Structural and Biochemical Analysis of HutD from *Pseudomonas fluorescens* SBW25

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Abstract

Pseudomonas fluorescens SBW25 is a gram-negative soil bacterium capable of growing on histidine as the sole source of carbon and nitrogen. Expression of histidine utilization (*hut*) genes is controlled by the HutC repressor with urocanate, the first intermediate of the histidine degradation pathway, as the direct inducer. Recent genome sequencing of *P. fluorescens* SBW25 revealed the presence of *hutD* in the *hut* locus, which encodes a highly conserved hypothetical protein. Previous genetic analysis showed that *hutD* is involved in *hut* regulation, in such a way that it prevents overproduction of the *hut* enzymes. Deletion of *hutD* resulted in a slow growth phenotype in minimal medium with histidine as the sole carbon and nitrogen source. While the genetic evidence supporting a role of *hutD* in *hut* regulation is strong, nothing is known of the mechanism of HutD action.

Here I have cloned and expressed the *P. fluorescens* SBW25 *hutD* in *E. coli*. Purified HutD was subjected to chemical and structural analysis. Analytic size-exclusion chromatography indicated that HutD forms a dimer in the elution buffer. The crystal structure of HutD was solved at 1.80 Å (R = 19.3% and R_{free} = 22.3%) by using molecular replacement based on HutD from *P. aeruginosa* PAO1. *P. fluorescens* SBW25 HutD has two molecules in an asymmetric unit and each monomer consists of one subdomain and two β-barrel domains. Comparative structural analysis revealed a conserved binding pocket. The interaction of formate with a highly conserved residue Arg61 via salt-bridges in the pocket suggests HutD binds to small molecules with carboxylic group(s) such as histidine, urocanate or formyl-glutamate.

The hypothesis that HutD functions via binding to urocanate, the *hut* inducer, was tested. Experiments using a thermal shift assay and isothermal titration calorimetry (ITC) analysis suggested that HutD binds to urocanate but not to histidine. However, the signal of HutD-urocanate binding was very weak and detected only at high urocanate concentration (53.23 mM), which is not physiologically relevant. The current data thus does not support the hypothesis of HutD-urocanate binding *in vivo*. Although the HutD-urocanate binding was not confirmed, this work has laid a solid foundation for further testing of the many alternative hypotheses regarding HutD function.

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Å	angstorms
ºC	degrees Celsius
µl	microlitre
µM	micromolar
APS	ammonium persulphate
BLAST	basic local alignment search tool
bp	base pairs
Da	Dalton
DNase	deoxyribonuclease I
dNTP	deoxynucleotide triphosphate
EDTA	ethylenediamine tetraacetic acid
g	gravitational force
h	hour
HEPES	n-2-hydroxylethylpiperazine-n'-2-ethanesulphonic acid
IPTG	isopropyl-β-D-thiogalactoside
ITC	isothermal titration calorimetry
kb	kilobase pairs
kDa	kiloDaltons
LB	luria-bertaini
LLG	log likelihood gain
M	molar
mg	milligram
ml	millimetre
min	minute
mM	millimolar
MR	molecular replacement
MW	molecular weight
MWCO	molecular weight cut-off
nm	nanometre
OD	optical density
ORF	open reading frames

PAGE	polyacrylamide gel electrophoresis
PCR	polymerase chain reaction
PISA	Protein Interfaces, Surfaces and Assemblies
PDB	Protein Data Bank
PMSF	phenylmethylsulfonyl fluoride
RNase	ribonuclease A
rpm	revolution per minute
SCOP	Structural Classification of Proteins
SEC	size exclusion chromatography
SDS	sodium dodecyl sulphate
SSM	Secondary Structure Matching
TAE	tris-acetate-EDTA
TEMED	N,N,N',N'-Tetramethylethylenediamine
TFZ	translation function Z-score
UV	ultraviolet