Delineation of functional roles of parasite-specific inserts in the malarial S-adenosylmethionine decarboxylase / ornithine decarboxylase

By

Marni Williams
25026519

Submitted in partial fulfilment of the requirements for the degree MSc. Biochemistry in the Faculty of Natural and Agricultural Science

Department of Biochemistry
University of Pretoria
Pretoria
South Africa

February 2008

I declare that the thesis/dissertation, which I hereby submit for the degree MSc. Biochemistry at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

SIGNATURE: ……..

DATE: …….

Acknowledgements

- Prof A.I. Louw, University of Pretoria, South Africa (Promoter)
- Dr L. Birkholtz, University of Pretoria, South Africa (Co-promoter)
- Prof R.D. Walter, Dr C. Wrenger, Dr I.B. Müller, M. Eschbach, B. Bergmann, Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany (Collaborative research visit in 2006)
- Dr P.F.G. Sims, Manchester Interdisciplinary Biocentre, Manchester, United Kingdom (Mass spectrometric analysis)
- Dr M. Rautenbach, University of Stellenbosch, South Africa (Design of synthetic peptides)
- G.A. Wells, University of Pretoria, South Africa (Secondary structure predictions)
- National Research Foundation (Grand holder's bursary)
- Family and friends

Contents

List of Figures

List of Tables

List of Abbreviations

ACC	Acetyl-CoA carboxylase
ACT	Artemisinin-based combination therapy
ADA	Adenosine deaminase
AdoDATO	S-adenosyl-1,8-diamino-3-thio-octane
AdoMet	S-adenosylmethionine
AdoMetDC	S-adenosylmethionine decarboxylase
AdoMetDC/ODC	S-adenosylmethionine decarboxylase/ornithine decarboxylase
AHT	Anhydrotetracycline
AMA-1	Apical membrane antigen 1
AMP	Adenosine 5'-monophosphate
APA	3-Aminooxy-1-aminopropane
AZ	Antizyme
bp	Base pair
BSA	Bovine serum albumin
bZIP	Basic-leucine-zipper
CCMB	Calcium-manganese based
CPM	Counts per minute
CSP	Circumsporozoite protein
DDT	bis (4-chlorophenyl)-1,1,1-trichloroethane
DFMO	α-Difluoromethylornithine
DHFR	Dihydrofolate reductase
DHFS	Dihydrofolate synthase
DHNA	Dihydroneopterin aldolase
DHPS	Dihydropteroate synthase
DHS	Deoxyhypusine synthase
DMSO	Dimethyl sulfoxide
DOHH	Deoxyhypusine hydroxylase
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide 5'-triphosphate
DTT	Dithiothreitol
EDTA	Ethylenediamine tetraaceticacid
eIF5A	Eukaryotic translation initiation factor 5A
ENR	Enoyl-ACP-reductase
FMP-1	Falciparum merozoite protein 1
FP	Fowl pox
FPGS	Folylpolyglutamate synthase
FPLC	Fast protein liquid chromatography
Glc6PD/6PGL	Glucose-6-phosphate dehydrogenase/6-phosphoglucolactonase
GluK	Glutamate-5-kinase
Glu-PRed	Glutamylphosphate reductase
GLURP	Glutamine-rich protein
GluS	Glutamate synthase
GMP	Guanosine 5'-monophosphate
GpA	Glycophorin A
GTP	Guanosine 5'-triphosphate
GTPCH	Guanosine 5'-triphosphate cyclohydrolase I

HABA	4-Hydroxy azobenzene-2-carboxylic acid
HBsAg	Hepatitis B surface antigen
HCA	Hydrophobic cluster analysis
HDM2	Human double minute 2
HGXPRT	Hypoxanthine-guanine-xanthine phosphoribosyl transferase
HIV/AIDS	Human immunodeficiency virus/acquired immunodeficiency syndrome
HPLC	High performance liquid chromatography
Hrs	Hours
HSP	Heat shock protein
HSS	Homospermidine synthase
IMP	Inosine 5'-monophosphate
kb	Kilobase
kDa	Kilodalton
LB	Luria Bertani
LCRs	Low-complexity regions
LSA-1	Liver stage antigen 1
4MCHA	*trans*-4-methylcyclohexylamine
MDM2	Mouse double minute 2
ME-TRAP	Multi-epitope thrombospondin-related adhesive protein
MMV	Medicines for Malaria Venture
MS	Mass spectrometry
MSP	Merozoite surface protein
MTA	5'-Methylthioadenosine
MTI	5'-Methylthioinosine
MVA	Modified vaccinia virus Ankara
MVI	Malaria Vaccine Initiative
MW	Molecular weight
NaCl	Sodium chloride
NADH	Nicotinamide adenine dinucleotide
nt	nucleotide
OAc	Acetate
OAT	Ornithine aminotransferase
ODC	Ornithine decarboxylase
pABA	*p*-aminobenzoic acid
PAGE	Polyacrylamide gel electrophoresis
PAO	Polyamine oxidase
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PCRed	1-Pyrroline-5-carboxylate reductase
PdxK	Pyridoxal kinase
PfEMP1	*Plasmodium falciparum* erythrocyte membrane protein 1
Pfs	*P. falciparum* surface antigens
PNP	Purine nucleoside phosphorylase
PLP	Pyridoxal-5'-phosphate
PMSF	Phenylmethylsulfonyl fluoride
PPPK	Hydroxymethyldihydropterin pyrophosphokinase
QCM	QuickChange™ site-directed mutagenesis

RBC	Red blood cell
RBM	Roll Back Malaria
RE	Restriction enzyme
rPfHinge-ODC	Recombinant *P. falciparum* ODC plus 144 amino acids of the hinge region
rPfODC	Recombinant *P. falciparum* ODC
RNA	Ribonucleic acid
rpm	Revolutions per minute
SA	Specific activity
SDS	Sodium dodecylsulphate
SDS-PAGE	Sodium dodecylsulphate polyacrylamide gel electrophoresis
SEC	Size-exclusion chromatography
SERCA	Sarcoplasmic-endoplasmic reticulum calcium adenosine triphosphatase
SHMT	Serine hydroxymethyltransferase
SNAP25	Synaptosome-associated protein 25
SNARE	Soluble *N*-ethylmaleimide-sensitive factor attachment protein receptors
SpdSyn	Spermidine synthase
SpmSyn	Spermine synthase
SSAT	Spermidine/spermine-N^1-acetyltransferase
TAE	Tris-acetate ethylenediamine tetraaceticacid
TEMED	N,N,N,N -Tetramethyl-Ethylenediamine
Tm	Melting temperature
TRAP	Thrombospondin-related adhesive protein
Tris-HCl	Trishydroxy (methyl-amino) methane/hydrochloric acid
TS	Thymidylate synthase
U	Units
UNICEF	United Nations Children's Fund
UV	Ultra Violet
WHO	World Health Organisation

Summary

The polyamines putrescine, spermidine and spermine play essential roles in the proliferation and differentiation of most eukaryotic cells. Inhibition of the polyamine pathway is known to have antitumour and antiparasitic effects and α-difluoromethylornithine (DFMO), a polyamine biosynthesis inhibitor, is clinically used in the treatment of African sleeping sickness caused by *Trypanosoma brucei gambiense*. Ornithine decarboxylase (ODC) and S-adenosylmethionine decarboxylase (AdoMetDC) are the rate-limiting enzymes in polyamine metabolism. Usually, these enzymes are individually regulated, however, in the malaria parasite, *Plasmodium falciparum*, these enzymes are part of a unique bifunctional PfAdoMetDC/ODC protein. In addition, compared to homologous proteins, this malarial protein contains six unique parasite-specific inserted regions, which can be targeted with novel drugs.

A modified restriction enzyme-mediated inverse PCR method was developed to delete the largest parasite-specific insert (411 bp) from the large *PfAdoMetDC/ODC* gene (4257 bp). The method was compared to existing deletion mutagenesis PCR protocols and was shown to be the most effective method (80% mutagenesis efficiency) as opposed to the 40% positively mutated clones obtained with the overlapping primer method in deleting a >100 bp region. The independent removal of all three the PfAdoMetDC domain parasite-specific inserts and subsequent activity analysis thereof showed that these inserts are essential for the catalytic activities of both the decarboxylase domains. *Plasmodia* conserved secondary structures within these inserts were identified and were also shown to be very important for domain activities, possibly through protein-protein interactions across and within the domains of the bifunctional complex for the efficient regulation of intracellular polyamine levels.

The N-terminally located O1 insert in the PfODC domain is a highly conserved and structurally distinct insert, which is essential for both domain activities. Previous studies showed that the deletion of this insert prevents dimerisation of the PfODC monomers and as a result influences association of PfODC with the PfAdoMetDC domain to form the bifunctional ~330 kDa complex. In addition, immobilisation of the insert via the mutagenesis of flanking Gly residues and the disruption of a single conserved α-helix within the insert severely affected both PfODC and PfAdoMetDC activities. It was thus hypothesised that the helix is involved in protein-protein interactions and the dimerisation of the PfODC domain. Size-exclusion chromatography of the monofunctional PfODC and bifunctional PfAdoMetDC/ODC proteins with disrupted helices resulted in the elution of only the monomeric (~85 kDa) and heterodimeric PfAdoMetDC/ODC (~160 kDa) proteins,

respectively. The mono- and bifunctional wild type and immobile proteins eluted as both dimeric PfODC (~170 kDa) and heterotetrameric (~330 kDa) fractions as a result of intact protein-protein interactions. These results were subsequently exploited in the design and application of a parasite-specific, mechanistically novel, inhibitory peptide specific for this non-homologous insert in the bifunctional protein. A 1000x molar excess of a synthetic peptide, complementary to the α-helix within the O1 insert but opposite in charge, resulted in a ~40% inhibition of the PfODC enzyme. This study thus provides a proof-of-principle for the use of an inhibitory peptide targeting a parasite-specific insert in the dimerisation interface of a uniquely bifunctional malarial protein.