

Structural model and properties of the AdoMetDC domain of  
the bifunctional *Plasmodium falciparum* S-adenosylmethionine  
decarboxylase/Ornithine decarboxylase

by

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# Contents

<b>Acknowledgements</b> . . . . .	i
<b>List of Figures</b> . . . . .	v
<b>List of Tables</b> . . . . .	vii
<b>Typographical conventions</b> . . . . .	viii
<b>List of Abbreviations</b> . . . . .	ix
<b>List of Computer Related Terms</b> . . . . .	xi
<b>Chapter 1. Introduction</b> . . . . .	1
1.1. The need for new anti-malarials . . . . .	1
1.2. Polyamines . . . . .	5
1.2.1. Functions of polyamines . . . . .	5
1.2.2. Polyamine metabolism . . . . .	6
1.2.3. Polyamines in malaria . . . . .	8
1.2.4. Polyamines as a drug target . . . . .	8
1.3. Properties of S-adenosylmethionine decarboxylase (AdoMetDC) . . . . .	9
1.3.1. AdoMetDC requires pyruvoyl . . . . .	9
1.3.2. Enzymatic mechanism . . . . .	12
1.3.2.1. Effects of putrescine on AdoMetDC . . . . .	12
1.3.3. Structure of AdoMetDC . . . . .	13
1.3.3.1. The AdoMetDC fold . . . . .	13
1.3.3.2. AdoMetDC structure, processing and enzyme activity . . . . .	14
1.3.3.3. AdoMetDC structure and putrescine stimulation . . . . .	16
1.3.4. Malarial AdoMetDC . . . . .	17
1.4. Aims . . . . .	18
<b>Chapter 2. Structural modelling of <i>P. falciparum</i> AdoMetDC</b> . . . . .	19
2.1. Introduction . . . . .	19
2.1.1. The need for Bioinformatics . . . . .	19
2.1.2. Computational protein modelling . . . . .	20
2.2. Methods . . . . .	22
2.2.1. Identification of other <i>Plasmodium</i> sequences . . . . .	22
2.2.2. Multiple alignment . . . . .	22

2.2.3.	Secondary structure prediction . . . . .	23
2.2.4.	Homology modelling . . . . .	23
2.3.	Results . . . . .	25
2.3.1.	Identification of other <i>Plasmodium</i> sequences . . . . .	25
2.3.2.	Alignment and motif identification . . . . .	26
2.3.3.	Secondary structure prediction . . . . .	27
2.3.3.1.	Inserts . . . . .	27
2.3.4.	Homology modelling . . . . .	28
2.3.4.1.	Overall model characteristics . . . . .	28
2.3.4.2.	Active site residues . . . . .	31
2.3.4.3.	Active site shape . . . . .	35
2.3.4.4.	Structure of insert 1 . . . . .	36
2.4.	Discussion . . . . .	37
2.4.1.	Identification of other <i>Plasmodium</i> sequences . . . . .	37
2.4.2.	Sequence properties of the <i>Plasmodium</i> AdoMetDC/ODC . . . . .	38
2.4.2.1.	Conservation of secondary structural elements . . . . .	38
2.4.2.2.	<i>Plasmodium</i> -specific inserts . . . . .	39
2.4.3.	Homology modelling . . . . .	41
2.4.3.1.	Overall model characteristics . . . . .	41
2.4.3.2.	Active site composition . . . . .	42
2.4.3.3.	Active site shape . . . . .	44
2.4.3.4.	Structure of insert 1 . . . . .	44
<b>Chapter 3. Model guided mutational analysis of malarial AdoMetDC . . . . .</b>		<b>46</b>
3.1.	Introduction . . . . .	46
3.2.	Methods . . . . .	48
3.2.1.	<i>In silico</i> putrescine docking . . . . .	48
3.2.2.	Construction of putrescine-like mutants . . . . .	48
3.2.2.1.	Mutagenesis of wild-type bifunctional AdoMetDC/ODC plasmid construct . . . . .	48
3.2.2.2.	Sequencing of putrescine-like mutants . . . . .	49
3.2.3.	Recombinant expression . . . . .	50
3.2.4.	Enzyme Assays . . . . .	51
3.3.	Results . . . . .	51
3.3.1.	Putrescine docking . . . . .	51
3.3.1.1.	Comparison with human and model residues . . . . .	51
3.3.1.2.	Putrescine docking . . . . .	52
3.3.2.	Mutagenesis of wild-type bifunctional AdoMetDC/ODC . . . . .	53
3.3.3.	Expression of mutant AdoMetDC/ODC . . . . .	54
3.4.	Discussion . . . . .	54
3.4.1.	Putrescine docking . . . . .	54

3.4.2. Mutagenesis . . . . .	56
<b>Chapter 4. Model guided inhibitor screening of malarial AdoMetDC . . . . .</b>	<b>58</b>
4.1. Introduction . . . . .	58
4.1.1. <i>In silico</i> ligand docking . . . . .	58
4.2. Methods . . . . .	60
4.2.1. <i>In silico</i> inhibitor screening . . . . .	60
4.2.2. Test compound solutions . . . . .	60
4.2.3. Assays . . . . .	61
4.3. Results . . . . .	61
4.3.1. <i>In silico</i> inhibitor screening . . . . .	61
4.3.2. Solubility of potential inhibitors . . . . .	61
4.3.3. Inhibition of AdoMetDC . . . . .	63
4.4. Discussion . . . . .	65
<b>Chapter 5. Concluding Discussion . . . . .</b>	<b>67</b>
<b>Summary . . . . .</b>	<b>72</b>
<b>Opsomming . . . . .</b>	<b>73</b>
<b>Bibliography . . . . .</b>	<b>74</b>
<b>Appendix A. Supplementary data for chapter 2 . . . . .</b>	<b>85</b>
CLUSTALX protein colouring: . . . . .	85
Swissprot accession numbers for multiple sequence alignment . . . . .	85
<b>Appendix B. Supplementary data for chapter 4 . . . . .</b>	<b>91</b>



## List of Figures

1.1.	The life cycle of <i>P. falciparum</i> . . . . .	2
1.2.	Current global status of malaria resistance . . . . .	3
1.3.	The main polyamines . . . . .	5
1.4.	The generic polyamine pathway . . . . .	7
1.5.	Formation of the AdoMetDC pyruvoyl residue . . . . .	10
1.6.	AdoMetDC reaction mechanism . . . . .	12
1.7.	Topology of human AdoMetDC . . . . .	13
1.8.	Dimer interface of human AdoMetDC . . . . .	14
1.9.	Known AdoMetDC inhibitors . . . . .	15
1.10.	Putrescine-binding and active-sites in the human enzyme . . . . .	16
2.1.	A brief overview of homology modelling . . . . .	22
2.2.	Predicted ORFs from <i>P. berghei</i> and <i>P. yoelii</i> . . . . .	25
2.3.	AdoMetDC fragments from <i>P. chabaudi</i> and <i>P. knowlesi</i> . . . . .	25
2.4.	Homology of human secondary structural elements . . . . .	26
2.5.	Final modelling alignment . . . . .	27
2.6.	Predicted secondary structures of AdoMetDC insert 2 . . . . .	28
2.7.	Consensus secondary structure predictions for AdoMetDC insert 3 . . . . .	29
2.8.	Ramachandran plots of the initial and final models . . . . .	30
2.9.	Topology of the final model . . . . .	31
2.10.	C- $\alpha$ trace superimposition of model on human template . . . . .	31
2.11.	Important model-active site residues . . . . .	32
2.12.	Active site substitutions . . . . .	33
2.13.	Ligand interactions for the model and human active sites with MeAdoMet . . . . .	34
2.14.	Active site shapes of AdoMetDC . . . . .	35
2.15.	Cavity near the sulphonium group . . . . .	36
2.16.	Charge network associated with insert 1 . . . . .	37
3.1.	Putrescine charge networks . . . . .	47
3.2.	Orientation of putrescine . . . . .	52
3.3.	<i>Xba</i> I and <i>Hind</i> III restriction of AdoMetDC/ODC . . . . .	53
3.4.	<i>Xba</i> I and <i>Hind</i> III restriction of mutants . . . . .	53
3.5.	Effect of mutations on AdoMetDC activity. . . . .	54

4.1. Orientations of the top 6 potential NCI inhibitors . . . . .	62
4.2. Overview of effect of identified inhibitors and solvent controls on AdoMetDC activity . . . . .	64
A.1. Multiple alignment . . . . .	87
A.2. Conserved motifs . . . . .	90

## List of Tables

1.1. Effects of key mutations in human AdoMetDC . . . . .	11
2.1. Secondary structure prediction algorithms applied to <i>Plasmodium</i> AdoMetDC/ODC sequences . . . . .	24
2.2. Backbone deviations . . . . .	30
2.3. Model and human active site composition . . . . .	33
3.1. AdoMetDC/ODC mutant primers . . . . .	49
3.2. Residues associated with putrescine stimulation . . . . .	52
3.3. Average relative activities . . . . .	54
4.1. Potential AdoMetDC inhibitors . . . . .	63
5.1. Summary of main differences between the malarial (model) and host enzymes. . . . .	68
B.1. LUDI BIOSYM virtual screening hits . . . . .	91
B.2. ACD virtual screening hits . . . . .	92
B.3. NCI virtual screening hits . . . . .	93



## Typographical conventions

- Computer related abbreviations and terms are given in PROGRAM CODE (usually uppercase) type in order to distinguish them from wet-bench and biological terms.
- Residues are referred to using the standard three letter code followed directly by the residue number of the organism in question. The organism follows directly in italics: *hum: Homo sapiens*, *pot: Solanum tuberosum* (potato). For example Ser68*hum* would refer to serine 68 of the human enzyme. When no species is given in the residue name or in the text, *P. falciparum* is assumed.
- Amino acid substitutions and mutations are indicated using the standard three letter code for the original residue, followed directly by it's position, which is in turn followed by the replacement amino acid, e.g.: Ser68Ala would indicate the replacement of serine 68 with alanine.

## List of Abbreviations

AdoMetDC	S-adenosylmethionine decarboxylase
CGP4884A	4-amidinoindan-1-one-2'-amidinohydrazone
CPM	Counts per minute
DFMO	$\alpha$ -Difluoromethyl ornithine
DDT	Dichlorodiphenyltrichloroethane
DHFR-TS	Dihydrofolate reductase-thymidylate synthase
DHPS	Dihydropteroate synthase
DMF	Dimethyl formamide
DMSO	Dimethyl sulphoxide
DNTP	Deoxynucleotide triphosphate
DTT	Dithiothreitol
EC	Enzyme Commission
EDTA	Ethylene diamine tetra-acetic acid
Glc6PD-6PGL	Glucose-6-phosphate dehydrogenase-6-phosphogluconolactonase
kb	Kilo base
LB	Luria-Bertani
MAOEA	5'-deoxy-5'-[N-methyl-N-[(2-aminooxy)ethyl]amino]adenosine
MeAdoMet	Methyl ester of S-adenosylmethionine
MGBG	Methylglyoxal bis(guanylhydrazone)
MHZPA	5'-deoxy-5'-[N-methyl-N-(3-hydrazinopropyl)amino]adenosine
MW	Relative molecular mass
NMR	Nuclear Magnetic Resonance
ODC	Ornithine decarboxylase
ORF	Open Reading Frame
PCR	Polymerase chain reaction
Pfu	<i>Pyrococcus furiosus</i>
PLP	Pyridoxal-5-phosphate
PMSF	Phenylmethylsulphonyl fluoride
PVL	Pyruvoyl
RMSD	Root Mean Square Deviation

SDS	Sodium dodecylsulphate
TEMED	N,N,N',N'-tetramethylethylenediamine
Tris-HCl	Trishydroxy (methyl-amino) methane / Hydrochloric acid
Wt	Wild-type

## List of Computer Related Terms

ACD	Available Chemicals Directory
BLAST	Basic Local Alignment Sequence Tool
CFF	Consistent force field
CHARMM	Chemistry at HARvard Molecular Mechanics
CLUSTALX	Cluster Alignment (for X windows)
EMBL	European Molecular Biology Laboratory
EMBOSS	European Molecular Biology Open Source Software
FASTA	Fast Alignment
GONNET	Amino acid substitution matrix
GRID	Program from the DOCK suite for generating scoring grids
LIGPLOT	Free program for automatically plotting protein-ligand interactions
MEME	“Multiple Em (Expectation maximisation) for Motif Elicitfication”
MODELLER	Homology modelling based on satisfaction of spatial restraints
NCI	National Cancer Institute (USA)
PAM	Point accepted mutation amino acid substitution matrix
PASS	Prediction of Activity Spectra for Substances
PDB	Protein Data Bank
PERL	Practical extraction and report language
PHRAP	“phragment assembly program” for assembling overlapping DNA segments into contiguous stretches
PLASMODB	<i>Plasmodium</i> genome database
PROCHECK	A useful protein structure validation program
PYMOL	Molecular graphics viewer implemented in PYTHON
SWISS-MODEL	Server for homology modelling
SWISS-PROT	High quality annotated database of protein sequences

## Summary

Malaria affects nearly 500 million people every year. The constant evolution of resistance to existing therapies calls for the identification of new drugs and strategies to fight this disease. One way to facilitate this is the characterisation of novel parasite metabolic pathways and their exploitation. The bifunctional S-adenosylmethionine decarboxylase/Ornithine decarboxylase (AdoMetDC/ODC) enzyme, represents one such target. Within this enzyme reside the two main regulatory activities for the biosynthesis of polyamines. Furthermore, the bifunctional arrangement does not occur in the human host, and is presently unique to *Plasmodium*. This uniqueness therefore represents a potential target for the identification of new *Plasmodium*-specific drugs.

The exploitation of parasitic drug targets can be aided immensely by knowledge of its atomic 3D structure. However, malarial proteins are often reluctant to yield to traditional experimental methods for gathering this information. In this study, a computational approach was followed to gain further insight into the structure of the AdoMetDC domain of the bifunctional enzyme. The AdoMetDC domain was modelled on X-ray crystal structure templates of the human and plant equivalents.

The model revealed a number of differences compared to the human structure. Amino acid substitutions and active site shape differences suggest this enzyme is worthwhile exploiting for the discovery of new drugs. The model also revealed possible reasons for the lack of putrescine stimulation, as seen in humans, and suggested a possible replacement mechanism in the form of internal residues assuming the putrescine's function. The presence of such a replacement mechanism was partially verified experimentally by site-directed mutagenesis and recombinant expression of mutant enzymes.

The model was also used to conduct *in silico* screens against databases of small molecules for the identification of potential inhibitors. Some of these compounds were subsequently subjected to preliminary screening with recombinantly expressed enzyme. No promising inhibitors were found, however, the results provided insights for further inhibitor identification.



## Opsomming

Malaria affekteer nagenoeg 500 miljoen mense per jaar. Die konstante evolusie van weerstand-biedendheid teenoor bestaande terapeutiese middels noodsaak die identifisering en karakterisering van unieke parasietpadweë. Die bifunksionele S-adenosylmetionien dekarboksilase/Ornitien dekarboksilase (AdoMetC/ODC) proteïen verteenwoordig een so 'n teiken. Die bifunksionele ensiem verteenwoordig die twee hoof regulatoriese aktiwiteite vir die biosintese van poliamiene. Verder kom die bifunksionele rangskikking nie voor in die menslike gasheer nie, en is tans uniek tot *Plasmodium*. Hierdie unieke kenmerk verteenwoordig a potensiële teiken vir die identifisering van nuwe *Plasmodium*-spesifieke geneesmiddels.

Die ontwikkeling van parasiet geneesmiddeltekens word aansienlik bevorder deur die kennis van drie-dimensionele atoomstrukture. Malaria proteïene is dikwels moeilike tekens vir tradisionele eksperimentele metodes om hierdie inligting te bekom. In hierdie studie is 'n rekenaargestesteunde benadering gevolg om verdere insig in die struktuur van AdoMetDC van die bifunksionele proteïen te bekom. Die AdoMetDC domein is gemodelleer op grond van die kristalstruktuur template van die menslike en plant ekwivalente.

Die model het 'n aantal verskille opgewys in vergelyking met die menslike struktuur. Aminosuur substitusies en vormverskille in die aktiewe setel dui aan dat die ensiem waarskynlik geskik is vir ontwikkeling van nuwe geneesmiddels. Die model het ook 'n moontlike verklaring gebied vir die afwesigheid van putresien stimulasie, soos wat by mense aangetref word, en het gedui op 'n moontlike vervangende meganisme in die vorm van interne residue wat die funksie van putresien oorneem. Die teenwoordigheid van so 'n vervangingsmeganisme is gedeeltelik eksperimenteel bevestig deur middel van setel-gerigte mutagenese en rekombinante uitdrukking van mutante ensieme.

Die model is ook gebruik om *in silico* sifting teen kleinmolekuul databasisse uit te voer, met die oog op die indentifikasie van nuwe potensiële inhibitore. Sommige van die middels is daarna gebruik vir voorlopige toetsing teenoor die rekombinante ensiem. Geen belowende inhibitore is gevind nie, alhoewel, die resultate verskaf insig vir verdere inhibitor identifikasie.