Analysis of the ATCase catalysis within the amino acid metabolism of the human malaria parasite Plasmodium falciparum

Bosch, Soraya Soledad

Analysis of the ATCase catalysis within the amino acid metabolism of the human malaria parasite *Plasmodium falciparum*

Soraya Soledad Bosch

university of groningen

UDD_
Unit for Drug Discovery

Analysis of the ATCase catalysis within the amino acid metabolism of the human malaria parasite *Plasmodium falciparum*
Soraya Soledad Bosch

PhD Thesis
University of Groningen, The Netherlands
University of São Paulo, Brazil
March 2019

The research described in this thesis was carried out at the Unit for Drug Discovery, Department of Parasitology, Institute of Biomedical Sciences at the University of São Paulo, Brazil and at the Structural Biology Unit, Department of Drug Design, Groningen Research Institute of Pharmacy at the University of Groningen, The Netherlands and was financially supported by an Ubbo Emmius and a FAPESP (project number 2013/17577-9) fellowship, further by the CAPES/Nuffic MALAR-ASP network and Marie Sklodowska-Curie grant Agreement No. 675555, Acelerated Early stage drug discovery (AEGIS).

Printing of this thesis was financially supported by the University Library and the Graduate School of Science, Faculty of Science and Engineering, University of Groningen, The Netherlands.

Printing: Zalsman Groningen B.V.

ISBN: 978-94-034-1414-0 (Printed version)
ISBN: 978-94-034-1413-3 (Electronic version)

Layout: Soraya Soledad Bosch
Cover design: Soraya Soledad Bosch. The image used for the cover page was took during the experiments performs in the production of this thesis. It is an image take of *in vivo* culture of *Plasmodium falciparum*.

university of groningen

Analysis of the ATCase catalysis within the amino acid metabolism of the human malaria parasite Plasmodium falciparum

Phd thesis

to obtain the degree of PhD of the
University of Groningen
on the authority of the
Rector Magnificus Prof. E. Sterken
and in accordance with
the decision by the College of Deans

and

to obtain the degree of PhD of the
University of São Paulo
on the authority of the
Rector Prof. Dr. V. Agopyan
and in the accordance with
the decision by the College of Deans

Double PhD degree

This thesis will be defended in public on

Monday 11 March 2019 at 12:45 hours

by

Soraya Soledad Bosch

born on 18 February 1988
in Avellaneda, Santa Fe, Argentina

Supervisors

Prof. A.S.S. Dömling
Prof. C. Wrenger

Co-supervisor

Prof. M.R. Groves

Assessment Committee

Prof. W.J. Quax
Prof. F.J. Dekker
Prof. E. Liebau
Prof. G. Wunderlich

Paranymph(s)

Fernando A. Batista, MSc
Sergey Lunev, PhD

Esta tesis esta dedicada a mi familia, por todo el esfuerzo que hicieron desde el primer día en que decidí venir a San Pablo para construir este camino, por dejarme abrir las alas y volar lejos de casa, por todos los viajes que hicieron para visitarme alrededor del mundo, por todas las despedidas, por todo el cariño y amor que les tengo, pero sobre todo por lo mucho que los extraño todos los dias.

This thesis is dedicated to my family, for all the effort they give me since day 1 when they bring me, literally, to São Paulo to start this new life, for let me open my wings, for all the trips they made to visit me, all around the world, for the goodbyes, for all the love I have for them, and most important for all I miss them every day.

Table of Contents

Abstract (Dutch)

BOSCH, S.S. **Analyse van de ATCase katalyse binnen het aminozuurmetabolisme van de *Plasmodium falciparum* malariaparasiet.** 2019. 103p. PhD (Parasitology) − Institute of Biomedical Sciences, University of São Paulo and University of Groningen, São Paulo, 2019.

Malaria, veroorzaakt door de Plasmodium parasiet, blijft met over 600,000 doden per jaar één van de meest verwoestende ziektes van onze tijd. Plasmodium falciparum, die de tropische variant van malaria veroorzaakt, is de meest gevaarlijke soort binnen het genus. Het doel van dit proefschrift is het evalueren van het belang van het enzym aspartaat carbamoyltransferase (ATCase) binnen het aspartaatmetabolisme van de P. falciparum parasiet. Het Open Reading Frame dat voor het eiwit codeert is geïdentificeerd en gekloneerd. Na het gecodeerde eiwit recombinant tot expressie te brengen konden we conformationeel en kinetisch inzicht verkrijgen met behulp van kristallisatie-experimenten, en konden we de kristalstructuur van het eiwit ophelderen in “T” (tense, gespannen) en “R” (relaxed, ontspannen) vorm. Daarnaast laten we het belang van PfATCase zien voor de proliferatie van de malariaparasiet aan de hand van mutagene studies en eiwit-interferentie experimenten. Zoals voorspeld door bio-informatica instrumenten heeft het eiwit een apicoplast-targeting sequence, een aantal aminozuren die ervoor zorgen dat het eiwit in de apicoplast belandt. Hiermee is de lokalisatie van het eiwit in de apicoplast bewezen.
Voorts richt dit werk zich op het onderzoeken van ATCase als geneesmiddeltarget. De resultaten van de dosis-response studies en in vivo eiwitinterferentie experimenten bewijzen dat het eiwit een goede kandidaat is als geneesmiddeltarget.

Kernwoorden: *Plasmodium falciparum*, Kristalstructuur, Pyrimidine, Geneesmiddeltarget-validatie.

Abstract (English)

BOSCH, S.S. **Analysis of the ATCase catalysis within the amino acid metabolism of the human malaria parasite *Plasmodium falciparum*** 2019. 103p. Ph.D. (Parasitology) − Institute of Biomedical Sciences, University of São Paulo and University of Groningen, São Paulo, 2019.

Malaria, caused by *Plasmodium* spp., remains with more than 400.000 deaths per year one of the devastating diseases of our time. *Plasmodium falciparum*, which causes tropical malaria, is the most dangerous one leading to severe malaria. The aim of this thesis was to evaluate the necessity of the aspartate carbamoyltransferase (ATCase) within the aspartate metabolism of the human malaria parasite *Plasmodium falciparum*. The respective open reading frame has been identified and was cloned; with the encoded enzyme recombinantly expressed we could get conformational and kinetic insights by crystallization experiments, we could resolve the crystal structure of the enzyme, in "T' (tense) and "R' (relaxed) states. Moreover, in this work, we show the importance of the *Pf*ATCase for the proliferation of the malaria parasite by mutagenic studies and protein interference experiments.

As predicted by bioinformatic tools the protein bears an apicoplast-targeting sequence and therefore its localization was determined here. Furthermore, this work is focusing on the ATCase as a drug target, dose-response experiments and protein interference studies with *in vivo* parasites, proves our hypothesis and the drugability of the enzyme.

Keywords: *Plasmodium falciparum*, Crystal structure, Pyrimidine, Drug target validation.

Resumo

BOSCH, S.S. **Análise da catalises da Aspartato Carbamoyltransferase dentro do metabolismo de amino ácidos do parasita efetor da malária humana *Plasmodium falciparum*.** 2019. 103f. Tese (Doutorado em Parasitologia) - Instituto de Ciências Biomédicas, Universidade de São Paulo e Universidade de Groningen, São Paulo, 2019.

A malária, causada por *Plasmodium* spp., continua sendo uma das doenças mais devastadoras do nosso tempo, com mais de 600.000 mortes por ano. O *Plasmodium falciparum*, é o parasita mais perigoso que produze à malária severa. O objetivo desta tese foi avaliar a necessidade da aspartato carbamoiltransferase (ATCase) no metabolismo do aspartato do parasita da malária humana *Plasmodium falciparum*. O respectivo ORF foi identificado e clonado; com a enzima recombinante expressa, conseguiu-se obter informações conformacionais e cinéticas. Por meio de experimentos de cristalização obteve-se a estrutura tridimensional da enzima, nos estados "T" (tenso) e "R" (relaxado). Além disso, neste trabalho, mostramos a importância do *Pf*ATCase para a proliferação do parasita da malária através de estudos mutagênicos e experimentos de interferência de proteínas. Como previsto por ferramentas bioinformáticas, a proteína possui uma seqüência de direcionamento de apicoplasto e, portanto, sua localização foi determinada em este trabalho.

Os ensaios de drogas, assim como, os ensaios de proliferação dos parasitas *in vivo*, demonstrou que a ATCase é um alvo terapêutico no parasita.

Palavras−chave: *Plasmodium falciparum*. Pirimidinas. Sínteses de pirimidinas. Estrutura cristalográfica. Alvo terapêutico.