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Biochemical and structural characterization of novel drug targets regulating polyamine biosynthesis in the human malaria parasite, *Plasmodium falciparum*

By

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Summary

Malaria is prevalent in over 100 countries which is populated by half of the world's population and culminates in approximately one million deaths per annum, 85% of which occurs in sub-Saharan Africa. The combined resistance of the mosquitoes and parasites to the currently available pesticides and antimalarial chemotherapeutic agents requires the concerted effort of scientists in the malaria field to identify and develop novel mechanisms to curb this deadly disease.

In this study, a thorough understanding of the role players in the polyamine pathway of the parasite was obtained, which could aid future studies in the development of novel inhibitory compounds against these validated drug targets. The uniquely bifunctional *S*-adenosylmethionine decarboxylase/ornithine decarboxylase (AdoMetDC/ODC) of *Plasmodium falciparum* forms an important controlling node between the polyamine and methionine metabolic pathways. It has been speculated that the unique bifunctional association of the rate-limiting enzymes allows for the concerted regulation of the respective enzyme activities resulting in polyamine synthesis as per requirement for the rapidly proliferating parasite while the methionine levels are strictly controlled for their role in the methylation status. The results of this study showed that the enzyme activities of the bifunctional complex are indeed coordinated and subtle conformational changes induced by complex formation is suggested to result in these altered kinetics of the individual AdoMetDC and ODC domains. Studies also showed that the identification of the interaction sites between the domains, which allows for communication across the complex, may be targeted for specific interference with the enzyme activities. Furthermore, these studies showed that the current knowledge on the different subclasses of the AdoMetDC family should be re-evaluated since *P. falciparum* AdoMetDC shows diverse properties from orthologues and therefore points towards a novel grouping of the plasmodial protein. The extensive biochemical and biophysical studies on AdoMetDC has also provided important avenues for the crystallisation and solving of this protein's 3D structure for subsequent structure-based identification of drug-like lead compounds against AdoMetDC activity.

The application of structure-based drug design on malarial proteins was additionally investigated and consequently proved that the rational design of lead inhibitory compounds can provide important scaffold structures for the identification of the key aspects that are required for the successful inhibition of a specific drug target. Spermidine synthase, with its intricate catalytic mechanism involving two substrate binding sites for the products of the reactions catalysed by

AdoMetDC/ODC, was used to computationally identify compounds that could bind within its active site. Subsequent testing of the compounds identified with a dynamic receptor-based pharmacophore model showed promising inhibitory results on both recombinant protein and *in vitro* parasite levels. The confirmation of the predicted interaction sites and identification of aspects to improve inhibitor interaction was subsequently investigated at atomic resolution with X-ray protein crystallography.

The outcome of this doctoral study shows the benefit in applying a multidisciplinary and multinational approach for studying drug targets within the malaria parasite, which has led to a thorough understanding of the targets on both biochemical and structural levels for future drug design studies.

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List of Abbreviations

AbeAdo:	5'-([(Z)-4-amino-2-butenyl]methylamino)-5'-deoxyadenosine
AdoDATO:	*S*-adenosyl-1,8-diamino-3-thio-octane
ACT:	artemisinin-based combination therapy
AdoMet:	*S*-adenosyl-L-methionine
AHT:	anhydrotetracycline
aIEX:	anion exchange chromatography
AMA-1:	apical membrane antigen 1
APA:	3-aminooxy-1-aminopropane
APE:	5-amino-1-pentene
ASU:	asymmetric unit
CD:	circular dichroism
CGP48664:	4-amidinoindan-1-one-2'-amidinohydrazone
CHA:	cyclohexylamine
CSP:	circumsporozoite protein
2D:	two-dimensional
3D:	three-dimensional
Da:	Dalton
dcAdoMet:	decarboxylated *S*-adenosyl-L-methionine
DDT:	bis(4-chlorophenyl)-1,1,1-trichloroethane
DEAE:	diethylaminoethyl-cellulose
DFMO:	D,L-α-difluoromethylornithine
DHFR:	dihydrofolate reductase
DHPS:	dihydropteroate synthase
DLS:	differential light scattering
DMSO:	dimethyl sulfoxide
DPM:	dynamic pharmacophore model
DSF:	differential scanning fluorimetry
DTT:	dithiothreitol
EDTA:	ethylenediaminetetraacetic acid
eIF-5A:	eukaryotic translation initiation factor 5A
GLURP:	glutamine-rich protein
G6PD:	glucose 6-phosphate dehydrogenase
HBA:	hydrogen bond acceptor
HBD:	hydrogen bond donor
HEPES:	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HRP:	horseradish peroxidase
Hsp70:	heat shock protein 70 kDa
HYD:	hydrophobic
IC_{50}:	inhibitory concentration at 50%
IRS:	indoor residual spraying
ITN:	insecticide-treated mosquito net
JCSG:	Joint Structural Genomics Consortium

kDa:	kilodalton
LB:	Luria-Bertani
LC-MS:	liquid chromatography-mass spectrometry
LSA-1:	liver stage antigen 1
MALDI-MS:	matrix-assisted laser desorption/ionisation-mass spectrometry
4MCHA:	*trans*-4-methylcyclohexyl amine
MD:	molecular dynamics
MDL73811:	5'-([(Z)-4-amino-2-butenyl]methylamino)-5'-deoxyadenosine
MES:	2-(*N*-morpholino)ethanesulfonic acid
MGBG:	methylglyoxal bis(guanylhydrazone)
MIF:	molecular interaction field
MSP-1:	merozoite stage protein 1
MTA:	5'-methylthioadenosine
MWCO:	molecular weight cut-off
NAC:	*N*-(3-aminopropyl)-cyclohexylamine
NACD:	*N*-(3-aminopropyl)-*trans*-cyclohexane-1,4-diamine
Ni-NTA:	nickel-nitrilo triacetic acid
OD:	optical density
pABA:	*p*-aminobenzoic acid
PBS:	phosphate buffered saline
PdI:	polydispersity index
*Pf*AdoMetDC:	*Plasmodium falciparum S*-adenosylmethionine decarboxylase
*Pf*AdoMetDC/ODC:	*Plasmodium falciparum S*-adenosylmethionine decarboxylase/ornithine decarboxylase
*Pf*CRT	*Plasmodium falciparum* chloroquine transporter
*Pf*DHFR/TS:	*Plasmodium falciparum* dihydrofolate reductase/thymidylate synthase
*Pf*EMP1:	*Plasmodium falciparum* erythrocyte membrane protein 1
*Pf*ODC:	*Plasmodium falciparum* ornithine decarboxylase
*Pf*s:	*Plasmodium falciparum* surface antigen
*Pf*SpdS:	*Plasmodium falciparum* spermidine synthase
*Pf*TIM:	*Plasmodium falciparum* triosephosphate isomerase
Pgh1:	P-glycoprotein homologue 1
PhFs:	pharmacophore features
PLP:	pyridoxal-5'-phospate
PMSF:	phenylmethylsulphonyl fluoride
PPPK:	hydroxymethyldihydropterin pyrophosphokinase
PVDF:	polyvinylidene fluoride
qPCR:	quantitative PCR
RMSD:	root mean square deviation
RT:	room temperature
SDS-PAGE:	sodium dodecyl sulphate polyacrylamide gel electrophoresis
SEC:	size-exclusion chromatography
S.E.M:	standard error of the mean

SGC:	Structural Genomics Consortium
TEV:	tobacco etch virus
TFA:	trifluoroacetic acid
Tm:	melting temperature
TOF:	time-of-flight
TRAP:	thrombospondin-related adhesive protein
TS:	thymidylate synthase
UTR:	untranslated region
UV:	ultra violet
WHO:	World Health Organisation
wt:	wild-type