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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 $R_{\text{free}} = 22.3\%$) 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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Table of Contents

Abstract.....	ii
Acknowledgements.....	iii
Table of Contents.....	iv
List of Figures.....	vii
List of Tables.....	viii
List of Abbreviations.....	ix
Introduction.....	1
1.1 Histidine Degradation Pathway.....	1
1.2 Organization of <i>hut</i> operon.....	3
1.3 Regulation of histidine utilization (<i>hut</i>) genes.....	4
1.4 The role of HutD in histidine utilization.....	5
1.5 The ‘governor’ model of HutD action.....	7
1.6 Deducing protein function from structural information.....	8
1.7 Objectives of this study.....	8
Materials and Methods.....	9
2.1 Reagents.....	9
2.2 Bioinformatics.....	9
2.2.1 Nucleotide and protein information.....	9
2.2.2 Sequence homology search and alignment.....	9
2.3 Bacterial strains, plasmids and growth conditions.....	9
2.4 DNA manipulation.....	10
2.4.1 Primer design.....	10
2.4.2 Polymerase chain reaction.....	11
2.4.3 DNA transformation.....	12
2.4.4 Preparation of electrocompetent <i>E. coli</i> cells.....	12
2.4.5 Glycerol-saline stock.....	12
2.4.6 Electroporation.....	13

2.4.7 Preparation of plasmid DNA.....	13
2.4.8 Restriction enzyme digestion.....	13
2.4.9 Agarose gel electrophoresis.....	13
2.4.10 DNA purification.....	14
2.4.11 DNA ligation.....	14
2.5 Protein expression and solubility tests.....	14
2.5.1 Small-scale protein expression.....	14
2.5.2 Large-scale protein expression.....	14
2.5.3 Cell lysis and protein solubility tests.....	15
2.6 Sodium Dodecyl Sulfate-PolyAcrylamide Gel Electrophoresis.....	15
2.6.1 Gel preparation.....	15
2.6.2 Sample preparation, gel running and staining.....	16
2.7 Purification of hexa-histidine-tagged hutD.....	17
2.8 Concentration of protein and buffer exchange.....	17
2.9 Size exclusion chromatography.....	17
2.10 Determination of protein concentration.....	18
2.10.1 Determination of protein concentration by Bradford assay.....	18
2.10.1 Determination of protein concentration by Beer-Lambert equation....	18
2.11 Protein function tests.....	19
2.11.1 Thermal shift assay.....	19
2.11.2 Isothermal titration calorimetry.....	19
2.12 Protein crystallization.....	20
2.12.1 Initial crystallization trials.....	20
2.12.2 Optimization of crystallization conditions.....	21
2.13 Protein diffraction data collection.....	21
2.13.1 Cryoprotectant test.....	21
2.13.2 Flash freezing crystals.....	21
2.13.3 X-ray diffraction data collection.....	22
2.14 Diffraction data processing.....	22
2.14.1 Indexing, integration and scaling of diffraction data.....	22
2.14.2 Unit cell content analysis.....	22
2.14.3 Phase determination using molecular replacement.....	23
2.14.4 Model building and refinement.....	23

2.15 Structural analysis.....	23
Results.....	24
3.1 Cloning of <i>hutD</i> to the expression vector pTrec99A.....	24
3.2 Protein expression and purification.....	26
3.2.1 Small-scale expression and solubility of HutD.....	26
3.2.2 Large-scale expression and purification of HutD.....	30
3.3 Analytical size-exclusion chromatography.....	31
3.4 Crystallization of HutD.....	33
3.4.1 Initial crystallization trials.....	33
3.4.2 Crystal optimization.....	33
3.5 Structure solution of HutD.....	35
3.5.1 Diffraction data collection and processing.....	35
3.5.2 Number of molecules in the asymmetric unit.....	35
3.5.3 Phase determination by molecular replacement.....	35
3.5.4 Model building, refinement and quality assessment.....	35
3.6 Description of HutD structure.....	40
3.6.1 Overall topology of HutD structure.....	40
3.6.2 Structural comparison of HutD monomer.....	41
3.7 Protein functional tests.....	45
3.7.1 Thermal shift assay.....	46
3.7.2 Isothermal titration calorimetry.....	49
Discussion.....	52
4.1 The effect of growing temperature on HutD expression.....	52
4.2 Determination of molecular weight of HutD.....	52
4.3 Comparative structural analysis of HutD.....	52
4.4 Function of HutD.....	54
Conclusion and Future Studies.....	56
References.....	58

List of Figures

Figure 1.1	The histidine degradation pathway.....	2
Figure 1.2	Genetic and transcriptional organization of <i>hut</i> genes in <i>Pseudomonas</i> species.....	4
Figure 1.3	Multiple sequence alignment of HutD sequence homologues.....	6
Figure 1.4.	Relative fitness of $\Delta hutC$ $\Delta hutD$ and $\Delta hutCD$ mutants.....	7
Figure 3.1	The map of pTrc99A expression vector.....	24
Figure 3.2	Construction of HutD expression plasmid pTrc99A- <i>hutD</i>	25
Figure 3.3	Restriction digestion of pTrc99A- <i>hutD</i>	26
Figure 3.4	SDS-PAGE analysis of HutD expression at 37°C.....	27
Figure 3.5	SDS-PAGE analysis of the solubility of HutD expressed at 37°C.....	28
Figure 3.6	SDS-PAGE analysis of HutD expression at 25°C.....	29
Figure 3.7	SDS-PAGE analysis of the solubility of HutD expressed at 25°C.....	29
Figure 3.8	Purification of His ₆ -HutD by Ni-NTA affinity chromatography.....	30
Figure 3.9	Gel filtration analysis of HutD.....	32
Figure 3.10	Initial crystallization of HutD.....	33
Figure 3.11	Optimization of crystallization conditions.....	34
Figure 3.12	Ramachandran plot for the final model of HutD.....	38
Figure 3.13	Cartoon representation of HutD dimer.....	40
Figure 3.14	Cartoon representation and topology diagram of HutD monomer.....	41
Figure 3.15	Conservation pattern obtained using ConSurf for HutD.....	43
Figure 3.16	Conservation pattern obtained using ConSurf for HutD in <i>Pseudomonas</i>	44
Figure 3.17	Cartoon representation of putative binding pocket.....	45
Figure 3.18	Recording of fluorescence intensity for different concentrations of HutD..	46
Figure 3.19	Analysis of interaction of HutD with histidine and urocanate.....	48
Figure 3.20	Isothermal titration calorimetry of histidine and HutD.....	50
Figure 3.21	Isothermal titration calorimetry of urocanate and HutD.....	51

List of Tables

Table 2.1	Bacterial strains and plasmids used in this study.....	10
Table 2.2	Primers used in this study.....	10
Table 2.3	Reagents for a 50 µl PCR reaction.....	11
Table 2.4	Typical PCR reaction conditions.....	11
Table 3.1	A summary of processing statistics of HutD crystal.....	36
Table 3.2	Summary of refinement and model statistics.....	39
Table 3.3	Structural similarity of HutD monomer found using Dali and SSM.....	41

List of Abbreviations

Å	angstroms
°C	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
<i>g</i>	gravitational force
h	hour
HEPES	n-2-hydroxyethylpiperazine-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	phenylmethanesulfonyl 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