Heme oxygenases catalyze the oxidation of heme to biliverdin, CO, and free iron. For pathogenic microorganisms, heme uptake and degradation are critical mechanisms for iron acquisition that enable multiplication and survival within hosts they invade. Here we report the first crystal structure of the pathogenic *Escherichia coli* O157:H7 heme oxygenase ChuS in complex with heme at 1.45 Å resolution. When compared with other heme oxygenases, ChuS has a unique fold, including structural repeats and a β-sheet core. Not surprisingly, the mode of heme coordination by ChuS is also distinct, whereby heme is largely stabilized by residues from the C-terminal domain, assisted by a distant arginine from the N-terminal domain. Upon heme binding, there is no large conformational change beyond the fine tuning of a key histidine (His-193) residue. Most intriguingly, in contrast to other heme oxygenases, the propionic side chains of heme are orientated toward the protein core, exposing the α-meso carbon position where O₂ is added during heme degradation. This unique orientation may facilitate presentation to an electron donor, explaining the significantly reduced concentration of ascorbic acid needed for the reaction. Based on the ChuS-heme structure, we converted the histidine residue responsible for axial coordination of the heme group to an asparagine residue (H73A) for comparison purposes. We employed spectral analysis and CO measurement by gas chromatography to analyze catalysis by ChuS, H193N, and H73A, demonstrating that His-193 is the key residue for the heme-degrading activity of ChuS.

Iron is an essential cofactor whose role as a biocatalyst or electron carrier enables organisms to fulfill many vital biological processes including respiration, the citric acid cycle, oxygen transport and utilization, gene regulation, and DNA biosynthesis (1). For bacteria invading mammalian hosts, iron acquisition, transport, and utilization are essential for survival and pathogenesis. However, due to the low solubility of iron(III) salts at physiological pH in the presence of oxygen, iron uptake, storage, and transport are considerable obstacles for invading bacteria to overcome. Enterohemorrhagic pathogens such as *Escherichia coli* O157:H7 may induce hemolytic lesions to gain access to host nutrient stores, such as heme, the most abundant source of invertebrate host iron (2). Furthermore, growth of O157 isolated from human patients is stimulated in the presence of heme and hemoglobin, suggesting that heme may be an important signaling molecule for the up-regulation of factors contributing to host colonization and virulence (2).

The proteins involved in heme internalization by enterohemorrhagic pathogens have been shown to involve homologues to members of the heme utilization locus (3). Using a laboratory strain of *E. coli* (1017 (ent::Tn5)) that was unable to import heme into the cytoplasm, it was established that the outer membrane receptor ChuA was required for heme uptake along with TonB, an energy-transducing protein associated with ExbB and ExbD, forming a complex that uses the proton motive force of the cytoplasmic membrane for the transport of ligands into the periplasm (4). Four other members of the heme uptake operon from *Yersinia enterocolitica* were shown to be required to complete heme uptake, transport, and use as an iron source by *E. coli* K-12 (3) including: the periplasmic heme-binding protein HemT, the heme permease protein HemU, the ATP-binding hydrophilic protein HemV, and the protein HemS, whose role was supposed to have been involved in regulating the amount of non-protein-associated heme in the cell (5). These *Y. enterocolitica* are homologous to members of the *E. coli* O157:H7 heme utilization locus chuT, chuU, chuV, and chuS, respectively.

We recently reported the crystal structure of apo-ChuS and confirmed via spectral analysis and CO measurement that ChuS was an HO, the first to be identified in any strain of *E. coli* (6). A *Shigella dysenteriae* chromosomal knock-out of *shuS*, a homologue to *chuS*, was shown to be defective in utilizing heme as an iron source, and expression of *ShuS* was up-regulated when challenged with iron-limited conditions (7). Furthermore, *shuS* was implicated to have a cytoprotective role against heme toxicity in that growth of the *shuS* knock-out mutant was impaired at intermediate concentrations of heme (15 μM) and...
that shuS was absolutely required for colony growth at high heme concentrations (40 μM) (7). Together, these studies suggest that ChuS and its homologues are up-regulated under iron-limited conditions and that ChuS activity enables pathogenic E. coli O157:H7 to use heme as an iron source, whereas conferring a cytoprotective role in the prevention of heme toxicity. It appears very probable that ChuS is an essential protein in heme utilization by various pathogenic, enterohemorrhagic bacteria.

The crystal structures of HOs from other Gram-negative bacteria such as HemO (8), HmuO (9), and PigA (10) all share a high structural similarity with human HO-1 (11); they are mainly α-helical, and the heme is sandwiched between a histidine residue and a glycine residue. However, the structure of ChuS revealed a unique architecture when compared with other HOs in that it contains a central set of antiparallel β-sheets and that the N- and C-terminal halves are structural repeats. In light of the identification of ChuS as a novel HO and its unique overall structure, it was of interest to elucidate the mechanism of its heme binding and degradation. To this end, we have determined the x-ray crystal structure of ChuS in complex with heme to 1.45 Å resolution. We have further performed site-directed mutagenesis of the histidine residue (His-193) (shown in the crystal structure to be responsible for axial coordination of the heme group within ChuS) to an asparagine (H193N) and of another conserved histidine residue (His-73) to an alanine (H73A) as a control. Using spectral analysis and CO measurement by gas chromatography to analyze heme degradation catalyzed by ChuS, H193N, and H73A, we have demonstrated that His-193 is a key residue for the heme-degrading activity of ChuS.

**EXPERIMENTAL PROCEDURES**

**Protein Expression and Purification, Site-directed Mutagenesis**—ChuS fused to an N-terminal His$_6$-tag was subcloned into a pET expression vector. Based on the complex structure and sequence conservation analysis, the mutations of H73A and H193N of ChuS were selected (6). Using the QuikChange® site-directed mutagenesis kit (Stratagene, La Jolla, CA), wild-type ChuS was mutated at these two histidine positions using the following primers, where base pair changes have been underlined: H73A, sense, 5′-CGTAATGAAATATGCAGTC-GCGGAGCAAGTTGGTACG-3′, antisense, 5′-GTACCAAGCCTTGCTCCGCGACTGCATATTCATTACG-3′; and H93N, sense, 5′-GGGCCATGACCCGACTTATCAAGG-TAATCACTGATTTATTTA-CGTTGCTCAA-3′, antisense, 5′-GAGGAGCTGAGATAATGAGAATGAAA-CTGATTAACGTCCGATCGTACGCCC-3′. Briefly, polymerase chain reaction conditions and cycling parameters recommended by Stratagene (12) were followed using Pfu Turbo polymerase for 30 cycles between 30 s, 95°C melt, 60 s, 55°C anneal, and 9 min, 68°C extend. Methylated (non-PCR amplified) DNA was then digested with DpnI restriction enzyme for 1 h, and the digested DNA was heat shock-transformed into MC1061 cells. Transformed cells were subsequently plated onto LB-agar plates supplemented with 100 μg/ml ampicillin. Single colonies were grown overnight at 37°C in 5 ml of Terrific Broth (BioShop, Burlington, Ontario, Canada) supplemented with 100 μg/ml ampicillin (TB-amp). Plasmid DNA was purified by means of a QIAprep spin miniprep kit (Qiagen, Mississauga, Ontario, Canada) and the desired mutations were confirmed by sequence analysis (Robarts Research Institute, London, Ontario, Canada).

Cultures (1 liter) of BL21(DE3) cells carrying plasmids for the respective recombinant protein were grown at 37°C in TB-amp. Protein expression was induced at a culture A$_{600}$ between 0.6 and 1.0 using 0.4 mM isopropyl-1-thio-β-D-galactopyranoside and grown for 5 h. ChuS, H73A, and H193N were then purified via nickel nitrolotriacetate agarose batch purification in phosphate buffer (pH 8.0) and dialyzed overnight in 30 mM Tris, 5 mM NaCl, pH 8.0. Overexpression of ChuS and H73A resulted in cells exhibiting a blue-green pigment that is characteristic of biliverdin accumulation (8, 13, 14). ChuS, H73A, and H193N all expressed at high levels (>20 mg/liter culture depending on batch) and were soluble in Tris (pH 8.0) or phosphate (pH 7.0) buffers following nickel nitrolotriacetate agarose purification. Purification of each protein was monitored by SDS-PAGE, and each was estimated to be >95% pure prior to spectral analysis, CO measurement, or heme reconstruction. H193N and H73A were subsequently dialyzed twice more to eliminate any residual imidazole. Protein concentrations were determined by Bio-Rad protein assay (Bio-Rad, Mississauga, Ontario, Canada) in 96-well format, using bovine-γ-globulin as a protein standard.

**ChuS-Heme Reconstitution**—The ChuS-heme complex was prepared by dissolving heme (ferriprotoporphyrin IX chloride) (Sigma, Oakville, Ontario, Canada) into 0.5% (v/v) ethanolamine for 30 min and then into 30 mM Tris, and the pH was adjusted to 8.0. Small volumes of this mixture were then added to dialyzed ChuS until a final ratio of 4:1 heme to protein was reached. The ChuS-heme mixture was then incubated for 15 min, centrifuged at 16,000 rpm for 15 min in a JA-20 Beckman Coulter rotor (Beckman Coulter, Mississauga, Ontario, Canada), and purified using a Resource Q column on an ATKA Explorer fast protein liquid chromatography system with 30 mM Tris, 1 M NaCl (pH 8.0) as the elution buffer. Collected fractions were examined by SDS-PAGE, and those that were evaluated to contain pure protein were pooled. Pure ChuS was concentrated using an Amicon Ultra 15-kDa cutoff centrifugal filtration device (Fisher, Mississauga, Ontario, Canada) and used immediately for crystallization. Reconstituted ChuS-heme complex concentrations were determined by using the extinction coefficient reported for ShuS (ε$_{410}$) of 159 mm$^{-1}$ cm$^{-1}$ for ChuS (15).

**Crystallization of ChuS-Heme**—ChuS-heme crystals were obtained only by means of the sitting drop vapor diffusion method, in 96-well plates (Greiner Bio-One, Monroe, NC), using freshly prepared protein and reagents. Crystallization was achieved by mixing 1.75 μl of 20 mg/ml ChuS-heme with 1.75 μl of chilled reservoir solution consisting of 12–15% (w/v) polyethylene glycol 3350, 0.15–0.20 M magnesium formate, and 10–20 mM NAD. Crystallization plates were incubated in the dark at 4°C, with both hexagonal and triangular prism crystals appearing after 1–3 days. For cryoprotection, crystals were soaked in the reservoir solution containing ethylene glycol (concentration increased stepwise to 30% (v/v)), picked up using a nylon loop, and flash-cooled in the N$_2$ cold stream at 100 K. Two crystal forms belonging to space groups P6$_3$ and R$_3$ were
Crystal Structure of ChuS-heme

obtained whose morphologies were hexagonal and triangular prism, respectively.

Data Collection and Structure Determination—Diffraction data of the P6₃ and R₃ crystals were collected at beam line X29 equipped with an ADSC Quantum-315, nine-quadrant, CCD detector at the National Synchrotron Light Source, Brookhaven National Laboratory. For both the P6₃ and the R₃ crystals, data were collected for 180° (0.5 and 1.0° oscillations, respectively) at the wavelength 1.100 Å. Data were processed with HKL2000 (16) (see Table 1), and the structure of the R₃ crystal was solved via molecular replacement using the program Phaser (17) with apo-ChuS (Protein Data Bank ID code 1U9T) as the initial probe structure. Although the P6₃ crystals diffracted beyond 2.0 Å resolution, due to the long unit cell dimensions and resulting spot proximity, diffraction was only collected to 2.7 Å. The heme moiety was manually placed in the ChuS structure according to the difference density in both space groups. The ChuS-heme complex structure in the R₃ space group was completed by iterative cycles of manually fitting using XtalView/ Xfit (18) and refinement using Refmac5 (19, 20) at a final resolution of 1.45 Å (see Table 1). Coordinates and structure factors for ChuS-heme have been deposited in the RSCB Protein Data Bank under accession code 2HQ2. Due to lower resolution and poor data quality in regions along the long axes, the P6₃ complex structure was not further refined beyond confirming the heme position.

Spectral Analysis and CO Measurements—Reconstitution of ChuS, H73A, and H193N with heme was achieved by dissolving heme into 0.5% (v/v) ethanolamine and diluted into phosphate buffer just prior to mixing with 10 μM protein samples to a final ratio of 1:1 protein to heme. Absorbance data were collected after a 15-min incubation at room temperature in 100 mM phosphate buffer (pH 7.0) using a microplate spectrophotometer (Bio-Tek Instruments, Inc., Winooski, VT). Heme degradation was initiated in parallel by adding ascorbic acid to a final concentration of 50 μM, and spectra were recorded between 300 and 700 nm at 5-nm intervals.

For CO measurement, in 2-ml amber vials, 250-μl reaction volumes of 10 μM heme and 10 μM ChuS, H73A, or H193N in 100 mM phosphate buffer (pH 7.0) were incubated with constant shaking at room temperature for 15 min, at which time enzymatic heme degradation was initiated by adding ascorbic acid to a final concentration of 50 μM. The amber vials were then sealed with screw caps and blue silicone rubber septa, the headspace above each was immediately purged with CO-free air, and the vials were incubated for 25 min at 37 °C with constant shaking. The reaction was stopped by placing the vials on powdered dry ice (−78 °C), where they remained for ~30 min. The amount of liberated CO in the headspace of each vial was measured using a gas chromatograph equipped with an HgO reduction detector (Trace Analytical). The amount of CO resulting from ChuS-catalyzed breakdown of heme was determined by comparing n = 3 peak area measurements for CO against linear CO standard curves (n = 2 determinations; average correlation coefficient, 0.9859). Technical details of CO detection are described elsewhere (21, 22).

RESULTS

Structure Determination and Analysis of ChuS-Heme—Crystallization of ChuS reconstituted with heme using the hanging drop vapor diffusion method turned from red to blue-green following room temperature incubation periods of over 1 week. In the sitting drop vapor diffusion setup, two different crystal forms of ChuS-heme were obtained from the same set of crystallization conditions over the same time period, but they differed dramatically in their morphology. In the case of the P6₃ crystals, which appeared more frequently at room temperature, the crystals appeared as broad (>1 mm), flat, hexagonal plates. Crystals belonging to the R₃ space group appeared as clusters of long (>1 mm) triangular prisms and diffracted to 1.45 Å resolution. Crystals from the P6₃ space group contained two molecules in the asymmetric unit, whereas those belonging to the R₃ space group contained only one.

The final structure of ChuS-heme complex contains residues 0–337 of a total 354 amino acids including the Hisᵦ, affinity tag for the R₃ space group (Fig. 1). In total, 100% of residues are within the allowed region of the Ramachandran plot. Only one residue, Ala-96, was in the generously allowed region and resides just prior to a surface-exposed β-turn. Final refinement statistics are summarized in Table 1.

The ChuS-heme complex structure is very close to that of apo-ChuS (all-atom root mean square deviation, 1.69 Å). The overall structure comprises a central core of two large pleated α-helices, each consisting of nine anti-parallel β-strands sandwiched together and bowing outward in a saddle motif (Fig. 1). Each β-sheet is flanked at its N terminus by one pair of parallel α-helices and at the C terminus by three α-helices in an α-loop-α-loop-α motif, forming a structural repeat. When ChuS-heme is superimposed with apo-ChuS, the only notable structural change is that the two α-helices N-terminal of His-193 have shifted toward the core of the protein (e.g. C-α of His-193 has shifted by 3.58 Å), resulting in the appropriate orientation of this key histidine residue for heme coordination (see following section and Fig. 1, inset).

Heme Binding—The heme group is coordinated in a cleft region delineated between His-193 at the base of a C-terminal α-helix and Arg-100 from the central set of β-sheets at the core of ChuS (Fig. 1). A network of hydrogen bonds is formed between Arg-100 and the iron atom of the heme group via two water molecules with distances of 2.93, 2.17, and 1.93 Å, respectively (Fig. 2). In this cleft, the heme group is stabilized by the non-polar residues Leu-90, Leu-92, Phe-102, Val-192, and Phe-243, and the polar residues Arg-100, His-193, Arg-206, Met-241, Lys-291, Gln-313, Tyr-315, and Arg-318 (Fig. 2). In contrast to other HOs, the propionic side chains of the heme moiety in ChuS are rotated ~110°, pointing toward the interior of one of the structural lobes formed between the two halves of ChuS, and are stabilized by the positively charged side chains Arg-206, Lys-291, and Arg-318 (Fig. 2). These interactions are probably important in maintaining the correct orientation of heme within ChuS. The burying of the propionic side chains exposes the α-meso carbon of the heme group, which is where O₂ is selectively added during heme degradation in HO reactions. Although the P6₃ data were of lower quality, the identical
The heme orientation in ChuS is unique when compared with other HOs, such as HO-1, in which the propionic side chains point outward (23), although the heme-degrading enzymes IsdG/IsdI may bind heme with the propionic side chains pointing inward (24).

Essential to the heme coordination is His-193, which makes axial interaction with the central iron atom of the heme group. In ChuS, the interaction distance is 2.03 Å between the NE2 atom of His-193 and the center of the iron atom, allowing His-193 to clamp the heme firmly into place. Arg-100, from the N-terminal domain, interacts with heme via two water molecules and originates from the protein core beneath the heme group on one of the central β-sheets (Fig. 2B). These water molecules were clearly visible even at early refinement stage across multiple data sets and well defined in the final high resolution structure.

Spectroscopic Properties of ChuS, H73A, and H193N in Complex with Heme—His-193 resides at the end of an α-helix, pointing into a cleft delineated by the central set of β-sheets. Thus, the mutation of His-193 was expected to inactivate the protein. On the other hand, His-73 is within the central β-sheets, pointing between the N- and C-terminal halves, and does not make contact with the heme group. Since His-73 is fully conserved but does not interact with heme, it provides a good control for His-193. As previously reported, the absorbance spectrum of native ChuS exhibits a clear Soret peak at 410 nm together with the common characteristic α/β-bands of HOs around 550 and 580 nm (6, 15, 25). The H73A mutation was not observed to instigate any spectral changes when compared with native ChuS. However, the spectrum of the H193N mutant broadened, showed a decrease in the magnitude of absorbance, and shifted the peak absorbance to 390 nm when compared with the Soret absorption observed for ChuS and H73A. A similar trend of spectral flattening and peak shift to 625 nm was also observed for H193N in the α/β-band region.

Heme Degradation by ChuS—Bacterial and mammalian HOs use the cytochrome P450 reductase-NADPH system, ferredoxin, flavodoxin, and/or ascorbic acid as reducing partners in vitro (8, 26–30). We have previously demonstrated the ability of ChuS to use either cytochrome P450 reductase-NADPH or ascorbic acid as an electron donor for heme degradation (6). It is
important to note that in our analysis, we employed only 50 μM ascorbic acid, a concentration between 100- (32) and 350-fold (30), less than that used for characterizing other HOs, thereby minimizing the non-enzymatic degradation of heme through coupled oxidation. Furthermore, catalase and superoxide dismutase were used for additional controls. To examine the heme-degrading activity of ChuS, H73A, and H193N, we monitored the reaction by UV-visible spectroscopy for 1 h after the addition of ascorbic acid. As the reaction proceeded, the Soret peak for ChuS and H73A at 410 nm decreased, the small broad peak at 560 nm increased, and the absorption at 680 nm initially increased and then decreased (Fig. 3). When ascorbic acid was added to H193N reconstituted with heme, the profile of the spectrum did not change but was observed to increase slightly in absorbance. In the case of native ChuS, the addition of catalase and superoxide dismutase did not affect the observed spectral change, demonstrating specific enzyme activity.

When HOs degrade heme to biliverdin, CO is released as a byproduct. Using gas chromatography, we measured CO release for wild-type ChuS, as well as the two mutants. As shown in Fig. 4, whereas wild-type ChuS and H73A were able to generate CO as expected, the H193N mutation inactivated the enzyme. However, based on the appearance of a broad peak for H193N near the Soret region of native ChuS, this mutant seems to retain some interaction with heme.

**DISCUSSION**

The x-ray crystal structure of the *E. coli* O157:H7 HO ChuS-heme complex has revealed that ChuS employs a unique mode of heme coordination. Although the structures of other HOs characterized to date coordinate heme between a set of α-helices, ChuS coordinates the central iron atom of the heme group between His-193 and the end of an
Crystal Structure of ChuS-heme

Despite the evidence that genes homologous to chuS have been shown to be up-regulated under iron-limiting conditions, that these homologues prevented heme toxicity in S. dysenteriae (7), and that no other heme-degrading enzyme has been identified in any E. coli strain, the precise role for ChuS and its homologues has been downplayed in recent reports to be that of a cytoplasmic trafficker of heme or a nonspecific oligomeric complex that binds to DNA (15, 25). By carefully examining the spectral progression and CO measurement of ChuS-catalyzed degradation of heme in the presence of minimal amounts of ascorbic acid, we conclude that ChuS specifically degrades heme and suggest that His-193 is the essential amino acid responsible, similar to His-25 in HO-1 (11). The confirmation of His-193 as an integral catalytic residue is affirmed through its sequence conservation, its role in coordination of the heme moiety in the crystal structure, as well as the comparison of the H73A control mutant. The spectral shifts observed for ChuS and H73A are similar to those observed for other HOs, which suggest the formation of biliverdin and free iron rather than the Fe$^{3+}$-biliverdin complex formed by HO-1 (35). Because the addition of catalase and superoxide dismutase did not affect the trends of the spectral change, the observed activity of ChuS can be attributed only to specific heme degradation (6).

It is difficult to extrapolate the pathophysiological significance of the heme-degrading ability of ChuS to its homologues in other bacteria due to the fact that other redundant systems have been identified for iron acquisition and utilization from heme sources including proteins from Pseudomonas aeruginosa (PigA and BphP) and S. aureus (IsdG and IsdI). Furthermore, redox enzymes are notoriously nonspecific with respect to their reaction with oxygen species (36). This has led to a lack of straightforward characterization of other bacterial cytoplasmic heme-binding proteins outside those with sequence homology to HO-1. Therefore, understanding the interplay between sequence and structural motifs in heme binding and utilization are of particular interest to clarify our understanding of heme utilization by pathogenic bacteria. As the propionic acid homologues prevent heme toxicity in E. coli strain, the precise role for ChuS and its homologues (37). The distinct orientation of heme may further explain the minimal amount of ascorbic acid needed for the degradation reaction because the α-meso carbon atom is exposed to facilitate interaction with an electron donor.

During our investigation, a variety of practical obstacles were overcome that could help in future studies of ChuS and its homologues. As previously reported, ChuS and its homologues form high molecular weight aggregates following incubation over a few days at 4 °C (15, 25, 31). To overcome aggregation, fresh, unfrozen preparations of pure ChuS were absolutely necessary for crystallization with heme and the heme degradation reaction. This mechanism of inhibition for imidazole is likely the forma-
tion of a distal heme axial ligand, thus precluding substrate oxygenation (38). Purification of ChuS must therefore ensure complete removal of imidazole from reaction buffers.

In conclusion, we have provided a first view of the heme-bound structure of ChuS, a novel pathogenic HO with a unique structure. The heme binding site in ChuS is very different from those of other known HO-heme complexes. His-193 plays a key role in anchoring the heme group by tightly interacting with the central iron ion. The majority of the heme stabilization is contributed by the C-terminal half, along with Arg-100 from the N-terminal half that also interacts with iron via two fully conserved water molecules. The exposed α-meso region, which is unique among known HO-heme complexes, may facilitate the R3 crystals of ChuS-heme. Excellent equipment management was also contributed by Hans Metz.

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REFERENCES

1. McHugh, J. P., Rodriguez-Quinones, F., Abdul-Tehrani, H., Svistunenko, D. A., Poole, R. K., Cooper, C. E., and Andrews, S. C. (2003) J. Biol. Chem. 278, 29478–29486

2. Law, D., and Kelly, J. (1995) Infect. Immun. 63, 700–702

3. Stojiljkovic, I., and Hantke, K. (1992) Mol. Microbiol. 6, 1695–16960

4. Suits, M. D., Pal, G. P., Nakatsu, K., Matte, A., Cygler, M., and Jia, Z. (2005) Proc. Natl. Acad. Sci. U. S. A. 102, 16955–16960

5. Wyckoff, E. E., Lopreato, G. F., Tipton, K. A., and Payne, S. M. (2005) J. Bacteriol. 187, 5658–5664

6. Zhu, W., Wilks, A., and Stojiljkovic, I. (2000) J. Bacteriol. 182, 6783–6790

7. Hirotsu, S., Chu, G. C., Unno, M., Lee, D. S., Yoshida, T., Park, S. Y., Shiro, Y., and Ikeda-Saito, M. (2004) J. Biol. Chem. 279, 11937–11947

8. Friedman, J., Lad, L., Wilks, A., and Poulos, T. L. (2004) Biochemistry 43, 5239–5245

9. Schuller, D. J., Zhu, W., Stojiljkovic, I., Wilks, A., and Poulos, T. L. (2001) Biochemistry 40, 11552–11558

10. Liu, Q., Swiderski, P., and Sommer, S. S. (2002) BioTechniques 33, 129–132, 134–136, 138

11. Wegele, R., Tasler, R., Zeng, Y., Rivera, M., and Frankenfeld-Dinkel, N. (2004) J. Biol. Chem. 279, 45791–45802

12. Maki, H., Matsura, K., Shimada, K., and Nagashima, K. V. (2003) J. Biol. Chem. 278, 3921–3928

13. Wilks, A. (2001) Arch. Biochem. Biophys. 387, 137–142

14. Otwinowski, Z., and Minor, W. (1997) Methods Enzymol. 267, 307–326

15. Stojiljkovic, I. C., McCoy, A. I., and Read, R. J. (2004) Acta Crystallogr. Sect. D Biol. Crystallogr. 60, 432–438

16. Otwinowski, Z., and Minor, W. (1997) Methods Enzymol. 267, 307–326

17. Schuller, D. J., Wilks, A., Ortiz de Montellano, P. R., and Poulos, T. L. (1999) Nat. Struct. Biol. 6, 860–867

18. Skaar, E. P., Gaspar, A. H., and Schneewind, O. (2004) J. Biol. Chem. 279, 436–443

19. Lanksy, I. B., Lukat-Rodgers, G. S., Block, D., Rodgers, K. R., Ratliff, M., and Wilks, A. (2006) J. Biol. Chem. 281, 13652–13662

20. Wilks, A., and Ortiz de Montellano, P. R. (1993) J. Biol. Chem. 268, 22357–22362

21. Matera, K. M., Zhou, H., Migita, C. T., Hobert, S. E., Ishikawa, K., Katakura, K., Maeshima, H., Yoshida, T., and Ikeda-Saito, M. (1997) Biochemistry 36, 4909–4915

22. Ishikawa, K., Takeuchi, N., Takahashi, S., Matera, K. M., Sato, M., Shibahara, S., Rousseau, D. L., Ikeda-Saito, M., and Yoshida, T. (1995) J. Biol. Chem. 270, 6345–6350

23. Blattner, F. R., Plunkett, G., III, Bloch, C. A., Perna, N. T., Burland, V., Riley, M., Collado-Vides, J., Glasner, J. D., Rode, C. K. Mayhew, G. F., Gregor, J., Davis, N. W., Kirkpatrick, H. A., Goeden, M. A., Rose, D. J., Mau, B., and Shao, Y. (1997) Science 277, 1453–1474

24. Park, H., Suquet, C., Satterlee, J. D., and Kang, C. (2004) Biochemistry 43, 2738–2746

25. Caignan, G. A., Deshmukh, R., Wilks, A., Zeng, Y., Huang, H. W., Moenne-Loccoz, P., Bunce, R. A., Eastman, M. A., and Rivera, M. (2002) J. Am. Chem. Soc. 124, 14879–14892

26. Hao, B., Isaza, C., Arndt, J., Soltis, M., and Chan, M. K. (2002) Biochemistry 41, 12952–12958

27. Baker, H. M., Anderson, B. F., and Baker, E. N. (2003) Proc. Natl. Acad. Sci. U. S. A. 100, 3579–3583

28. Wilks, A., Ortiz de Montellano, P. R., Sun, J., and Loehr, T. M. (1996) Biochemistry 35, 930–936

29. Imlay, J. A. (2003) Annu. Rev. Microbiol. 57, 395–448, 395–418

30. Friedman, J., Lad, L., Deshmukh, R., Li, H., Wilks, A., and Poulos, T. L. (2003) J. Biol. Chem. 278, 34654–34659

31. McMillan, K., and Masters, B. S. (1993) Biochemistry 32, 9875–9880