doi:10.1016/j.jmb.2010.08.051 J. Mol. Biol. (2010) 403, 405–419

Contents lists available at www.sciencedirect.com

Journal of Molecular Biology

journal homepage: http://ees.elsevier.com.jmb

The 2.2-Å Structure of the HP0958 Protein from *Helicobacter pylori* Reveals a Kinked Anti-Parallel Coiled-Coil Hairpin Domain and a Highly Conserved Zn-Ribbon Domain

Delphine L. Caly[1], Paul W. O'Toole[1]* and Stanley A. Moore[2]*

[1]*Department of Microbiology, University College Cork, Cork, Ireland*
[2]*Department of Biochemistry, University of Saskatchewan, Saskatoon, SK, Canada S7N 5E5*

Received 1 June 2010;
received in revised form
24 August 2010;
accepted 26 August 2010
Available online
6 September 2010

Edited by R. Huber

Keywords:
flagellum biogenesis;
mRNA chaperone;
Zn ribbon;
coiled-coil hairpin;
Helicobacter pylori

We have determined the 2.2-Å structure of the HP0958 protein from the human gastric pathogen *Helicobacter pylori*. HP0958 is essential for flagellum formation and motility. It functions as a chaperone for RpoN (σ^{54}) and also controls the stability and translation of mRNA for the major flagellin subunit FlaA. The protein is composed of a highly elongated and kinked coiled-coil hairpin domain (residues 1–170), followed by a C_4 Zn-ribbon domain (residues 174–238). The Zn-ribbon domain is rich in aromatic and positively charged amino acid residues. Electrophoretic mobility shift assays identified residues in a positively charged region of the Zn-ribbon domain of HP0958 whose mutation alters the mobility of an HP0958–*flaA* mRNA complex. Mutation of surface residues in the coiled-coil domain did not result in an observable change in the mobility of the HP0958–*flaA* transcript complex. The data thus suggest the arrangement of HP0958 into distinct structural and functional domains.

Introduction

Helicobacter pylori is a motile Gram-negative bacterium that belongs to the ε-proteobacterial group, which includes *Campylobacter* spp. Infection with *H. pylori* causes inflammation that may or may not be symptomatic, depending on strain genotype; chronic infection with the most pathogenic subtypes can lead to gastritis, peptic and duodenal ulcer diseases, and, in the most severe cases, gastric adenocarcinoma.[1–5] *H. pylori* cells are motile due to the action of multiple sheathed polar flagella. Significantly, motility is an essential factor for the colonization and persistence of *H. pylori* in the human gastric mucosa.[6,7] The overall structure of the *H. pylori* flagellum is similar to that of enteric bacteria, except that it is sheathed by an extension of the outer membrane phospholipid bilayer and lipopolysaccharide, and flagella are located at the cell poles.[8–10] Over 40 proteins are required for the assembly of a functional bacterial flagellum, which is made up of three major structures: a basal body and rod composed of the flagellar motor and the export apparatus, a hook that acts as the universal joint for torque transmission, and a flagellar filament. The hook is composed of multiple copies of the FlgE protein; in *H. pylori*, the filament is composed of two flagellin proteins: the major flagellin FlaA and the minor flagellin FlaB.[8,11] In the well-studied *Enterobacteriaceae*, flagella are assembled in a hierarchical fashion, starting with the expression of the genes of the master operon

**Corresponding authors.* E-mail addresses: pwotoole@ucc.ie; stan.moore@usask.ca.

Abbreviations used: MAD, multiwavelength anomalous dispersion; PDB, Protein Data Bank; EMSA, electrophoretic mobility shift assay; GST, glutathione *S*-transferase; PBS, phosphate-buffered saline; RT, room temperature.

FlhDC.[12–14] This master operon has not been annotated in available *H. pylori* genomes.[9,15] In *H. pylori*, flagellar genes are grouped into three classes based on their transcriptional regulation by one of three RNA polymerase σ factors (σ^{80}, σ^{54}, and σ^{28}), and by the anti-σ^{28} factor FlgM.[15–20] Early flagellar genes (class I) are under the control of σ^{80}.[15] Class II flagellar genes, including the genes encoding the minor flagellin FlaB and the hook-length control protein FliK, are under the control of σ^{54} (RpoN).[15] σ^{28} (FliA) mediates the expression of late flagellar genes (class III), including the gene encoding the major flagellin FlaA, and is regulated by its anti-σ factor FlgM.[15–17]

HP0958 is the product of a recently characterized *H. pylori* flagellar gene, which has no known homologues in enteric bacteria and is essential for the assembly of a functional flagellum.[21] Homologues of HP0958 are distributed throughout the ε-proteobacteria, chlamydiae, spirochetes, *Bacteroidetes*, δ-proteobacteria, Chlorobia, deinococci, and many extremophiles; thus, characterization of the functions of this protein is warranted. Previous studies have established that *H. pylori hp0958* mutant strains are aflagellate and exhibit lower levels of *flgE* and *flaB* mRNA transcripts.[21] HP0958 is also required for the normal accumulation and stability of RpoN, the σ factor controlling the expression of class II flagellar genes.[18] An *H. pylori* genomewide yeast two-hybrid analysis demonstrated that the *H. pylori* HP0958 protein interacts with both RpoN and FliH.[22] FliH is a highly conserved flagellar export apparatus protein that binds to, and presumably regulates the activity of, the flagellar export ATPase FliI and furthermore inhibits the flagellum export pathway in the absence of FliI.[23–25] Since HP0958 interacts with a number of molecules intimately associated with flagellum biogenesis, HP0958 may itself have a key role in flagellum biogenesis in *H. pylori*. Bioinformatic analysis of the HP0958 amino acid sequence revealed the presence of a conserved Zn-ribbon motif consisting of four cysteine residues with the consensus sequence $CXGCX_{20}CPHCGR$ (where X is any amino acid), suggesting the involvement of HP0958 in nucleic acid interactions.[21] We recently demonstrated that HP0958 regulates flagellin protein production via interactions with *flaA* mRNA.[26] An *hp0958*-null mutant exhibited increased levels of flagellin gene (*flaA*) transcription; however, the amount of FlaA protein was lower in the *hp0958* mutant than in the wild-type strain.[26] It was proposed that this paradox could be explained by HP0958 acting to promote *flaA* mRNA translation. This suggestion was supported by the fact that HP0958 bound to *flaA* mRNA *in vitro* and that the interaction was partially inhibited by DTT and ethylenediaminetetraacetic acid—agents that are capable of disrupting Zn–S covalent bonds in the HP0958 Zn-ribbon domain.[26] The aim of this study was to gain insight into the function of HP0958 by elucidating its three-dimensional structure using X-ray crystallography. Specifically, we sought to understand how the structure of HP0958 could provide insight on known interactions with *flaA* mRNA, RpoN (σ^{54}), and FliH in the process of flagellum assembly.

Results

Overall structure of HP0958

The structure of *H. pylori* HP0958 was solved by multiwavelength anomalous dispersion (MAD) using the endogenous Zn anomalous scattering signal and has been fully refined to 2.2-Å resolution ($R_{work}=0.248$; $R_{free}=0.294$) (Tables 1 and 2). The refined model consists of residues 2–235 of HP0958,

Table 1. Data collection and phasing statistics

		Zn[a]		
	Native (C2)	Peak	Inflection	Remote
Unit cell parameters *a*, *b*, *c* (Å)	172.72, 37.950, 66.056			
Unit cell parameters α, β, γ (°)	90, 110.66, 90			
Wavelength (Å)	0.984	1.28386	1.28410	1.2579
Resolution (Å)	50–2.2 (2.28–2.2)	50–2.6 (2.69–2.60)	50–2.6 (2.69–2.60)	50–2.6 (2.69–2.60)
Number of unique reflections	19,056 (1327)	12,364 (1025)	12,411 (1041)	12,455 (1047)
Redundancy	5 (3.4)	3.1 (2.4)	3.5 (2.7)	3.4 (2.6)
Completeness (%)	91.9 (65.7)	96.9 (80.7)	97.2 (82.0)	97.5 (83.0)
Mosaicity (°)	0.540	0.543	0.545	0.496
Average I/σ	33.6 (3.5)	18.2 (3.0)	20.4 (3.5)	19.6 (3.1)
R_{merge}[b]	0.059 (0.22)	0.076 (0.21)	0.068 (0.20)	0.073 (0.22)

Values in parentheses correspond to the highest-resolution shell. Data processing statistics were calculated using Denzo/HKL2000.[27]

[a] The anomalous pair of $F(+hkl)$ and $F(-hkl)$ was not merged during data processing. Data were collected over 180° (180 frames). An identical orientation matrix was used to process the three data sets.

[b] $R_{merge}=\sum_{hkl}\sum_i |\langle I(hkl)_{obs}\rangle - I(hkl)_{obs,\ i}| / \sum_{hkl,\ i} I(hkl)_{obs,\ i}$, where $I(hkl)_{obs,\ i}$ is the individual measurement of an *hkl* intensity and $\langle I(hkl)_{obs}\rangle = \sum_i I(hkl)_{obs,i}/N$, where $i=1$–N individual reflections are measured.