Biochemical and Molecular Characterisation of FliI and FliH from *Helicobacter pylori*

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Abstract

The bacterium *Helicobacter pylori* is a human pathogen that infects a large proportion of the world's population and is associated with serious diseases such as gastric ulcers and adenocarcinoma. The motility of this organism, by virtue of sheathed polar flagella is essential to colonisation and persistence in the human host.

The sequencing of the *H. pylori* genome in 1996 identified homologues of the majority of the flagellar genes found in *S. enterica* serovar *typhimurium.* These included genes encoding the flagellum ATPase, FliI and FliH a presumptive inhibitor, the primary focus of this study. Sequencing did not originally identify an *H. pylori* homologue of the flagellar chaperone FliJ, and this is also considered in this study.

Bioinformatic analysis and modeling suggests a structural and functional relationship between FliI and homologues such as F_1-ATPase α- and β-subunit. In particular, residues 2-91 of FliI resemble the N-terminal domain of the F_1-ATPase α- and β-subunits. Biochemical analyses reported in this thesis showed that a truncated FliI-(2- 91) protein was folded, although the N-terminal 18 residues were likely unstructured. Furthermore, deletion mutagenesis showed that this disordered segment of the protein mediates interaction with FliH and very likely forms an amphipathic α-helix upon forming of the FliI-FliH complex. The scanning mutagenesis of this interaction segment of FliI identified a cluster of conserved hydrophobic residues that was critical for the interaction with FliH. Thus, the interaction between FliI and FliH has similarities to the interaction between the N-terminal α-helix of the α-subunit and the globular domain of the δ-subunit of the F_1-ATPase. This similarity suggests that FliH, by analogy with the δ-subunit of the F_1-ATPase, may function as a molecular stator of the flagellum. The findings presented above have been published (96).

The function of a putative *H. pylori* FliJ homologue, HP0256, was also investigated by knock-out mutagenesis. Disruption of this gene does not abolish flagellar assembly, however further research continued beyond this thesis showed that the knock-out mutant results in impaired motility.

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List of Abbreviations

ADP adenosine diphosphate
AMP adenosine monophosphate
AMP-PNP adenylyl imidodiphosphate
Ap Ampicillin
APS ammonium persulphate
ATP adenosine triphosphate
BLAST Basic Local Alignment Search Tool
BSA bovine serum albumin
CAPS 3-(cyclohexylamino)-1-propanesulfonic acid
CD Circular dichroism
Cm Chloramphenicol
colE1 colicin E1
DLS Dynamic light scattering
DNA deoxyribonucleic acid
dNTP deoxynucleoside triphosphate
DTT dithiothreitol
EDTA ethylenediaminetetraacetic acid
FP forward primer
FPLC Fast protein liquid chromatography
FSB final sample buffer
GSP general secretory pathway
GEP general export pathway
GST Glutathione-S-transferase
HEPES N-(2-hydroxyethyl)-piperazine-N'-2-ethanesulfonic acid
HIC Hydrophobic interaction chromatography
IEC Ion exchange chromatography
IPTG isopropyl-β-D-galactoside
Kan Kanamycin
LB Luria-Bertani broth
LBA Luria-Bertani broth agar
MALT mucosa-associated lymphoid tissue
MCS multiple cloning site
na not applicable
NCBI National Centre for Biotechnology Information
OD Optical density
PBS Phosphate buffered saline
PCR polymerase chain reaction
PHI-BLAST Pattern Hit Initiated BLAST
pI isoelectric point
RP reverse primer
Rpm revolutions per minute
PSI-BLAST Position Specific Iterative BLAST
RT room temperature

SDS sodium dodecyl sulphate
SDS-PAGE Sodium dodecyl sulphate-Polyacrylamide gel electrophoresis
SEC Size exclusion chromatography
T3SSs Type III secretion systems
T4SSs Type IV secretion systems
TEMED N,N,N',N'-Tetramethylethylenediamine
TIGR The Institute for Genomic Research
Tris Tris(hydroxymethyl)aminomethane hydrochloride
TSB tryptic soy broth
U unit
w/v weight per volume

Amino acid abbreviations used:
A, Ala, Alanine
C, Cys, Cysteine
D, Asp, Aspartic acid
E, Glu, Glutamic acid
F, Phe, Penylalanine
G, Gly, Glycine
H, His, Histidine
I, Ile, Isoleucine
K, Lys, Lysine
L, Leu, Leucine
M, Met, Methionine
N, Asn, Asparagine
P, Pro, Proline
Q, Gln, Glutamine
R, Arg, Arginine
S, Ser, Serine
T, Thr, Threonine
V, Val, Valine
W, Trp, Tryptophan
Y, Tyr, Tyrosine

Deoxyribonucleosides: A, deoxyadenylate, C, deoxycytidylate, G, deoxyguanylate, T, deoxythymidylate

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