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

by

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Submitted in partial fulfilment of the requirements for the degree *Magister Scientiae*

in the Faculty of Natural and Agricultural Sciences

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Pretoria

January 2004

Acknowledgements

- My supervisor Prof. A. I. Louw, and cosupervisor Dr Fourie Joubert of the University of Pretoria Biochemistry Department for enabling me to pursue this project and enter the world of structural biology.
- My cosupervisor Dr Lyn-Marie Birkholtz for always being prepared to give much needed advice and criticism.
- Prof. Walter of The Bernhard Nocht Institute (BNI) for Tropical Medicine, Hamburg, Germany for giving me the opportunity to visit his laboratory. This enabled me to perform the experiments described herein and allowed me to gain invaluable experience.
- My fellow students of the University of Pretoria and the students of the BNI for helping a self-confessed and incurable computer geek to get his hands wet doing real Biochemistry.
- Prof S. Ealick (Cornell University, USA) for providing the crystal structure of the potato enzyme prior to publication.
- The National Research Foundation (NRF) of South Africa and the University of Pretoria for awarding me the bursaries which enabled me to continue my studies.
- No Microsoft® employees were directly harmed in the making of this production: I am indebted to the Open Source Community for providing much of the software that has enabled me to do my work over the last three years[1].
- My family for their support and patience. Specifically, my parents for making the invaluable purchase of a 486 DX2 50 Mhz computer 9 years ago.

[1] This manuscript and almost all figures were prepared using Open Source Software. Typesetting was done in LaTeX 2_ε, using version 1.3 of the LyX editor.

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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	α-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	*Pyroccocus furiosis*
PLP	Pyridoxal-5-phosphate
PMSF	Phenylmethylsulphonyl fluoride
PVL	Pyruvoyl
RMSD	Root Mean Square Deviation

SDS	Sodium dodecylsulphate
TEMED	N,N,N',N'-tetramethylenediamine
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	*Plasmodium* 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 weerstandbiedendheid 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 geneesmidddels.

Die ontwikelling van parasiet geneesmiddelteikens word aansienlik bevorder deur die kennis van drie-dimensionele atoomstrukture. Malaria proteïene is dikwels moeilike teikens vir tradisionele eksperimentele metodes om hierdie inligting te bekom. In hierdie studie is 'n rekenaargesteunde 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 ontwikelling 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 duer 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.