Helicobacter pylori Csd4 requires a glutamine zinc ligand

Anson C. K. Chan¹, Kris M. Blair²,³, Yanjie Liu⁴, Emilisa Fridrich⁴, Erin C. Gaynor¹, Martin E. Tanner⁴, Nina R. Salama²,³, Michael E. P. Murphy¹

¹From the Department of Microbiology and Immunology, University of British Columbia, Vancouver, BC, V6T 1Z3, Canada
²Division of Human Biology, Fred Hutchinson Cancer Research Center, Seattle, WA, 98109, USA
³Program in Molecular and Cellular Biology, University of Washington, Seattle, WA, 98195, USA
⁴Department of Chemistry, University of British Columbia, Vancouver BC, V6T 1Z1, Canada

*Running title: Helicobacter pylori Csd4 requires a glutamine zinc ligand

#These authors contributed equally to this work

To whom correspondence should be addressed: Michael E. P. Murphy, Department of Microbiology and Immunology, 2350 Health Sciences Mall, The University of British Columbia, Vancouver, BC, Canada, V6T 1Z3; Telephone: (604) 822-8022; Fax: (604) 822-6041; E-mail: michael.murphy@ubc.ca

Keywords: peptidoglycan; Helicobacter pylori; zinc; metalloenzyme; carboxypeptidase; cell wall; tripeptide; meso-diaminopimelic acid; cell shape

Background: Csd4 is required for the helical shape of Helicobacter pylori. 
Results: Csd4 activity relies on a Gln-zinc ligand to cleave cell wall tripeptides and produce helical shape. 
Conclusion: Carboxypeptidase activity can be achieved with a Gln, His, and Glu zinc coordination. 
Significance: Csd4 represents a new subfamily of carboxypeptidases.

ABSTRACT

Peptidoglycan (PG) modifying carboxypeptidases (CPs) are important determinants of bacterial cell shape. Here, we report crystal structures of Csd4, a three-domain protein from the human gastric pathogen Helicobacter pylori. The catalytic zinc in Csd4 is coordinated by a rare H+E+Q configuration that is conserved amongst most Csd4 homologs, which form a distinct subfamily of CPs. Substitution of the glutamine to histidine, the residue found in prototypical zinc carboxypeptidases, resulted in decreased enzyme activity and inhibition by phosphate. Expression of the histidine variant at the native locus in a H. pylori csd4 deletion strain did not restore the wild-type helical morphology. Biochemical assays show that Csd4 can cleave a tripeptide PG substrate analog to release m-DAP. Structures of Csd4 with this substrate analog or product bound at the active site reveal determinants of peptidoglycan specificity and the mechanism to cleave an isopeptide bond to release m-DAP. Our data suggest that Csd4 is the archetype of a new CP subfamily with a domain scheme that differs from this large family of peptide cleaving enzymes.

The peptidoglycan (PG) sacculus encases the cell and is responsible for maintaining cellular shape and protecting against osmotic stress in most bacteria (1). PG is a heteropolymer of glycan chains with attached short peptides that are cross-linked to form a rigid, mesh-like structure that retains cell shape when purified (2). This PG layer undergoes constant remodeling during cell growth, requiring enzymes that cleave (PG hydrolases) and...
grow (PG synthases) the existing PG structure. The combined effect of PG modifying enzymes leads to diverse cellular morphologies, such as stars, squares and spirals (3). However, much of our understanding of cell shape determination involves well-studied organisms that are cocci, rods or vibrioids and we are only beginning to identify and understand the components involved in determining other bacterial cell shapes.

The PG glycan chains consist of alternating β-(1-4) linked N-acetylglucosamine (GlcNAc) and N-acetylmuramic acid (MurNAc). The peptide component varies in sequence depending on the species, especially for Gram(+) bacteria (4). In most Gram(-) and some Gram(+) species, the PG precursor is composed of the pentapeptide L-Ala-γ-D-Glu-m-DAP-D-Ala-D-Ala covalently linked to the MurNAc moiety (where m-DAP is meso-1,6-diaminopimelate) (1). Once attached to the nascent PG, the pentapeptide can be trimmed sequentially to a tetra-, tri- and then dipeptide by DD-, LD-, and DL-carboxypeptidases, respectively. The m-DAP and D-Ala residues from two opposing peptide stems can be directly cross-linked by DD-transpeptidases with the subsequent removal of a terminal D-Ala resulting in slightly curved or straight rods that can be directly cross-linked by DD-transpeptidases (5,6). Periplasmic DD-carboxypeptidases are well characterized in many bacteria (5), a closely related human diarrheal pathogen, revealed that deletion of the csd4 homolog, pgp1, led to a similar straight rod phenotype with impaired chick colonization ability, a model representing the natural reservoir of this pathogen (9). HPLC analysis of H. pylori Δcsd4 PG sacculi or purified disaccharide-linked tripeptide digested with Csd4 demonstrated production of a disaccharide dipeptide (i.e. DL-carboxypeptidase activity) (8).

Sequence analysis suggests that H. pylori Csd4 contains an N-terminal zinc-containing carboxypeptidase domain of the M14 family and a C-terminal region with unpredicted structural features. Zinc hydrolases have been grouped into over 30 families by Barrett, Rawlings and Woessner (12). Family M14 consists of the carboxypeptidases, with the prototypical member being carboxypeptidase A (13). One hallmark feature of this family is the zinc binding motif HXXE+H (+H2O). Zinc coordination lowers the pKα of the bound catalytic water (14). A second glutamate residue forms a H-bond to the catalytic water molecule and is proposed to abstract a proton forming a hydroxide ion that undergoes a nucleophilic attack on the bound substrate (15). In this study, we show that Csd4 is a DL-carboxypeptidase with a modified zinc binding site containing a glutamine residue in place of a conserved histidine. To elucidate the hydrolytic mechanism, we have solved crystal structures of Csd4 with bound peptide substrate or product. Crystallographic studies, biochemical assays and bacterial morphological studies of mutant strains implicate a key role of the glutamine ligand of the active site zinc ion in the formation of helical cell shape. Based on sequence analysis of Csd4 homologs, we propose that Csd4 is the archetype of a new CP subfamily.

**EXPERIMENTAL PROCEDURES**

**Bacterial strains and growth conditions** – Strains used in this work, as well as primers and plasmids used in strain construction are described in Table 1. H. pylori were grown in Brucella broth (BB) with 10% fetal bovine serum (Gibco) without antimicrobials or on horse blood (HB) agar plates with antimicrobials as described (2). Bacteria were incubated at 37°C under microaerobic conditions in a trigas incubator (10% CO2, 10% O2, and 80% air). For resistance marker selection HB plates were supplemented with 15 μg ml⁻¹ chloramphenicol (Cm), 25 μg ml⁻¹ kanamycin or 60 mg ml⁻¹ sucrose. For plasmid selection and maintenance in E. coli, cultures were

---

**Note:** The content is a continuation of a scientific paper discussing the role of Csd4 in Helicobacter pylori and its implications on bacterial cell morphology. The text provides detailed descriptions of experimental procedures and findings related to the study of Csd4 and its potential role in bacterial cell shape determination. The paper references various studies and organisms, including H. pylori and C. jejuni, and discusses the mechanisms by which these organisms maintain their cellular structures. The document emphasizes the importance of understanding the components involved in cell shape determination and the role of zinc hydrolases in this process.
Helicobacter pylori Csd4 requires a glutamine zinc ligand

routinely grown in Luria-Bertani (LB) broth or agar at 37°C supplemented with 100 µg ml⁻¹ ampicillin.

Synthesis of tripeptide substrate – Chemicals were purchased from Aldrich Chemical Co., Alfa Aesar Co. or Fisher Scientific. Ion exchange resin was from Aldrich Co. and Bio-Rad Laboratories. Silica gel (230-400 mesh) was obtained from Silicycle Inc. The TLC silica gel (aluminum sheets) was from EMD Chemical Inc. CH₂Cl₂, MeOH and TEA were distilled under Ar from CaH₂. ¹H NMR and proton-decoupled ³¹P NMR was recorded on a Bruker AV400dir spectrometer or a Bruker AV400inv spectrometer at field strength of 400 MHz and 162 MHz, respectively. Mass Spectra obtained on a Waters 2965 HPLC-MS.

Ac-L-Ala-D-Glu(OtBu)-OH (Compound 1) (Fig. 1). To a solution of N-Acetyl-L-alanine (131 mg, 1.0 mmol) in DMF (10 ml), was added HOBt (135 mg, 1.0 mmol) and HBTU(380 mg, 1.0 mmol) and the mixture was stirred for 30 min at RT. D-Glu(OtBu)-OH (203 mg, 1.0 mmol) and DIPEA (0.15 ml, 1.0 mmol) were added and the reaction was stirred for another 2h at RT. The solution was evaporated to dryness under reduced pressure, and was extracted with H₂O/EtOAc. The resulting residue was purified by chromatography on silica gel (4% MeOH in CH₂Cl₂) to give compound 1 as a colorless oil (256 mg, 54%). ¹H NMR (400 MHz, MeOD) δ 4.46 – 4.25 (m, 2H), 2.52 – 2.30 (m, 2H), 2.23 – 2.10 (m, 1H), 1.99 (s, 3H), 1.96 – 1.87 (m, 1H), 1.48 (s, 9H), 1.35 (d, J = 6.8 Hz, 3H). MS (ESI) (m/z) 339.4 [M+Na]⁺.

Compound 2. To a solution of Ac-L-Ala-D-Glu(OtBu)-OH (Compound 1, 72 mg, 0.23 mmol) in DMF (5 ml), was added HOBt (34 mg, 0.25 mmol) and HBTU(96 mg, 0.25 mmol), and the mixture was stirred for 30 min at RT. Mono-Boc-protected meso-Dap dibenzyl ester (Compound 2, 98 mg, 0.21 mmol) and DIPEA (27 mg, 0.21 mmol) were added, the reaction was stirred for another 2 h at RT. Compound 2 was prepared as a mixture of two regioisomers according to a literature known method (16). The solution was evaporated to dryness under reduced pressure, and was extracted with H₂O/EtOAc. The organic layer was dried over Na₂SO₄, and evaporated to dryness under reduced pressure. The resulting residue was purified by chromatography on silica gel (4% MeOH in CH₂Cl₂), but the ¹H NMR spectrum showed that the fraction containing compound 2 still included significant amount of impurities. Therefore the impure compound 2 fraction was used for the next step without further purification. MS (ESI) (m/z) 791.5 [M+Na]⁺.

Compound 3. To a solution of compound 2 (70 mg, 0.91 mmol) in MeOH (20 ml) was added Pd/C (10%, 50 mg). The resulting mixture was stirred under hydrogen gas (1 atm) for 5 h, and then filtered through celite. The filtrate was evaporated in vacuo and dried under reduced pressure to give compound 3 as a colorless oil. The crude oil was dissolved in NaHCO₃ solution (10%) and the pH was adjusted to 8.0. This was loaded onto a column of AG 1-X8 resin (formate form, 100-200 mesh, 5 ml). The column was washed with water (50 ml) and formic acid (0.5 M, 100 ml). The fractions containing the product were combined and evaporated to dryness in vacuo to give compound 3 (mixture of two diastereoisomers) as a colorless oil (55 mg, 43% over 2 steps). ¹H NMR (400 MHz, MeOD) δ 4.41 – 4.32 (m, 2H), 4.30 – 4.18 (m, 2H), 2.71 (m, 2H), 2.38 – 2.30 (m, 2H), 2.02 (s, 3H), 1.90 – 1.84 (m, 4H), 1.78 – 1.63 (m, 2H), 1.48 (s, 9H), 1.45 (s, 9H), 1.40 – 1.35 (m, 3H). MS (ESI) (m/z) 611.3 [M+Na]⁺.

Ac-L-Ala-iso-D-Glu(OH)-meso-Dap-OH (Tripeptide Substrate). Compound 4 (55 mg, 0.91 mmol) was dissolved in TFA (9.5 ml)/H₂O (0.5 ml). The resulting solution was stirred for 3h at RT, and then was evaporated in vacuo. The residue was redissolved in H₂O (2.0 ml) and the pH of the solution was adjusted to 8 by adding NaHCO₃ (0.5 M). This was loaded onto a column of AG 1-X8 resin (formate form, 100-200 mesh, 5 ml). The column was washed with water (50 ml) and formic acid (0.5 M, 50 ml), and then was eluted by formic acid (4.0 M, 100 ml). The fractions containing the compound were combined and evaporated to dryness in vacuo to give the tripeptide substrate (mixture of two diastereoisomers) as a colorless oil (28 mg, 71%). ¹H NMR (400 MHz, MeOD) δ 4.40 (m, 3H), 4.12 – 3.94 (m, 1H), 2.49 – 2.28 (m, 3H), 2.27 – 2.16 (m, 1H), 2.08 – 2.03 (m, 2H), 1.99 (s, 3H), 1.99 – 1.86 (m, 2H), 1.85 – 1.72 (m, 1H), 1.67 – 1.52 (m, 1H), 1.44 – 1.35 (m, 3H). MS (ESI) (m/z) 433.4 [M+H]⁺.
Cloning and recombinant expression of Csd4 – Wild-type Csd4, consisting of the native H. pylori strain G27 sequence (HPG27_353) minus the first 20 N-terminal signal sequence residues, three Csd4 active-site variants (Q46H, Q46A and Q46E) and Corynebacterium glutamicum meso-diaminopimelate dehydrogenase (DAPDH) were used in this study. Cloning of csd4 into pET15 vector for recombinant expression has been described previously (8). Gln46 variants were made in pET15-csd4 using the Agilent QuickChange XL kit with primers A1-A6 described in Table 1 and verified by sequencing. All Csd4 variants were overexpressed in E. coli strain BL21(DE3). Each 1 L culture was inoculated with 2 ml of overnight culture and incubated at 37 °C with shaking. When the cell density reached an optical density at 600 nm of ~0.9, the temperature was reduced to 20 °C for 30 min and then induced with 0.25 mM isopropyl-β-D-thiogalactopyranoside (IPTG) and grown overnight. The cells were pelleted, resuspended and lysed at 4 °C in binding buffer (25 mM Tris pH 7.5, 500 mM NaCl and digested with thrombin overnight (500:1 w/w Csd4:thrombin ratio) at 4 °C to remove the His-tag. Benzanidine beads were used to remove the thrombin and the protein was further purified by gel-filtration chromatography (Superdex 200 16/60 column) equilibrated with 20 mM Tris pH 7.5, 500 mM NaCl. Q46H Csd4 used for crystallization was not additionally purified by gel-filtration but was buffer exchanged by ultrafiltration. The protein concentration was calculated using an extinction coefficient value of 36190 M⁻¹ cm⁻¹ for folded Csd4 protein (εapprox). εapprox was derived using the Beer-Lambert law and UV absorption values of Csd4 denatured in 6 M guanidium hydrochloride and predicted extinction coefficient values (εapprox) (17). Protein samples were then concentrated to ~15-20 mg ml⁻¹ and flash frozen in liquid nitrogen. Recombinant DAPDH protein was expressed and purified from E. coli BL21(DE3) (vector was a generous gift from Lilian Hor and Matthew Perugini, La Trobe University, Australia) as previously described (18).

Construction of H. pylori strains expressing tagged and mutant Csd4 – Plasmids containing wild-type and mutant derivatives of csd4 fused to the 3x-Flag epitope were used for complementation and creation of merodiploid strains, and are described in Table 1. To generate pKB20, the csd4 gene and the 3x-flag epitope were individually amplified by PCR (Phusion, NEB) using TRH1 (csd4:4xFLAG) genomic DNA as template, with oligos 53/54 (csd4) and 55/56 (3xFlag) which contained XhoI and EcoRI sequences appended. Strain TRH1 was generated using PCR SOEing (19) to add the 3x-FLAG sequences and a stop codon right before the endogenous csd4 stop codon, which was then used to replace csd4::catsacB in LSH18 using counter selection (8,20,21). The vector pLKS2 (8) was digested with XhoI and EcoRI (NEB) and gel purified (Qiagen). The vector backbone and purified PCR fragments were ligated together using the HD In-Fusion cloning kit (Clontech). Point mutant derivatives of csd4 were generated using a modified quick-change procedure (Stratagene) with the pKB20 master vector as template and oligos 94 (Q46A) and 95(Q46H). To generate pKB27 and pKB29, the csd4 truncation alleles and the 3xFlag epitope were individually amplified using pKB20 as template with primers 85/86 (Csd41-251), 85/87 (Csd4251-343) and 89/90 (3xFlag). The csd4 alleles and 3x-flag were gel purified and stitched together by PCR SOEing (19) using primers 85/90 which contained KpnI and SacI sequences appended. The vector pKB20 and the stitched PCR products were digested with KpnI and SacI and gel purified. The vector backbone and PCR fragments were ligated together using standard T4 ligase-based methodology and verified by Sanger sequencing of candidate clones (FHCRG Genomics Shared Resource). To generate pKB44 and pKB46 for integration of mutant alleles at the rdxA locus, the csd4-3xFlag alleles were PCR amplified from KBH33 (Csd4 Q46H:3xFlag) and KBH42 (Csd4 Q46A:3xFlag) genomic DNA using primers 120/121 with BamHI and EcoRI sequences appended, digested with BamHI and EcoRI and gel purified. The vector pLC292 (22) was digested with BamHI and EcoRI (NEB) and gel purified (Qiagen). The vector backbone and purified PCR
fragments were ligated together using standard T4 ligase-based methodology and verified by Sanger sequencing of candidate clones (FHCRC Genomics Shared Resource). Recipient *H. pylori* bacteria containing a *csd4::catsacB* (LSH18) or *rdxA::kansacB* (LSH108) cassette (LSH18) were transformed with 2-4 μg of the appropriate plasmid DNA (Qiagen) containing DNA sequences homologous to regions flanking the *rdxA::kansacB* or *csd4::catsacB* cassette. Genomic DNA was isolated from candidate clones and verified by Sanger sequencing of PCR amplified *csd4* (FHCRC Genomics Shared Resource).

**Crystallization and structure determination** – Initial phasing by molecular replacement using available structures of distantly-related carboxypeptidases did not lead to interpretable maps. Instead, a crystallization condition containing iodide was optimized to employ de novo phasing. The final structures of wild-type *Csd4* and its variants were crystallized in the P212121 space group using reservoir solution containing 16-20% PEG 3350, 0-100 μM Tris pH 8 and 0.3-0.4 M sodium iodide by hanging drop vapor diffusion. Crystals appear within a few days at room temperature but were allowed to grow to a sufficient size for approximately 2 weeks. The crystal structure was initially determined to 2.1 Å by single wavelength iodide phasing using data collected at the UBC Astrid X-ray homesource (1.54 Å wavelength) at 100K. Data was processed and phased using HKL3000 (23) (*Csd4-initial* in Table 2). The anomalous substructure determined by SHELXD (24) contained 14 iodide sites. A combination of autobuilding with ArpWarp (25) and manual rebuilding using Coot (26) were used to complete the structure, which included the manual placement of additional iodide sites. Zinc-containing crystal structures of WT and Q46H *Csd4* were obtained by sequentially soaking the crystals in freshly prepared 3% increments of PEG 3350, but with each soaking solution also supplemented with 1 mM ZnCl2, followed by a 30 min soak in the 33% solution supplemented with 2.5 mM tripeptide and 1 mM ZnCl2. An equal volume of 33% soaking solution supplemented with 4 mM ZnCl2 was then added and soaked for another 9 min, followed by a quick soak in the same cryoprotectant and flash frozen. Native (0.98-1.00 Å) and zinc-anomalous (1.26-1.28 Å) wavelength datasets on derivitized crystals were collected at the Canadian Lightsource beamlines 08B1-1 and 08ID-1 at 100 K and processed using XDS (27). The original *Csd4* structure was then used as a starting point for direct refinement using PHENIX and manual rebuilding with Coot. Poor electron density precluded modelling of the following loop region on domain 3: residues 389-394 (TriZn- *Csd4*); residues 389-392 with Lys393 modeled as Ala (Q46H-Csd4 and Zn-Csd4). All structures have excellent stereochemistry, with 96.9-97.4% of residues in the favored region of the Ramachandran plots and no outliers. The atomic coordinates for the crystal structures of Apo- *Csd4*, Zn- *Csd4*, TriZn- *Csd4* and Q46H-Csd4 are available in the Research Collaboratory for Structural Bioinformatics Protein Databank under PDB # 4WCK, 4WCL, 4WCN and 4WCM, respectively.

**Sequence alignments** – Homologs of *Csd4* were identified from the non-redundant database at the National Center for Biotechnology Information utilizing BLASTP (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and an E-value cutoff of 1×10-7. Identical protein sequences derived from different strains of the same species and proteins with short alignment coverage were removed. The sequences were aligned with Clustal Omega (28) and a bootstrapped tree (with 500 replicates, subtree pruning and regrafting and five random starts) was generated using PhyML (29) within Seaview (30). The aligned sequences were also used to generate Fig. 2C using Consurf (31) to identify the degree of amino acid conservation.

**UV-Visible spectroscopy** – *C. glutamicum* DAPDH was used to determine the cleavage rates by *Csd4* and its variants by consuming the predicted product of the *Csd4* reaction, m-DAP, and NADP+. The consumption of NADP+ to produce NADPH is detectable as an increase in absorbance at 340 nm. To determine the activity profile of DAPDH over various pH values, various
concentrations of DAPDH was incubated with 100 mM buffer (Bis-tris pH 5.6 and 6.5, MES pH 6, and Tris pH 7.5 and 8), 500 mM NaCl, 0.3 mM NADP$^+$ and 1.2 mM m-DAP. Due to differing DAPDH activity at varying pH, an uncoupled assay was used to examine the pH effects on Csd4 activity. 5 μM Csd4 was incubated in 20 mM Na/K phosphate (pH 4.8, 5, 5.5, 6, 6.5, 7, 7.5 and 8), 500 mM NaCl and 7 μM EDTA for 5 min at 30 °C, followed by the addition of 17 μM ZnCl$_2$ and incubating for another 5 min. The reaction was then initiated by the addition of 1 μM tripeptide, incubated for 20 min and stopped by boiling the sample for 10 min at 98 °C. The sample was then centrifuged to remove precipitated protein, mixed with Tris pH 8 (100 mM final concentration), 2.5 mM NADP$^+$ and 2.5 μM DAPDH and the relative activity was determined based on final absorption values at 340 nm. Assays that coupled the reactions of Csd4 and DAPDH involved 100 mM buffer (Na/K phosphate or Bis-tris pH 6.5), 0.5 mM NaCl, 0.25 mM ZnCl$_2$, 2.5 mM NADP$^+$, 25 μM DAPDH and 5 μM Csd4. After 10 min incubation at 30 °C, the reaction was initiated by adding 1 mM peptide substrate. An initial lag phase is observed due to the coupling of detection by DAPDH consumption of NADP$^+$ to the production of m-DAP by Csd4. Reaction rates were calculated using the maximal slope of the change in A$_{340}$ and the molar absorptivity $\varepsilon_{340}$ values.

**H. pylori morphological analyses** – Phase-contrast microscopy was performed as described (2) and resulting images were thresholded using the ImageJ software package. Quantitative analysis of threshold images of bacteria (300-350 cells/strain) to measure side curvature and central axis length was performed with the CellTool software package as described (2). Side curvature is the reciprocal of the radius of a circle tangent to a curve at any point; as such, a straight line has zero curvature while a point on a bent line has a curvature proportional to the tightness of the bend. Cell length was estimated using the central axis length calculated by CellTool. Statistical comparison of cell shape distributions were performed using a CellTool module that calculates a bootstrap distribution of Kolmogorov–Smirnov (KS) statistics, as described (8).

**Immunoblotting** – *H. pylori* whole-cell extracts were prepared by harvesting log-phase grown bacteria by centrifugation, 2 min max speed in a microcentrifuge and resuspending in 2x sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer at 10.0 optical density (600 nm) per ml and boiled for 10 min. Proteins were separated by 12.5% SDS-PAGE and transferred onto PVDF membranes using a semidry transfer system (I-blot; Invitrogen) according to the manufacturer’s instructions. Membranes were blocked for 1 h at RT or overnight at 4°C with 5% non-fat milk-Tris-buffered saline with Tween 20 (TBS-T; 0.5 M Tris, 1.5 M NaCl, pH 7.6, plus 0.05% Tween 20), followed by incubation overnight at 4°C with primary antibody at 1:2500 dilution for anti-Flag M2 (Sigma) or 1:20,000 dilution for anti-Cag3, in TBS-T (10). Four 10 min washes with TBS-T were followed by a 1 h incubation at RT with appropriate horseradish peroxidase-conjugated anti-immunoglobulin G (Santa Cruz Biotechnology) antibody at 1:20,000 dilution in TBS-T (anti-rabbit for Cag3 blots, anti-mouse for 3xFlag blots). After four more TBS-T washes, antibody detection was performed with ECL Plus immunoblotting (Cag3) or Millipore Immobilon (3xFlag) detection kits, following the manufacturer’s protocol (GE Healthcare).

**RESULTS**

**Domain structure of Csd4** – The 1.40 Å resolution Csd4 crystal structure reveals a monomer with an N-terminal carboxypeptidase domain, followed by two smaller domains of unknown function (domains 2 and 3; Fig. 2A; Table 2). The CP domain, consisting of residues 1-251 of the native sequence, is globular with a mixed $\alpha/\beta$ fold containing a 9-stranded antiparallel $\beta$-sheet core sandwiched by groups of 4 and 5 $\alpha$-helices. A structural similarity search using DaliLite (32) found significant similarity between the CP domain and the family of funnelin-type carboxypeptidases (e.g. human carboxypeptidase B2, PDB ID: 3LMS, Z-score 19.1, r.m.s.d. 2.3 Å over 192 aligned residues), a family of metallopeptidases that exhibits CP activity (15). Although the overall funnelin fold was conserved, sequence identities with Csd4 are under 20% over the aligned regions. Domains 2 (residues 252-343)
and 3 (residues 343-438) consist primarily of β-
strands with one and two half-turn helices, re-
spectively, in an immunoglobulin-like fold (Fig.
2A). Neither domain 2 nor 3 shares significant
structural similarity with components found in
other carboxypeptidases. Instead, domain 2 shares
low structural similarity to a binding domain of
human RhoGDI (PDB ID: 1HH4, Z-score 3.2,
r.m.s.d. 2.9 Å over 65 residues). Domain 3 shares
some structural similarity to the periplasmic, non-
sugar bound domain of a heparin-sensing two
component system (BT4663) of the human gut
symbiont Bacteroides thetaiotaomicron (PDB ID:
4A2M, Z-score 4.9, r.m.s.d. 2.9 Å over 79
residues). Due to weak structural and sequence
similarity, domains 2 and 3 are not likely to have
similar functions as their top Dali hits. Instead, we
predict that these domains participate in protein-
protein or protein-PG interactions.

Domains 2 and 3 are required for stable
Csd4 expression as replacement of the endogenous
csd4 gene with a 3x-Flag-tagged allele truncated at
the end of either domain 1 or 2 at the native locus
resulted in no detectable expression within whole
cell lysates (using an anti-Flag antibody) and
showed a straight rod phenotype similar to the null
allele (Fig. 3). In contrast, replacement with full
length Csd4 containing the same 3x-Flag epitope
revealed the robust expression of the epitope-
tagged full length allele and showed normal
morphology. From inspection of the crystal
structures, the two domain interfaces (~945 Å
between domains 1 and 2; ~820 Å between 2 and
3) are largely hydrophobic, suggesting that both
domains are required to stabilize the protein within
cells.

**PG peptide binding to the active site of
Csd4** – In contrast to all other characterized
members of the CP family, the catalytic zinc site
of Csd4 consists of Glu, His and an atypical Gln
residue. Zn
2+ was soaked into Csd4 crystals to
obtain a structure with zinc bound at 90% occu-
pancy (Zn-Csd4). The zinc site is situated along
the inner edge of a shallow, positively-
charged substrate-binding pocket located on the
surface of the CP domain (Fig. 2B and 2D). The
metal ion is coordinated by His128 N81 (2.0 Å),
the side-chain carboxylate of Glu49 in a
symmetric, bidentate manner (2.3/2.3 Å) and
Gln46 through the carboxamide Oε (2.2 Å) in Zn-
Csd4 (Fig. 2E and 2F). Two additional density
peaks that are best modeled as either water or
hydroxide ions (2.2 and 2.3 Å) are also observed
(Table 2), together forming an infrequently
observed six-coordinate zinc site (33).

The initial crystal structure of Csd4 (Csd4-
initial) did not contain bound metal in the active
site, as observed for other carboxypeptidases (34),
suggesting weak affinity or insufficient metal
availability during protein purification. Zinc
binding results in structural changes to the active
site ligands. His128 exhibits an 18° rotation about
Cβ to bring the imidazole ring closer to the zinc
ion. In the absence of a bound metal, Gln46 has
elevated B-factors and the carboxamide side chain
is primarily directed away from the active site.
Upon zinc binding, Gln46 reorients toward the
ezinc ion, strongly supporting formation of a
Gln46-Zn
2+ ligand interaction. No significant
displacement of Glu49 is observed.

In the substrate-binding pocket of Csd4-
initial, apo-Csd4 and Zn-Csd4, positive electron
density was observed matching the proposed
tripeptide cleavage product, m-DAP, based on
comparisons between wild-type and Δcsd4 PG
from *H. pylori* (8). As no exogenous *m*-DAP was
included during the purification or crystallization
of Csd4, affinity for *m*-DAP was sufficiently high
for co-purification from *E. coli* lysate. The *m*-DAP
forms direct interactions with Asn93, Arg94,
His126, the Zn
2+ ligand His128, Thr208 and
Glu222 (Fig. 2E and 2F). Asp91 directly forms an
H-bond to the Zn
2+ ligand Gln46 and to a water
molecule that is H-bonded to both Gln46 and the
outer carboxylate oxygen of *m*-DAP. Three
hydrophobic residues also line one side of the
substrate-binding pocket (Trp148, Ile153 and
Met203) to interact with the alkyl portion of
*m*-DAP. Additionally, a buried network of water
molecules is present that form interactions
between *m*-DAP and both Csd4 backbone and
side-chain residues.

To examine the interactions of Csd4 with
substrate, Csd4 crystal soaking experiments were
performed with Zn
2+ and a tripeptide representing
a portion of the PG substrate (Ac-L-Ala-γ-D-Glu-
*m*-DAP; Fig. 1). The substrate was synthesized as
a mixture of two stereoisomers; one with the (R)-
stereocenter of *m*-Dap attached to γ-D-Glu and one
with the (S)-stereocenter of *m*-Dap attached to γ-
D-Glu. The crystal structure of tripeptide-Zn
2+.
Csd4 complex (TriZn-Csd4) was solved to 1.75 Å (Tables 2 and 3). No significant difference in the overall fold was observed when compared to Zn-Csd4 (r.m.s.d. 0.2 Å over all Cα atoms). Electron density at the metal binding site was weaker and a zinc ion was modeled at 50% occupancy. As in Zn-Csd4, Gln46, His128 and Glu49 are coordinated to the zinc ion; additionally, a single solvent molecule with well-defined electron density is observed coordinated to the zinc and is modeled as a water at full occupancy (Table 3; Fig. 4A). This water is H-bonded to the backbone carbonyl group of Asp129 (2.8 Å) and the side chain of Glu222 (2.9 and 3.1 Å, bidentate) and is situated 3.3 Å from C7 of the tripeptide scissile bond. Therefore, it is poised to be the catalytically essential water (15), though conformational changes at the active site may be required during the catalytic process. The second zinc coordinated water molecule in Zn-Csd4 appears to have been displaced by the bound tripeptide.

The tripeptide substrate analog was modeled at full occupancy in TriZn-Csd4 (Fig. 4). Under the crystal soaking conditions, tripeptide cleavage is sufficiently impaired that the substrate and not the product was observed. Only the cleavage is sufficiently impaired that the substrate is observed in the active site indicating that the enzyme selectively bound the preferred isomer from solution. The m-DAP moiety of the tripeptide overlays with the product structure and the interactions with Csd4 are conserved (Fig. 4B). The tripeptide substrate extends outward past the binding pocket and only the carbonyl O of D-Glu provides an additional significant interaction with Arg86 (Fig. 4A and 5). Accordingly, the B-factors of the buried m-DAP moiety are low (~20 Å²) and increase towards the surface-exposed end of the tripeptide (Fig. 4C).

Plotting the degree of amino acid conservation amongst the homologs of Csd4 (see below) on the surface of the structure reveals highest conservation at the substrate binding site (Fig. 2C). Although the majority of these conserved residues are part of the extensive zinc and substrate binding network, additional conserved residues are present at the CP domain surface surrounding the active site pocket. This surface is composed primarily of loops, the largest of which consists of residues Tyr133–Trp148 that are poised to interact with the polysaccharide backbone of the PG substrate.

**Q46 is required for full Csd4 activity** – To examine the role of Gln46 in the catalytic activity of Csd4, three active site variants were constructed (Q46H, Q46A and Q46E) and assayed for m-DAP release from the tripeptide substrate. Wild-type Csd4 demonstrated highest activity in either Na/K phosphate or Bis-tris buffer (Fig. 6A). The peptidase activity of the Q46H variant was half that of wild-type in the Bis-tris-buffered system but was ten-fold reduced in the presence of phosphate. The complete loss of a zinc ligand (Q46A variant) retained ~20% of wild-type activity in phosphate but no significant activity in Bis-tris buffer. Substitution of Gln with acidic Glu resulted in no significant activity and appeared to disrupt the active site. Wild-type Csd4 had highest activity at pH 6 whereas the Q46H optimal was at pH 5.5 but was much less pH sensitive (Fig. 6B).

To explore the structural basis for altered activity by the Q46H variant, the crystal structure of Zn-bound Q46H was solved to 1.75 Å resolution (Fig. 7A). As expected, the His-His-Glu metal site bound Zn²⁺ with modest changes in ligand geometry (Table 3). A tetragonal-shaped density was observed near the zinc ion in a Fc-Fc map (Fig. 7B). Based on the presence of phosphate in the protein purification buffer, the density was modeled as a phosphate molecule at 90% occupancy. Structural alignment between TriZn-Csd4 and the Q46H variant revealed that the phosphate molecule occupies the space of the zinc-coordinated solvent molecule. Arg86 and Glu222 have rotated to form H-bonds with the phosphate group. Density for the product, m-DAP, is observed in the structure of Q46H and is situated in the same location as in the wild-type Csd4 structures.

To determine if Gln46 is required for normal helical cell shape, we generated strains of *H. pylori* expressing Q46H and Q46A variants fused to a C-terminal 3x-Flag epitope integrated at the native locus. Both mutants exhibited non-helical cell morphology consistent with a csd4 null phenotype of slightly curved or straight rods with occasional kinks or bends (Fig. 8A and 8B) (8). Western blot analysis with anti-FLAG monoclonal antibodies indicated no detectable differences in protein expression between the wild-type and mutant variants of Csd4 (Fig. 8D).
Since the Q46H mutant retained some mDAP cleavage activity in vitro, we generated a merodiploid strain expressing a second copy of csd4 Q46H-3xFlag (KBH60) at the rdxA locus (used for csd4 complementation in previous studies) (8) to explore whether overexpression of the Q46H variant might rescue helical shape. While we observed a 2.4 fold higher protein expression in the strain expressing two copies of csd4Q46H (Fig. 8D), we saw no restoration of helical morphology (Fig. 8A and 8B). In contrast, a strain expressing two copies of the Q allele (KBH65) supports normal morphology in most cells (Fig. 8A), but has an increased population of straight cells that have side curvature values less than 4 compared to the strain with a single copy of the Q allele (Fig. 8B), as was reported previously (10). The perturbation of cell shape during overexpression was suggested to result from a requirement of precise asymmetric localization of Csd4 activity to generate proper helical curvature. In this model, loss of expression prevents the induction of curvature while extra protein expression at additional sites may break asymmetry and again lead to loss of curvature. We then created a strain expressing the wildtype csd4 allele at the native locus and the csd4Q46H allele at rdxA (KBH66), and observed a dominant-negative effect of the csd4Q46H allele with a complete loss of helical cell morphology (Fig. 8A and 8B). This unexpected result may suggest that in cells Csd4 acts cooperatively in a complex. Previously we observed an increase in cell length in a wildtype csd4 merodiploid strain (10). Interestingly, all strains containing two copies of csd4 show increased cell length regardless of whether one or both copies contain Q46H (Fig. 8D). Taken together with the in vitro activity data, these results suggest that the Q46H variant is unable to generate helical cell morphology due to a perturbation of enzyme activity but enzymatic activity is not required for Csd4’s effects on cell length (Fig. 8C).  

Csd4 as an archetype for a new family of CPs – A sequence analysis of Csd4 homologs was performed to identify conserved features. Bacterial homologs containing all three domains of Csd4 were identified based on a sequence similarity search; the sequences were aligned and a phylogenetic tree was constructed (Fig. 9 and 10). Homologs were identified primarily within the δ and ε Proteobacteria, Deferrribacteres, and Aquificae (9). Although primarily found in helical- or curved rod-shaped organisms, homologs of Csd4 were identified in a number of rod-shaped bacteria, including ones isolated from deep sea hydrothermal vents and coastal sediments. Homologs of Csd4 generally cluster based on bacterial class and by the presence of the Gln zinc ligand versus an equivalent His. However, Helicobacter homologs fall into two distinct branches. The first branch includes H. pylori and other ε-Proteobacteria (Group “Epsilon – Q”). All members of this branch contain a Gln at position equivalent to Gln46 except for Helicobacter hepaticus and Helicobacter cinaedi, which contain a histidine but are still helical. Additionally, five of these Helicobacter species, including H. hepaticus and H. cinaedi, contain an extended C-terminal region of approximately 200-300 residues as compared to Csd4. The second branch contains ε-Proteobacteria and have the His variation at position 46 (“Epsilon – H”).  

Whether the Csd4 homologs in those organisms play an active role in determining their shape is not currently known. The zinc ligands Glu49 and His128 are absolutely conserved amongst the identified Csd4 homologs. Although Gln46 is required for full activity and helical shape of H. pylori, approximately one quarter of the species identified have a histidine in the equivalent position. All of the bacteria isolated from deep sea vents, whether they belong to the phylum Aquificae or are ε-proteobacteria, have such a histidine. Although His46-containing organisms include spiral-shaped species, the majority of deep sea vent isolates appear to be rod-like; however, whether these species have classical rod morphology is unknown.  

DISCUSSION  
Our product and substrate-bound crystal structures with an active site zinc gives further insight into cell wall substrate recognition and hydrolysis by enzymatically active Csd4 and expands our knowledge on the larger family of related funnelin CPs (15). In the Csd4 resting state, as represented by the Zn-Csd4 crystal structure, two water molecules are bound to the active site zinc. Substrate binding (i.e. TriZn-
Csd4) displaces one of these two water molecules, leaving the other water positioned for nucleophilic attack of C7 of the substrate scissile amide bond. Glu222 is the conserved glutamate positioned to act as the essential general base that abstracts a proton from the catalytic water to enhance nucleophilic attack on the amide carbonyl carbon (C7). Both zinc and Arg86 are positioned to stabilize a negatively charged gem-diolate intermediate that is proposed in the generally accepted mechanism of CPs (15). In contrast to other funnelins, no Tyr residue is present to H-bond to the amide nitrogen. Protonation of the amide nitrogen by Glu222 is associated with cleavage of the peptide bond. The PG dipeptide moiety appears to easily dissociate; however, the m-DAP remains bound and is likely displaced by the next PG tripeptide to repeat the cycle. Typically, funnelin family CPs hydrolyze the C-terminal residues of folded proteins in contrast to the isopeptide bond of the PG tripeptide. The unique structural features in Zn-Csd4 and TriZn-Csd4 may be a consequence of interacting with PG as the substrate. Recently, the crystal structures of muramyltripeptide and m-DAP bound Csd4 have been solved with calcium in the active site (35). As expected, the sugar moiety was not observable in the electron density (PDB ID:4Q6N).

Most residues that bind the catalytic zinc or that interact with the substrate are highly conserved amongst the homologs of Csd4. Glu222 is absolutely conserved in accordance with its proposed direct role in the Csd4 reaction mechanism. Moreover, mutation of Glu222 in Csd4 alters H. pylori cell shape (8). Further insight into the Csd4 reaction may be gained by monitoring kinetics of residue variants, substrate analogs, and inhibitors such as phosphate. The two notable exceptions to the strict conservation of active site residues are the zinc ligand Gln46 and His126. His126 interacts with m-DAP and is often replaced by polar uncharged residues, which may fulfill the same role. In some Csd4 homologs Gln46 is substituted by His, as observed in most funnelin CPs. Although the substitution of Gln to His requires a single point mutation, this residue is required for function and is largely conserved within branches of the Csd4 family. Additionally, the specific spatial positioning of a glutamine at position 46 appears to be required, since glutamine is not found in place of the other histidine zinc ligand, His128.

The binding of phosphate at the zinc site in the structure of the Q46H variant may be due to structural similarity to the tetrahedral gem-diolate intermediate of the catalytic cycle. The rearrangement of Arg86 and Glu222 to accommodate this phosphate molecule may reflect the role of these residues in stabilization of the intermediate formed during normal catalysis. The observed inhibition of the activity of the Q46H variant by phosphate together with phosphate bound in the variant crystal structure suggest that coordination of the zinc by Gln in Csd4 may serve to prevent phosphate inhibition. Notably, phosphate was observed bound to Bacillus subtilis LdcB (34) and Streptococcus pneumoniae DacB (36), two LD-carboxypeptidases that remove the fourth amino acid from PG peptides and have a His-His-Asp zinc-binding motif. Although LD-CPs are also found in H. pylori (Csd6) (10) and C. jejuni (Pgp2) (37), neither are related to the Gram(+) LD-CPs, nor to DL-CPs such as Csd4.

Gln is required for Csd4 function, yet is an uncommon zinc ligand. Zinc ligands play a key role in modulating the pKa and nucleophilicity of the bound catalytic water and therefore the activity of the enzyme. Gln is a polar amino acid that has a similar size and chemical properties as histidine; yet substitution of Gln46 by His results in the loss of both CP activity and in vivo function. Zinc binding sites in proteins are typically formed by His, Cys, Glu and Asp residues (33). A previous analysis in the PDB database found 6200 zinc-containing sites (38). From this list (provided by C. Andreini as a Personal Communication), we have found 12 wild-type entries, representing 6 proteins that are observed with Gln as a zinc ligand, none of which are peptidoglycan peptidases (Table 4). One well-characterized example is human glyoxalase I, an unrelated zinc enzyme with a zinc site composed of a His, two Glu and a Gln whereas glyoxalase I from other organisms have a second His ligand instead of Gln. A Q33E variant of human glyoxalase I was not examined and therefore whether a similar deleterious effect as the Q46H substitution in Csd4 is not clear.
Typical to the members of the M14 family, the tripeptide binding pocket of Csd4 forms a characteristic cul-de-sac that determines substrate length and specificity. At the bottom of this pocket in Csd4 is Thr208, an absolutely conserved residue that forms a H-bond to the terminal amine of the m-DAP moiety through the side chain Oγ (3.0 Å). The equivalent position is responsible for substrate specificity in other CPs. An example is subfamily M14A which is further subdivided into A-type (e.g. carboxypeptidase A) and B-type (e.g. carboxypeptidase B) enzymes (15). In A-type CPs, Thr208 is replaced by small hydrophobic residues to preferentially interact with aromatic or small aliphatic side chains whereas B-type CPs, which prefer substrates terminating in basic amino acids, have a negatively charged residue at the position equivalent to 208. The presence of Thr208 rather than an acidic residue like in B-type CPs in part explains the lack of detectable activity on hippuryl-Lys, a chromogenic substrate commonly used to assay the activity of B-type carboxypeptidases (data not shown). The Csd4 substrate binding pocket is designed to accommodate the stem tripeptide portion of PG, but does not have sufficient space for a fourth amino acid, explaining the lack of activity on a disaccharide tetrapeptide substrate analog reported previously (8).

Csd4 shares similar substrate preferences to members of subfamily M14C, characterized by two bacterial CPs involved in the cleavage of murein-derived substrates: endopeptidase I from Lysinibacillus sphaericus and the E. coli murein peptide amidase (MpaA). Though both enzymes are CPs and have a canonical zinc binding motif, neither has detectable sequence similarity to Csd4 by BLAST analyses (E-values > 0.1). Endopeptidase I cleaves m-DAP-D-Ala from the PG tetra-peptide (DL-endopeptidase activity) and also m-DAP from the tri-peptide (DL-carboxypeptidase activity) (40). The CP domain of endopeptidase I is preceded by two tandem copies of a LysM domain predicted to bind PG. Conversely, MpaA is a single domain cytoplasmic CP involved in the catabolic usage of murein-derived peptides as a nutrient source. Unlike Csd4, MpaA cleaves murein tripeptides but has little to no activity on MurNAc-tripeptides and tetrapeptides (41). The structure of the Vibrio harveyi MpaA homolog (solved only in the apo form) shows overall homology to other CPs including Csd4 but contains an extra loop region over the substrate binding site that is proposed to determine the preference for small, sugarless substrates. The absence of such a loop in Csd4 is consistent with its ability to interact with the large intact PG sacculus.

Csd4 is a structurally unique CP that hydrolyzes tripeptides to modify PG. We have shown that Csd4 contains an M14-type domain and has the requisite DL-carboxypeptidase activity to release m-DAP using a zinc site with a Gln ligand. Interestingly, the MEROPS database of CPs (12) currently lists Helicobacter as a genus lacking any members of the M14-type of CPs and a BLAST search of the Csd4 sequence against this database yields CP sequences with only weak significance scores (E-values > 1×10⁻³). In addition, Csd4 and its close homologs contain two additional C-terminal domains that are unlike those found in the other characterized M14 subfamilies. Therefore, we propose that Csd4 be defined as the prototypical member of a new subfamily tentatively named M14E.

Open questions include how Csd4 functions within the context of the bacterial cell. The C-terminal domains play a role in Csd4 stability within the bacterial cell but may have additional functions in coordinating Csd4 localization through interactions with other shape determining proteins or the PG itself. While overexpression of the Q46H variant did not support helical cell shape generation, it did promote the increased cell length previously observed during overexpression of wild-type Csd4 (10), suggesting additional functions for the protein that do not require enzymatic activity. H. pylori Csd3, an unrelated M23B family metallopeptidase, was shown to have DD-carboxypeptidase activity and can produce tetrapeptides from uncross-linked pentapeptides (42). In turn, the tetrapeptide-cleaving LD-carboxypeptidase Csd6 was recently shown to provide the tripeptide substrate for Csd4. Overexpression (as well as loss) of either Csd4 or Csd6 perturbs helical morphology, thus peptide hydrolysis by Csd4 to achieve a helical bacterial shape is likely to require precise spatial coordination within the PG layer (10).
Helicobacter pylori Csd4 requires a glutamine zinc ligand

REFERENCES

Helicobacter pylori Csd4 requires a glutamine zinc ligand


Acknowledgements – Research described in this paper was performed using beamlines 08B1-1 and 08ID-1 at the Canadian Light Source, which is supported by the NSERC, the National Research Council Canada, the Canadian Institutes of Health Research, the Province of Saskatchewan, Western Economic Diversification Canada, and the University of Saskatchewan.

FOOTNOTES

*This work was supported by Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery grants to M.E.P.M and M.E.T., and a Canadian Institutes of Health Research operating grant (MOP-68981) to E.G. This material is based upon work supported by the US National Institutes of Health under award numbers RO1A1094839 and T32CA009657 and by the National Science Foundation Graduate Research Fellowship to K.B. under Grant No. DGE-1256082. Support for infrastructure for structural biology was provided by the Canadian Foundation for Innovation to M.E.P.M. The content is solely the responsibility of the authors and does not necessarily represent the official views of the funding agencies.

1To whom correspondence should be addressed: Michael E. P. Murphy, Department of Microbiology and Immunology, 2350 Health Sciences Mall, Life Sciences Centre, The University of British Columbia, Vancouver, BC, Canada, V6T 1Z3; Telephone: (604) 822-8022; Fax: (604) 822-6041; E-mail: michael.murphy@ubc.ca

The abbreviations used are: PG, peptidoglycan; CP, carboxypeptidase; Cm, chloramphenicol; LB, Luria-Bertani; DAPDH, diaminopimelate dehydrogenase; IPTG, isopropyl-β-D-thiogalactopyranoside; KS, Kolmogorov–Smirnov; TBS-T, Tris-buffered saline with Tween 20.

The atomic coordinates for the crystal structures of Apo-Csd4, Zn-Csd4, TriZn-Csd4 and Q46H-Csd4 are available in the Research Collaboratory for Structural Bioinformatics Protein Databank under PDB #4WCK, 4WCL, 4WCN and 4WCM, respectively.
FIGURE LEGENDS

FIGURE 1. The synthetic scheme for the tripeptide substrate.

FIGURE 2. The crystal structure of Csd4 (PDB ID: 4WCL). (A) The overall monomeric structure of Csd4 with Zn$^{2+}$ and m-DAP bound. The catalytic domain and domains 2 and 3 are colored blue, grey and green, respectively. (B) Overall and (D) active-site magnified electrostatic surface potential of Csd4 contoured at $\pm 3 k_B T/e$. Electropositive regions are colored blue; electronegative regions are colored red; position of buried Zn$^{2+}$ indicated with a star. (C) Distribution of conserved residues mapped onto the surface of Csd4. Most conserved regions are colored blue; least conserved is colored red. (E) 2D interaction map between Csd4 and the product m-DAP (light gray). The predicted catalytic water highlighted in black font. (F) Corresponding Zn-Csd4 active site with key ligands and an omit $F_o-F_c$ difference density map for the density of the bound m-DAP product contoured at 3 $\sigma$.

FIGURE 3. Complementation and western blot analysis of Csd4-3xFlag control and Csd4 C-terminal truncations. Strains used: LSH18 (∆csd4), LSH100 WT (no 3x-Flag tag), KBH19 (WT-3xFlag), KBH35 (T1-3xFlag), and KBH37 (T2-3xFlag). (A) 1000X Phase contrast images of wild-type and mutant H. pylori. (B) Smooth histograms displaying population cell curvature (x axis) as a density function (y axis). (C) Western blot analysis of Csd4 (detected by anti-Flag M2 antibody): LSH100 WT (-); KBH19 (+) predicted M.w. 48 kDa; KBH35 (T1) predicted M.w. 39 kDa; KBH37 (T2) predicted M.w. 28 kDa. Cag3 predicted M.w. 55 kDa. Equivalent amounts of cell extract based on optical density of the culture were loaded for each strain.

FIGURE 4. Tripeptide substrate binding site (PDB ID: 4WCN). (A) Key zinc and substrate interactions with Csd4 are shown. Zinc ligands are colored white; tripeptide ligands are colored cyan; the predicted catalytic water is red and its ligands are orange; zinc is grey; key interactions are shown as dotted lines. Hydrophobic residues and other waters are not shown. (B) Structural alignment between substrate- and product-bound Csd4. View is of the active site in panel A with a 30 degree rotation about the X-axis. (C) Tripeptide omit $F_o-F_c$ difference map contoured at 2 $\sigma$ generated prior to the addition of the tripeptide to the model (purple) and from the final model (green) showing well-defined omit density for the deeply buried portions of the tripeptide substrate. Increased tripeptide flexibility in relation to the degree of surface exposure are indicated by a B-factor based coloring scheme (blue=\~20 Å$^2$ and green=\~60 Å$^2$).

FIGURE 5. 2D tripeptide-Csd4 interaction map (PDB ID: 4WCN). Tripeptide substrate is highlighted in grey; waters are colored cyan; iodide in green; red bristled arcs depict nearby hydrophobic interactions; zinc cofactor and its ligands are not shown. Drawn using LigPlot$^+$ (43).

FIGURE 6. Wild-type Csd4 exhibits higher catalytic activity on the tripeptide substrate than its active site variants. (A) Csd4 activity was continuously monitored via the activity of meso-diaminopimelate dehydrogenase, which consumes the Csd4 product m-DAP to produce NADPH. Buffer-based activity rate differences of Csd4 and its variants are shown. (B) The pH-based activity differences between wild-type Csd4 and the Q46H variant was examined by examining the amount of product produced after 20 min. Mean values are shown with error bars representing the standard deviation based on at least three experimental replicates. The P-value for all variants is < 0.0005 as compared to wild-type in their respective buffers utilizing the t test in panel A. P-values between wild-type and Q46H are < 0.0006 for all pH values except pH 4.8 in panel B.

FIGURE 7. Active site of the Zn-bound Q46H variant (PDB ID: 4WCM). (A) Zinc ligands are colored white; m-DAP is in yellow; phosphate is in orange; other residues interacting with the phosphate are
colored blue; phosphate interactions are shown as dotted lines. (B) Omit \( F_o - F_c \) difference density map contoured at 3 \( \sigma \) showing density for a bound phosphate.

**FIGURE 8.** Complementation and overexpression analysis of Csd4 active site variants. Strain labels indicate the copy number (1 or 2) and amino acid residue at position 46 (Q, A, H) of \( \text{csd} 4-3\times\text{Flag} \). Strains with 2 copies have one copy at the native locus and the second copy at the \( rdxA \) locus. Strains used: (-) LSH100 WT (no 3x-Flag tag), KBH54 (\( \Delta \text{csd} 4 \)), KBH19 (1Q), KBH33 (1H), KBH42 (1A), KBH60 (2H), KBH65 (2Q), and KBH66 (1Q, 1H). (A) 1000X phase contrast images of wild-type and \( \text{csd} 4 \) mutant \( H. pylori \). (B, C) Smooth histograms displaying population cell curvature (x axis, B) and population axis length (x axis, C) as a density function (y axis). (D) Western blot analysis of Csd4 (detected by anti-Flag M2 antibody, predicted M.w. 48 kDa) and Cag3 (periplasmic protein loading control, predicted M.w. is 55 kDa). Equivalent amounts of cell extract based on optical density of the culture were loaded for each strain. ImageJ software was used for densitometry analysis of Csd4 variant expression relative to Cag3 and is indicated below each lane.


**FIGURE 10.** A bootstrapped tree of the Csd4 family of CPs. Homologs of Csd4 were identified from the non-redundant database at the National Center for Biotechnology Information utilizing BLASTP and an \( E \)-value cutoff of \( 1 \times 10^{-7} \). Identical protein sequences derived from different strains of the same species and proteins with short alignment coverage were removed. The sequences were aligned with Clustal Omega (28) and a bootstrapped tree (with 500 replicates, subtree pruning and regrafting and five random starts) was generated using PhyML (29) within Seaview (30). (*) *Helicobacter hepaticus* and *Helicobacter cinaedi* form their own sub-branch and contain a His at the equivalent position of Gln46.
### TABLES

Table 1. Plasmids, strains and primers used in this study

<table>
<thead>
<tr>
<th>Plasmids</th>
<th>Relevant or Descriptive Genotype</th>
<th>Reference or Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>pLKS2</td>
<td>Csd4 in pBluescript II SK+</td>
<td>Sycuro 2012</td>
</tr>
<tr>
<td>pLC292</td>
<td>rDxA with ampicillin resistance marker</td>
<td>Sycuro 2012</td>
</tr>
<tr>
<td>pKB20</td>
<td>Csd4 WT:3xFlag (in pBluescript II SK+)</td>
<td>This work</td>
</tr>
<tr>
<td>pKB27</td>
<td>Csd4 1-251:3xFlag (in pBluescript II SK+)</td>
<td>This work</td>
</tr>
<tr>
<td>pKB29</td>
<td>Csd4 1-343:3xFlag (in pBluescript II SK+)</td>
<td>This work</td>
</tr>
<tr>
<td>pKB37</td>
<td>Csd4 Q46A:3xFlag (in pBluescript II SK+)</td>
<td>This work</td>
</tr>
<tr>
<td>pKB38</td>
<td>Csd4 Q46H:3xFlag (in pBluescript II SK+)</td>
<td>This work</td>
</tr>
<tr>
<td>pKB44</td>
<td>rdxA::Csd4 WT:3xFlag (in pLC292)</td>
<td>This work</td>
</tr>
<tr>
<td>pKB46</td>
<td>rdxA::Csd4 Q46H:3xFlag (in pLC292)</td>
<td>This work</td>
</tr>
<tr>
<td>pAC-C4H</td>
<td>Csd4 Q46H (in pET15)</td>
<td>This work</td>
</tr>
<tr>
<td>pAC-C4E</td>
<td>Csd4 Q46E (in pET15)</td>
<td>This work</td>
</tr>
<tr>
<td>pAC-C4A</td>
<td>Csd4 Q46A (in pET15)</td>
<td>This work</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Strains</th>
<th>Relevant or Descriptive Genotype</th>
<th>Reference or Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>LSH100</td>
<td>Wild-type <em>H. pylori</em> (G27 background)</td>
<td>Sycuro 2012</td>
</tr>
<tr>
<td>LSH18</td>
<td>csd4::catsacB</td>
<td>Sycuro 2012</td>
</tr>
<tr>
<td>LSH108</td>
<td>rdxA::kansacB</td>
<td>Sycuro 2010</td>
</tr>
<tr>
<td>LSH122</td>
<td>csd4::cat</td>
<td>Sycuro 2012</td>
</tr>
<tr>
<td>TRH1</td>
<td>Csd4 4xFlag (at native locus)</td>
<td>This work</td>
</tr>
<tr>
<td>KBH19</td>
<td>Csd4 WT:3x-Flag (at native locus)</td>
<td>This work</td>
</tr>
<tr>
<td>KBH33</td>
<td>Csd4 Q46H:3x-Flag (at native locus)</td>
<td>This work</td>
</tr>
<tr>
<td>KBH35</td>
<td>Csd4 1-343:3x-Flag (at native locus)</td>
<td>This work</td>
</tr>
<tr>
<td>KBH37</td>
<td>Csd4 1-251:3x-Flag (at native locus)</td>
<td>This work</td>
</tr>
<tr>
<td>KBH42</td>
<td>Csd4 Q46A:3x-Flag (at native locus)</td>
<td>This work</td>
</tr>
<tr>
<td>KBH47</td>
<td>Csd4 Q46H:3x-Flag, rdxA::kansacB</td>
<td>This work</td>
</tr>
<tr>
<td>KBH54</td>
<td>csd4::cat, rdxA::kansacB</td>
<td>This work</td>
</tr>
<tr>
<td>KBH60</td>
<td>Csd4 Q46H:3xFlag, rdxA::Csd4 Q46H:3xFlag</td>
<td>This work</td>
</tr>
<tr>
<td>KBH64</td>
<td>Csd4 WT:3xFlag, rdxA::kansacB</td>
<td>This work</td>
</tr>
<tr>
<td>KBH65</td>
<td>Csd4 WT:3xFlag, rdxA::Csd4 WT:3xFlag</td>
<td>This work</td>
</tr>
<tr>
<td>KBH66</td>
<td>Csd4 WT:3xFlag, rdxA::Csd4 Q46H:3xFlag</td>
<td>This work</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Primers</th>
<th>Description</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>53</td>
<td>Csd4FLAG_UP_F</td>
<td>cgccccccccctcgaatgatagaagctgcaaagctt</td>
</tr>
<tr>
<td>54</td>
<td>Csd4FLAG_UP_R</td>
<td>acttttcaacttatatatcttgctg</td>
</tr>
<tr>
<td>55</td>
<td>Csd4FLAG_DN_F</td>
<td>tatagattaaaagttgaatgtgatatctgcgttaagat</td>
</tr>
<tr>
<td>56</td>
<td>Csd4FLAG_DN_R</td>
<td>cgggtcaggaatattatcatcagcacttcacgatgtridge</td>
</tr>
<tr>
<td>94</td>
<td>Csd4-Q46A-SDM</td>
<td>ttgtgtttttagcaggtagtcagcagtccgccgggt</td>
</tr>
<tr>
<td>95</td>
<td>Csd4-Q46H-SDM</td>
<td>tgcggtttttacgaggttacctgccagtccggcggggggt</td>
</tr>
<tr>
<td>85</td>
<td>Csd4-3x_F_UP_KpnI_F</td>
<td>gaattgggtaccgggcccccc</td>
</tr>
<tr>
<td>86</td>
<td>Csd4-1-251_3xF_DNR</td>
<td>ataattcctattagctatcgtcttttaataatccaaaagggattattggtgatgatgattga</td>
</tr>
<tr>
<td>88</td>
<td>Csd4-1-343_3xF_DNR</td>
<td>ataattcctattagctatcgtcttttaataatccaaaagggattattggtgatgatgattgata</td>
</tr>
<tr>
<td>89</td>
<td>Csd4-3xF_DN_F</td>
<td>gattattattatcgagcataaagttgctgatgagttaa</td>
</tr>
<tr>
<td>90</td>
<td>Csd4-3xFDNSacI_R</td>
<td>aagcgggaggtcagcagtccggc</td>
</tr>
<tr>
<td>120</td>
<td>Csd4-3xFl-BamHI</td>
<td>aggagggtcatggtgttttaaatccgttggttatcaaatccct</td>
</tr>
<tr>
<td>121</td>
<td>Csd4-3xFl-EcoRI</td>
<td>ctctcttgttctaagtggcatgacttctttc</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>A1</td>
<td>Csd4-Q46H – For</td>
<td>gttcttttagcagggattgagcc</td>
</tr>
<tr>
<td>A2</td>
<td>Csd4-Q46H – Rev</td>
<td>ggctcatgccatgatctgctaaagcaac</td>
</tr>
<tr>
<td>A3</td>
<td>Csd4-Q46E – For</td>
<td>tttgcttttagcagggattgagcc</td>
</tr>
<tr>
<td>A4</td>
<td>Csd4-Q46E – Rev</td>
<td>gctcatgctcttcaatccctgctaaagcaacaa</td>
</tr>
<tr>
<td>A5</td>
<td>Csd4-Q46A – For</td>
<td>aggctcatgcctgaatccctgctaaagcaacaaatgg</td>
</tr>
<tr>
<td>A6</td>
<td>Csd4-Q46A – Rev</td>
<td>ccatttgttttagcagggattgagccgagcgtgcc</td>
</tr>
</tbody>
</table>
Table 2. Data collection and refinement statistics for Csd4

<table>
<thead>
<tr>
<th></th>
<th>Apo-Csd4</th>
<th>Zn-Csd4</th>
<th>TriZn-Csd4</th>
<th>Q46H-Csd4</th>
<th>Csd4-initial</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Data collection</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Space group</td>
<td>(P2_12_1)</td>
<td>(P2_12_1)</td>
<td>(P2_12_1)</td>
<td>(P2_12_1)</td>
<td>(P2_12_1)</td>
</tr>
<tr>
<td>Cell dimensions</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a, b, c (\AA))</td>
<td>53.28, 66.78, 145.53</td>
<td>53.02, 66.77, 145.05</td>
<td>53.23, 66.83, 145.05</td>
<td>53.60, 66.65, 145.05</td>
<td>53.11, 66.92, 146.02</td>
</tr>
<tr>
<td>Resolution (\AA)</td>
<td>42.99-1.40 (1.45-1.40)</td>
<td>42.76-1.85 (1.92-1.85)</td>
<td>49.15-1.75 (1.81-1.75)</td>
<td>49.13-1.75 (1.81-1.75)</td>
<td>50.00-2.10 (2.14-2.10)</td>
</tr>
<tr>
<td>(R_{merge})</td>
<td>0.070 (0.644)</td>
<td>0.062 (0.434)</td>
<td>0.058 (0.539)</td>
<td>0.078 (0.463)</td>
<td>0.148 (0.469)</td>
</tr>
<tr>
<td>(I/\sigma I)</td>
<td>17.5 (3.6)</td>
<td>21.4 (4.6)</td>
<td>21.2 (2.8)</td>
<td>15.4 (3.0)</td>
<td>21.2 (2.9)</td>
</tr>
<tr>
<td>Completeness (%)</td>
<td>100.0 (99.9)</td>
<td>99.8 (99.3)</td>
<td>99.6 (96.3)</td>
<td>99.8 (97.9)</td>
<td>99.9 (98.3)</td>
</tr>
<tr>
<td>Redundancy</td>
<td>7.1</td>
<td>7.1</td>
<td>7.0</td>
<td>7.0</td>
<td>27.4</td>
</tr>
<tr>
<td><strong>Refinement</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. unique reflections</td>
<td>102944</td>
<td>44613</td>
<td>52911</td>
<td>53300</td>
<td>31191</td>
</tr>
<tr>
<td>(R_{work}/R_{free})</td>
<td>0.14/0.16</td>
<td>0.17/0.20</td>
<td>0.18/0.21</td>
<td>0.18/0.21</td>
<td>0.17/0.22</td>
</tr>
<tr>
<td>No. atoms</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein</td>
<td>7002</td>
<td>6803</td>
<td>6803</td>
<td>6823</td>
<td>3428</td>
</tr>
<tr>
<td>Substrate/Product</td>
<td>25</td>
<td>25</td>
<td>55</td>
<td>25</td>
<td>13</td>
</tr>
<tr>
<td>Water</td>
<td>466</td>
<td>401</td>
<td>327</td>
<td>451</td>
<td>384</td>
</tr>
<tr>
<td>Average B-factors (\AA(^2))</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein</td>
<td>23.1</td>
<td>28.6</td>
<td>30.0</td>
<td>25.0</td>
<td>17.6</td>
</tr>
<tr>
<td>Substrate/Product</td>
<td>19.9</td>
<td>21.1</td>
<td>44.9</td>
<td>35.1</td>
<td>14.5</td>
</tr>
<tr>
<td>Water</td>
<td>35.1</td>
<td>35.2</td>
<td>37.2</td>
<td>34.5</td>
<td>26.0</td>
</tr>
<tr>
<td>R.m.s. deviations</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bond lengths (\AA)</td>
<td>0.013</td>
<td>0.007</td>
<td>0.011</td>
<td>0.012</td>
<td>0.010</td>
</tr>
<tr>
<td>Bond angles ((^\circ))</td>
<td>1.49</td>
<td>1.09</td>
<td>1.33</td>
<td>1.36</td>
<td>1.41</td>
</tr>
<tr>
<td>PDB Accession Code</td>
<td>4WCK</td>
<td>4WCL</td>
<td>4WCN</td>
<td>4WCM</td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Zinc ligand bond lengths in the crystal structures of Csd4

<table>
<thead>
<tr>
<th></th>
<th>Zn-Csd4</th>
<th>TriZn-Csd4</th>
<th>Q46H-Csd4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gln46/His46</td>
<td>2.2</td>
<td>2.0</td>
<td>2.1</td>
</tr>
<tr>
<td>Glu49</td>
<td>2.3/2.3</td>
<td>2.4/2.6</td>
<td>2.1/2.5</td>
</tr>
<tr>
<td>His128</td>
<td>2.0</td>
<td>2.5</td>
<td>2.1</td>
</tr>
<tr>
<td>H2O (catalytic)</td>
<td>2.3</td>
<td>2.9</td>
<td>-</td>
</tr>
<tr>
<td>H2O (other)</td>
<td>2.2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Phosphate</td>
<td>-</td>
<td>-</td>
<td>1.9</td>
</tr>
</tbody>
</table>
Table 4. Characterized Gln-containing Zn proteins identified from the Protein Data Bank.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Organism</th>
<th>PDB-ID</th>
<th>Zn ligands</th>
<th>Class</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphomannose isomerase</td>
<td>Candida albicans</td>
<td>1dp1</td>
<td>Q-H-E-H-H₂O</td>
<td>Isomerase</td>
</tr>
<tr>
<td>Glyoxalase I</td>
<td>human, mouse</td>
<td>1bh5, 1fro,</td>
<td>H-E-Q-E-H₂O</td>
<td>Lyase</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1qip, 1qin,</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2za0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ING4</td>
<td>human</td>
<td>2k1j</td>
<td>Q-H (and 2 weak C</td>
<td>Gene Regulation/</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>interactions)</td>
<td>Zinc-finger</td>
</tr>
<tr>
<td>L-Histidinol Dehydrogenase</td>
<td><em>Escherichia coli</em></td>
<td>1k2, 1k6,</td>
<td>Q-H-D-H</td>
<td>Oxidoreductase</td>
</tr>
<tr>
<td>Glucose Dehydrogenase I</td>
<td><em>Sulfolobus solfatarius</em></td>
<td></td>
<td>C-H-E-Q</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(archaea)</td>
<td>1k2, 1k6,</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5'-Nucleotidase</td>
<td><em>Escherichia coli</em></td>
<td>1hp1, 1ush,</td>
<td>D-H-D-Q</td>
<td>Hydrolase</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2ush</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
FIGURES

Figure 1

Helicobacter pylori Csd4 requires a glutamine zinc ligand

FIGURES

Figure 1

Helicobacter pylori Csd4 requires a glutamine zinc ligand

FIGURES

Figure 1

Helicobacter pylori Csd4 requires a glutamine zinc ligand

FIGURES

Figure 1

Helicobacter pylori Csd4 requires a glutamine zinc ligand

FIGURES

Figure 1

Helicobacter pylori Csd4 requires a glutamine zinc ligand

FIGURES

Figure 1

Helicobacter pylori Csd4 requires a glutamine zinc ligand

FIGURES

Figure 1

Helicobacter pylori Csd4 requires a glutamine zinc ligand

FIGURES

Figure 1

Helicobacter pylori Csd4 requires a glutamine zinc ligand

FIGURES

Figure 1

Helicobacter pylori Csd4 requires a glutamine zinc ligand

FIGURES

Figure 1

Helicobacter pylori Csd4 requires a glutamine zinc ligand

FIGURES

Figure 1

Helicobacter pylori Csd4 requires a glutamine zinc ligand

FIGURES

Figure 1

Helicobacter pylori Csd4 requires a glutamine zinc ligand

FIGURES

Figure 1

Helicobacter pylori Csd4 requires a glutamine zinc ligand

FIGURES

Figure 1

Helicobacter pylori Csd4 requires a glutamine zinc ligand

FIGURES

Figure 1

Helicobacter pylori Csd4 requires a glutamine zinc ligand

FIGURES

Figure 1

Helicobacter pylori Csd4 requires a glutamine zinc ligand

FIGURES

Figure 1

Helicobacter pylori Csd4 requires a glutamine zinc ligand

FIGURES

Figure 1

Helicobacter pylori Csd4 requires a glutamine zinc ligand

FIGURES

Figure 1

Helicobacter pylori Csd4 requires a glutamine zinc ligand

FIGURES

Figure 1

Helicobacter pylori Csd4 requires a glutamine zinc ligand

FIGURES

Figure 1

Helicobacter pylori Csd4 requires a glutamine zinc ligand

FIGURES

Figure 1

Helicobacter pylori Csd4 requires a glutamine zinc ligand

FIGURES

Figure 1

Helicobacter pylori Csd4 requires a glutamine zinc ligand

FIGURES

Figure 1

Helicobacter pylori Csd4 requires a glutamine zinc ligand

FIGURES

Figure 1

Helicobacter pylori Csd4 requires a glutamine zinc ligand

FIGURES

Figure 1

Helicobacter pylori Csd4 requires a glutamine zinc ligand

FIGURES

Figure 1

Helicobacter pylori Csd4 requires a glutamine zinc ligand

FIGURES

Figure 1

Helicobacter pylori Csd4 requires a glutamine zinc ligand

FIGURES

Figure 1

Helicobacter pylori Csd4 requires a glutamine zinc ligand

FIGURES

Figure 1

Helicobacter pylori Csd4 requires a glutamine zinc ligand

FIGURES

Figure 1

Helicobacter pylori Csd4 requires a glutamine zinc ligand

FIGURES

Figure 1

Helicobacter pylori Csd4 requires a glutamine zinc ligand

FIGURES

Figure 1

Helicobacter pylori Csd4 requires a glutamine zinc ligand

FIGURES

Figure 1

Helicobacter pylori Csd4 requires a glutamine zinc ligand

FIGURES

Figure 1

Helicobacter pylori Csd4 requires a glutamine zinc ligand

FIGURES

Figure 1

Helicobacter pylori Csd4 requires a glutamine zinc ligand
Helicobacter pylori Csd4 requires a glutamine zinc ligand

Figure 2

A

B

C

D

E

F
Helicobacter pylori Csd4 requires a glutamine zinc ligand
Helicobacter pylori Csd4 requires a glutamine zinc ligand
Helicobacter pylori Csd4 requires a glutamine zinc ligand
Figure 6

A

Activity (mDAP/Csd4/min)

B

Relative Activity

Buffer (100 mM, pH 6.5)

Na/K PO₄ Buffer (20 mM, pH 7.0)

Csd4 WT

Q46H

Q45A

Q46E
Figure 7

Helicobacter pylori Csd4 requires a glutamine zinc ligand
Helicobacter pylori Csd4 requires a glutamine zinc ligand

Figure 8

A

<table>
<thead>
<tr>
<th>WT (1Q)</th>
<th>Δcsd4</th>
<th>1H</th>
<th>1A</th>
<th>2Q</th>
<th>2H</th>
<th>1Q,1H</th>
</tr>
</thead>
</table>

B

<table>
<thead>
<tr>
<th>1Q</th>
<th>1H</th>
<th>1A</th>
<th>Δcsd4</th>
<th>2H</th>
<th>2Q</th>
<th>1Q,1H</th>
</tr>
</thead>
</table>

C

<table>
<thead>
<tr>
<th>1Q</th>
<th>1H</th>
<th>1A</th>
<th>Δcsd4</th>
<th>2H</th>
<th>2Q</th>
<th>1Q,1H</th>
</tr>
</thead>
</table>

D

<table>
<thead>
<tr>
<th>anti-Flag</th>
<th>anti-Cag3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Csd4 rel. exp.</td>
<td>0.6</td>
</tr>
</tbody>
</table>

M.w 55kD
Figure 9

* Helicobacter pylori Csd4 requires a glutamine zinc ligand *
Figure 10

Helicobacter pylori Csd4 requires a glutamine zinc ligand
Helical Shape of *Helicobacter pylori* Requires an Atypical Glutamine as a Zinc Ligand in the Carboxypeptidase Csd4
Anson C. K. Chan, Kris M. Blair, Yanjie Liu, Emilisa Frirdich, Erin C. Gaynor, Martin E. Tanner, Nina R. Salama and Michael E. P. Murphy

*J. Biol. Chem.* *published online December 12, 2014*

Access the most updated version of this article at doi: 10.1074/jbc.M114.624734

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/early/2014/12/12/jbc.M114.624734.full.html#ref-list-1