersham).
sterile glycerol and stored at −20 °C. Under these conditions, His$_6$-RocF retained catalytic activity following dialysis.

**Protein Gels—SDS-PAGE** was conducted by standard methods and stained with Coomassie Blue.

**Affinity Chromatography of Arginase in the Absence or Presence of the** *H. pylori* 70K Supe or 70K Pellet—XL1-Blue MRF$^+$ pQE30-rocF (5) was grown in L broth and induced 3 h with isopropyl thio-$\beta$-D-galactopyranoside (2 mM). French press (16,000 p.s.i.) lysates were clarified by centrifugation and the soluble fraction incubated with nickel-nitroliacetic acid-agarose resin (1 ml/10 mg of protein) (Qiagen). The resin was washed three times with 1 ml of wash buffer (50 mM Na$_2$HPO$_4$, 300 mM NaCl, 15 mM imidazole, pH 8.0) and then the 70K supe or pellet was added (~600 µg of protein). After 1 h of end-over-end incubation (4 °C), the resin was washed again five times with wash buffer. The proteins were coeluted with 275 µl of elution buffer (50 mM Na$_2$HPO$_4$, 300 mM NaCl, 250 mM imidazole, pH 8.0).

**Preparation of Peptides for Mass Spectrometric Analysis and Protein Sequencing**—Bands of interest were excised from the gel and destained (200 mM ammonium bicarbonate in 50% methanol). The gel fragments were dehydrated with acetonitrile and dried by centrifugation under vacuum, and the proteins were denatured and reduced (8 mM urea, 100 mM ammonium bicarbonate, 5 mM EDTA, 10 mM Tris (carboxyethyl) phosphine; Sigma–Aldrich) at 37 °C for 15 min. Iodoacetamide (50 mM; Sigma–Aldrich) was added as necessary to alkylate free sulphydrys, and incubation was continued for an additional 15 min. The alkylating reagent was removed by three rounds of acetonitrile dehydration followed by rehydration in 50 mM ammonium bicarbonate. The samples were dried in a SpeedVac centrifuge, followed by trypsinization (0.1 mM ammonium bicarbonate with 20 ng/µl trypsin; Promega) and incubated overnight (37 °C). Following digestion, the supernatant was retained, and additional peptides were extracted from the remaining gel fragments by agitation in 1% acetic acid, 1% acetic acid for 90 min. The combined supernatants were centrifuged (2% acetic acid) and then evaporated to dryness as above. The samples were rehydrated (15 µl of 2% acetonitrile, 1% acetic acid) prior to analysis by the University of South Alabama Proteomics/MS Spectrometry Laboratory. MS and MS/MS analyses were carried out using a Waters (Micromass) quadrupole time of flight Ultima$^\text{TM}$ API (Milford, MA) mass spectrometer. The samples were loaded into a Waters CapLC Autosampler for liquid chromatography-MS/MS. The data were acquired using the Masslynx software and converted into peak list files. These were searched with the MASCOT MS/MS ion search engine (www.matrixscience.com/cgi/search_form.pl?FORMVER = 26-SEARCH = MIS) against the NCBI nonredundant primary sequence data base, allowing up to two miscuts (peptide tolerance = 0.2 Da; MS/MS ion tolerance = 0.2 Da). Additional searches were conducted using the ebucas database and the *H. pylori* 26695 genome (1) using the BLASTp algorithm at www.tigr.org.

**Cloning of hsp60 into pQE30**—The hsp60 (groEL) coding region (bp 1–1641 of hp0010) was PCR-amplified from *H. pylori* strain 26695 using primers DM95-F1 (cgggatccATGGCAAAAGAAATCAAATTTTC; BamHI site underlined; non-hsp60 sequence in lowercase letters) and DM96-R1 (aactgcatTTACATCATGCCACCCTACG; PsiI site underlined; non-hsp60 sequence in lowercase letters). The PCR product was digested with BamHI and PsiI and cloned into pQE30 (Qiagen) predicted with the same enzymes to generate pQE30-hsp60. The construct was confirmed by sequencing, restriction enzyme digestion, and mini-protein expression analyses (data not shown). The fusion protein, His$_6$-Hsp60, has a predicted molecular mass of 59.2 kDa.

**Purification of Hsp60**—XL1-Blue MRF$^+$ pQE30-hsp60 (1.5 liters) was grown to mid-log phase and induced with isopropyl thio-$\beta$-D-galactopyranoside (5 mM). The cultures were harvested in wash buffer, lysed by two passages through a French press, and clarified by centrifugation, and the cytosolic portion was loaded onto polypropylene columns (8.5 × 2.0 cm) containing nickel-nitroliacetic acid resin at 4 °C. The washes and elutions were conducted according to the manufacturer’s specifications. The fractions were analyzed for the presence of His$_6$-Hsp60 by SDS-PAGE.

**Urease Activity Measurements**—Urease activity was measured by the phenol-hypochlorite assay as described previously (11).

**Arginase Activity Measurements**—Arginase activity was measured by colorimetric detection of ornithine in arginine buffer (15 mM MES, 10 mM arginine, pH 6.0) as described previously (5). The data are in units of pmol or nmol l-ornithine/min/mg protein. During this study it was revealed that purified His$_6$-RocF had catalytic activity when assayed without the 30-min 50–55 °C heat activation step, as long as cobalt (5 mM) was provided; therefore this extra step was omitted in the present study except where noted in the figure legends. The protein concentration was determined using the bicinchoninic acid assay (Pierce), following the manufacturer’s 30-min method, Bovine serum albumin was used as the standard. The unpaired, one-tailed t test was used to statistically analyze the data using Instat 3.05 (GraphPad Software, Inc.). p < 0.05 was considered significant.

**Fluorescence Spectra of Trx1**—Purified Trx1 was rendered fully oxidized or fully reduced, and its fluorescence emission at different times was determined in the presence or absence of purified His$_6$-RocF using previously established procedures (9).

**Chaperone Experiments**—Arginase was denatured in 1 M urea. Arginase was renatured in 750 ml of 10 mM glycine, 150 mM NaCl, pH 6.5, for 1 h in the presence or absence of Trx1 in Slide-A-Lyzer cassettes (7K; Pierce). The renaturation buffer was changed once and allowed to proceed another hour before the samples were removed and assayed for arginase activity.

**Treatment of Arginase with ROI/RNIs and Reversal or Protection by Trx1**—Arginase ROI/RNI protection was assessed by preincubating Trx1 for 15 min on ice with ROI/RNIs followed by the addition of purified His$_6$-RocF. Arginase activity was then immediately measured. Reversal of ROI/RNI-mediated damage to arginase by Trx1 was conducted by incubation of His$_6$-RocF with ROI/RNI for 15 min, followed by the addition of purified Trx1.

**RESULTS**

*H. pylori* Contains an Asf—To uncover proteins that affect arginase activity, we incubated purified His$_6$-RocF (5) with extracts from four *H. pylori* strains (J75, 43504, 26695, and SS1) in which the rocF gene encoding arginase had been disrupted. Although extracts from all four strains were completely devoid of arginase activity, these extracts still stimulated purified arginase activity (Fig. 1A), suggesting that the Asf is present in all *H. pylori* strains.

**Characterization of Asf and Identification of Asf as Thioredoxin**—Previous work established that urease was not involved in arginase activity (4). Urease is the most abundant protein of *H. pylori* (4). Urease activity (4). Urease is the most abundant protein of all *H. pylori* strains, accounting for 5–10% of the total cellular protein (12). The urease abundance makes it arduous to purify other proteins. To facilitate further characterization of Asf, we constructed an arginase-urease (rocF/ureA) double mutant of *H. pylori* strain 43504 (see “Experimental Procedures”). The rocF/ureA mutant was devoid of both urease and arginase activities (data not shown). Lysates of the rocF/ureA mutant were differentially ultracentrifuged to yield cytosolic and membrane-enriched fractions.
(70K supe and 70K pellet, respectively; see "Experimental Procedures"). The 70K pellet, but not the 70K supe, stimulated purified arginase activity (Fig. 1B). Fractionation experiments using a 10-kDa membrane filtration device revealed that Asf was probably a protein greater than 10 kDa (Fig. 1C).

The Asf was narrowed down to a handful of candidates by affinity chromatography. Purified arginase (H_{6}-RocF) bound to nickel-nitriloacetic acid-agarose was incubated with either the 70K pellet, the 70K supe, or vehicle control (0.9% NaCl) (see "Experimental Procedures"). After binding, the samples were extensively washed and then the proteins eluted (see "Experimental Procedures"). The 70K pellet coelution was found to have 3.5-fold higher arginase activity than the 70K supe coelution and 5-fold higher arginase activity than arginase eluted by itself. SDS-PAGE analysis revealed approximately eight H. pylori proteins that coeluted with arginase and the 70K pellet fraction, whereas only several faintly staining H. pylori proteins coeluted with arginase and the 70K supe fraction (Fig. 2A). Gel slices were excised from the 70K pellet lane or from the corresponding molecular weight regions in the 70K supe or H_{6}-RocF control lanes; the gel slices were analyzed by mass spectrometry (see "Experimental Procedures"). The proteins from the H. pylori 70K pellet sample were identified as several chaperone family members, fumarase, catalase, a putative thioester hydrolase, ferritin, neutrophil-activating protein (NapA; an iron-binding protein), and thioredoxin 1 (HP0824, Trx1) (Fig. 2A). Catalase (KatA) and NapA were not likely the Asf because katA or napA mutants had the same arginase activity as wild type H. pylori (data not shown). Hsp60 was also not likely involved because purified Hsp60 did not stimulate purified arginase activity (data not shown). Hsp60 and KatA may have coeluted because of their abundance in H. pylori. For the 70K supe sample, arginase coeluted with several faintly staining H. pylori proteins...
Thioredoxin-mediated Chaperoning of *H. pylori* Arginase

*H. pylori* has two thioredoxins, Trx1 (HP0824) and Trx2 (HP1450), that share 35% amino acid identity and 57% similarity. Purified Trx1, but not Trx2, not only stimulated arginase activity from wild type *H. pylori* extracts (data not shown) but also stimulated purified arginase activity (Fig. 2B). The data indicated that arginase specifically discriminates between the two closely related thioredoxins. Purified Trx1 and Trx2 alone did not exhibit arginase activity and the buffer in which the purified thioredoxins were stored had no effect on arginase activity (data not shown). Stimulation of arginase activity by purified Trx1 was dose-dependent (Fig. 2C). Although a mutation in *trxA1* coding for Trx1 reduced arginase activity by 50% (wild type, 6,044 ± 659 units; *trxA1* mutant, 2,601 ± 191 units), a mutation in *trxA2* encoding Trx2 had elevated arginase activity (19,035 ± 1,312 units). The reason for the arginase elevation in the *trxA2* mutant is unclear, because Western blot analysis using anti-Trx1 polyclonal sera (9) did not reveal an increase in Trx1 protein in the *trxA2* mutant versus the wild type strain (data not shown).

**Trx1 Stimulation of Arginase Is Likely Independent of Redox Status**—Thioredoxins normally target cysteolic proteins for cysteine reduction via disulfide reductase activity (13). Trx1 has the conserved active site motif typical of thioredoxins, 26WCGPCK31, where the Cys residues are able to cycle through oxidized and reduced redox states. We therefore hypothesized that Trx1 was serving a redox role for arginase. Trx1 and His6-RocF were incubated together in the presence or absence of the sulfhydryl alkylating agent iodoacetamide, and the disulfide bond statuses of the two proteins were monitored by mass spectrometry in comparison with either protein alone. No evidence was obtained for a change in sulfhydryl status of either protein, but the results did reveal that Trx1 Cys27 and Cys30 form a disulfide bond, as expected from the literature on other thioredoxins (13), and His6-RocF Cys66 and Cys73 form a disulfide bond, explaining the previous observation that *H. pylori* arginase is sensitive to reducing agents (5). *H. pylori* arginase is the first example of an arginase displaying a disulfide bond. No mixed disulfide bonds between Trx1 and His6-RocF were detected, and no evidence for contaminating proteins in purified His6-RocF or Trx1 was found.

The oxidized form of Trx1 (Trx1ox) displays much lower fluorescence intensity at its emission maximum (λem) than the reduced form (Trx1red) (9). If the cysteines of His6-RocF were being oxidized by Trx1ox, resulting in Trx1red, then an increase in the fluorescence emission of Trx1ox would occur similar to that of Trx1red alone. The addition of purified His6-RocF to fully oxidized or reduced forms of Trx1 elevated fluorescence, but the fluorescence of the Trx1ox did not reach that of Trx1ox alone (Fig. 3). Taken together with the mass spectrometry results, the data suggest that the mechanism of Trx1-mediated stimulation of arginase is not likely due to redox control of cysteines. However, we have not completely eliminated this possibility.

**Evidence That Trx1 Stimulates Arginase by Acting as a Chaperone**—Previous evidence revealed that purified His6-RocF rapidly loses catalytic activity when stored at 4 °C (<1 week) or −20 °C (−4 months) (5), making it difficult to use the same batch of purified His6-RocF in multiple experiments or for an extended period of time. Remarkably, Trx1, but not Trx2, completely restored arginase activity to a catalytically inactive batch of arginase (Fig. 4A, p < 0.05 comparing RocF[I] versus RocF[I] + Trx1). Mass spectrometry revealed that Cys66 and Cys73 were oxidized in both catalytically active and inactive arginase batches, suggesting that the sulfhydryl status of the protein was not involved. It was therefore reasoned that rather than acting in a redox role, Trx1 may play an alternative role as an arginase chaperone. Support for this hypothesis comes from recent evidence showing that *E. coli* thioredoxin is involved in refolding heterologous proteins containing cysteines (porcine citrate synthase, yeast α-glucosidase) as well as an *E. coli* protein devoid of cysteines (galactose receptor) (14). *E. coli* thioredoxin was able to restore only up to 30% of the enzymatic activity of the target protein.

**H. pylori** His6-RocF was 90% denatured (based on 90% loss of catalytic activity) in urea (1 M) (Fig. 4B). When arginase was dialyzed to remove the urea, little to no catalytic activity was restored (Fig. 4C, p < 0.05 comparing control versus denatured and dialyzed), suggesting very little renaturation of arginase can occur when the enzyme is by itself. However, if arginase was allowed to renature in the presence of purified Trx1, arginase activity was completely restored (Fig. 4D, p < 0.05 comparing denatured and dialyzed versus denatured and dialyzed + Trx1). If Trx1 was added to denatured His6-RocF that had not been permitted to renature, activity was only partially restored. These results suggest that Trx1-mediated stimulation of arginase activity is through a chaperone-like mechanism whereby Trx1 refolds arginase to the catalytically more active state.

To assess an independent type of denaturation, heat was chosen. Two types of experiments were conducted: (i) protection experiments, in which Trx1 and His6-RocF were co-incubated together in the presence of heat followed by measurement of arginase activity, and (ii) reversal experiments, in which His6-RocF was first heated, and then Trx1 was added, followed by measurement of arginase activity. It was discovered that Trx1-mediated stimulation of arginase was enhanced by preincubation of the His6-RocF-Trx1 complex at 55 °C (Fig. 4D), further suggesting a role of Trx1 in folding arginase to an improved catalytic three-dimensional conformation. Above 55 °C there was a steep decline in Trx1-mediated arginase stimulation suggestive of a loss of Trx1 chaperone function. Trx1 stimulation of arginase occurred optimally when both Trx1 and cobalt were present (Fig. 4E) and was rapid (Fig. 4F). If Trx1 or cobalt were added after heat treatment rather than during heat treatment, arginase activity was lower but still higher than arginase assayed without Trx1 (Fig. 4D and E). The Trx1-mediated chaperoning of arginase increased with increasing cobalt concentrations (Fig. 4G), but the identical shapes of the two curves did not lend support to a direct

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**FIGURE 3. Fluorescence spectra of oxidized or reduced Trx1 in the presence or absence of His6-RocF.** The fluorescence emission of Trx1 (13 mg/ml) in 2 ml of phosphate buffer (0.1 M, pH 7.4) containing EDTA (2 mM) at 337 nm was recorded over time (excitation, 280 nm). The arrows indicate the addition of purified His6-RocF (3.0 mg/ml) to either oxidized (Trx1ox) or reduced (Trx1red). The shift in the fluorescence intensity upon addition of His6-RocF is due to the presence of His6-RocF and not due to a change in the redox status of Trx1.
role of Trx1 as a cobalt chaperone. We reason that if Trx1 was a cobalt chaperone, there would have been a change in the shape of the curve from a hyperbolic for arginase alone to a sigmoidal curve for arginase plus Trx1.

**Trx1 Protects Arginase from Oxidative and Nitrosative Stresses**—Trx1 and Trx2 have recently been shown to play critical roles in the ability of *H. pylori* to overcome oxidative and nitrosative stresses (8), but the *H. pylori* target protein(s) for Trx1 (except for alkyl hydroperoxide reductase, AhpC (10)) and Trx2 are largely unknown. ROI/RNIs exhibiting distinct *in vivo* properties (15) (hydrogen peroxide, methyl viologen (a superoxide generator), hypoxanthine/xanthine oxidase (a superoxide generator), S-nitrosothiol (GSNO), a NO· (nitric oxide radical) and NO· donor), sodium nitroprusside (SNP, a nitrosating agent mainly producing NO·), 3-morpholinosydnonimine (SIN-1, a peroxynitrite generator), and diethylene triamine-nitric oxide (DETA/NO, slowly releases NO·) were coincubated with arginase and either Trx1 or buffer control, followed by assessment of arginase activity. This experiment assesses whether Trx1 protects arginase from ROI/RNIs. In the absence of Trx1, arginase was found to be exquisitely sensitive to hydrogen peroxide and SNP, moderately sensitive to GSNO, sensitive to DETA/NO and SIN-1 only at high concentrations, and completely resistant to methyl viologen and hypoxanthine/xanthine oxidase (Table 1). Both wild type and rocF mutant *H. pylori* were shown to be equally sensitive to methyl viologen in a disc diffusion assay (zone of inhibi-

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**FIGURE 4. Evidence that Trx1 acts as an arginase chaperone.** A, catalytically inactive purified arginase (His6-RocF [I], stored 6 months at −20 °C, 4.7 μg) was incubated in the absence or presence of either purified Trx1 (4.1 μg) or purified Trx2 (5.1 μg), and arginase activity was compared with a freshly isolated batch of arginase (His6-RocF [A], 3.1 μg). B, arginase (1.4 μg) was denatured with 1 μM urea and then left untreated or treated with purified Trx1 (Denat + Trx1, 1.3 μg), or dialyzed to remove the urea in the absence (Den, DZ) or presence of Trx1 (Den, DZ + Trx1, 1.3 μg). C, purified His6-RocF (1.4 μg) was assessed for arginase activity in the absence or presence of various concentrations of urea. No loss of activity occurred below 100 mM, suggesting that although urea is a product of the arginase reaction, urea is not itself an inhibitor at the enzyme activity level. Rather, urea inhibits only at high concentrations by denaturing arginase. D, purified His6-RocF (1.4 μg) was coincubated at different temperatures in the absence (No Trx1) or presence or Trx1 (Trx1 Coinc, 1.3 μg) (protein experiments). Purified His6-RocF was also treated at different temperatures in the absence of Trx1, but then Trx1 was added after the heat treatment (Trx1 post) (reversal experiments). E, purified His6-RocF (1.4 μg) was incubated for 10 min at 55 °C in the presence (Co during) or absence of cobalt (5 mM) in the presence or absence of Trx1 (1.3 μg). For samples incubated without cobalt, cobalt was added back following the heat treatment (Co post) prior to assay for arginase activity. For samples incubated without Trx1, Trx1 was added back to some samples (Trx1 post) or left out as a control. F, purified His6-RocF (1.4 μg) was incubated for various times at 55 °C in the presence or absence of Trx1 (1.3 μg, Trx1 Coinc). Following heat treatment, Trx1 was added back to one sample (1.3 μg, Trx1 post) or the sample was left alone as a control (no Trx1). G, purified His6-RocF (1.4 μg) was incubated with or without purified Trx1 at various concentrations of cobalt. The data for all of the figures are representative of at least three experiments, presented as mean arginase specific activity ± standard deviation from duplicate or triplicate measurements. *, p < 0.05 versus control.
duced in which His6-RocF was pretreated with ROI/RNIs followed by protection from GSNO and SNP-mediated damage was most dramatic (Fig. 5, B; p < 0.05 comparing ROI/RNI treatment of arginase versus ROI/RNI treatment plus Trx1); protection from GSNO and SNP-mediated damage was most dramatic (Fig. 5B). Reversal experiments were also conducted in which His<sub>e</sub>-RocF was pretreated with ROI/RNIs followed by the addition of Trx1. These experiments suggested that Trx1 completely reversed damage to arginase by DETA/NO, SIN-1, and hydrogen peroxide, but GSNO and SNP-treated arginase had lower activity after Trx1 addition than when Trx1 was present during GSNO or SNP treatment (Fig. 5C and D; p < 0.05 comparing ROI/RNI treatment of arginase versus ROI/RNI treatment plus Trx1; p < 0.05 comparing GSNO/Trx1 post vs. control (Ctrl)). DETA/NO (2 mM) and SNP (10 μM) were used as nitrosative stresses. Because copious ROI/RNIs are rapidly generated from infiltrating inflammatory cells following adherence of H. pylori to gastric epithelial cells (19), the bacterium must be equipped with an immediately available system to overcome this onslaught of potentially toxic redox compounds. In addition to the established role of Trx1 as a disulfide reductase that reduces H. pylori AhpC (10), Trx1 has two additional functions revealed in this study. First, Trx1 serves as a chaperone that converts denatured or suboptimally folded arginase into its optimal three-dimensional catalytically active structure. Second, Trx1 not only protects arginase against oxidative and nitrosative-mediated stress, it can detoxify the damage. Whether Trx1 acts as a guardian for other H. pylori enzymes is unknown. Post-translational modulation of H. pylori arginase by Trx1 may have evolved as a specialized adaptation for the unique gastric lifestyle of H. pylori. Because H. pylori urease is required for colonization (20), the urea substrate for urease may derive from the bacterial arginase when the host is faced with a deluge of ROI/RNIs. Supporting this model is the finding that expression of host arginases is responsible for mutations in genes that lead to stomach cancer (18, 19). Because copious ROI/RNIs are rapidly generated from infiltrating inflammatory cells following adherence of H. pylori to gastric epithelial cells (19), the bacterium must be equipped with an immediately available system to overcome this onslaught of potentially toxic redox compounds. In addition to the established role of Trx1 as a disulfide reductase that reduces H. pylori AhpC (10), Trx1 has two additional functions revealed in this study. First, Trx1 serves as a chaperone that converts denatured or suboptimally folded arginase into its optimal three-dimensional catalytically active structure. Second, Trx1 not only protects arginase against oxidative and nitrosative-mediated stress, it can detoxify the damage. Whether Trx1 acts as a guardian for other H. pylori enzymes is unknown. Post-translational modulation of H. pylori arginase by Trx1 may have evolved as a specialized adaptation for the unique gastric lifestyle of H. pylori. Because H. pylori urease is required for colonization (20), the urea substrate for urease may derive from the bacterial arginase when the host is faced with a deluge of ROI/RNIs. Supporting this model is the finding that expression of host arginases is

**TABLE 1**

Arginase activity inhibition by ROI/RNIs

| ROI/RNI                  | *IC<sub>50</sub>*<sup>a</sup> |
|--------------------------|-----------------------------|
| Hydrogen peroxide        | 3 μM                        |
| Methyl viologen          | No inhibition at 1 mM       |
| Hypoxanthine/xanthine oxidase | No inhibition at 2 mM hypoxanthine, 0.1365 units of XO |
| GSNO                     | 50 μM                       |
| Diethylene triamine-nitric oxide | 2 mm                     |
| Sodium nitroprusside     | 5 μM                        |
| SIN-1                    | 1 mM                        |

<sup>a</sup> Concentration that inhibits arginase activity by 50%. The values are the averages of at least three experiments.

**FIGURE 5**. Trx1 protects arginase from ROI/RNIs and reverses oxidative and nitrosative damage to arginase. A, protection experiments. Purified Trx1 (1.3 μg) was incubated for 15 min with hydrogen peroxide (147 μM) or SIN-1 (1 mM) followed by the addition of purified His<sub>e</sub>-RocF (1.4 μg) and assayed for arginase activity (Trx1 coinc). Controls lacked Trx1. B, same as A except DETA/NO (2 mM), GSNO (25 μM), or SNP (10 μM) was used as nitrosative stresses. C, reversal experiments. Purified His<sub>e</sub>-RocF was incubated for 15 min in the presence of ROI/RNIs, followed by the addition of Trx1 (Trx1 post). DETA/NO (2 mM) and SNP (10 μM) were used as stresses. D, same as C except GSNO (25 μM), hydrogen peroxide (29 μM), and SIN-1 (1 mM) were used. The data are representative of at least three experiments, presented as mean arginase specific activity ± standard deviation from duplicate or triplicate measurements. * p < 0.05 versus control (Ctrl).
diminished when NO is elevated (21). This delicate balance of host ROI/RNIs is also manipulated by *H. pylori* arginase itself, because arginase can decrease NO production by the host (3). Therefore, Trx1-mediated protection of arginase from ROI/RNIs may allow *H. pylori* arginase to remain active even in the face of elevated ROI/RNI levels and provide the necessary urea required by urease so that the ammonium generated can protect *H. pylori* from gastric acid.

*H. pylori* is equipped with a “renox guardian” comprised of Trx1 and arginase that helps the bacterium overcome reactive nitrogen and oxygen stresses. This allows the arginase to remain functional and provide an endogenous urea source for the bacterium when the *H. pylori* encounters host ROI/RNIs and meager host urea availability. This protective system could be responsible for the exceptional ability of *H. pylori* to persist for decades in the hostile gastric environment.

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