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Engineering Corticosteroid-Binding Globulin to Release New Compounds at New Sites

Wee Lee Chan, Aiwu Zhou, Randy J Read. *Department of Haematology, Cambridge Institute for Medical Research, Wellcome Trust/MRC Building, Addenbrooke's Hospital, University of Cambridge (United Kingdom).* E-mail: wlc29@cam.ac.uk

Corticosteroid-binding globulin (CBG) is a blood plasma protein that transports the weakly water-soluble hormone, cortisol, throughout the circulation. It is a member of the serine protease inhibitor (SERPIN) structural family; upon cleavage by human neutrophil elastase, it undergoes the canonical S-to-R transition, which results in a change in its binding affinity for cortisol, allowing it to release the hormone at sites of inflammation. We plan to redesign CBG to transport compounds other than its physiological ligand and, by harnessing the S-to-R transition, release them at specific sites in the body.

In preliminary experiments, we succeeded in altering the proteinase specificity of CBG, making it susceptible to cleavage by human α-thrombin. We plan to build on this and prove the principle that by altering the amino acid sequence of the reactive centre loop, CBG can be made to release its ligand in response to specific cleavage by various proteinases. We are especially interested in making it susceptible to proteinases that are tissue-specific so that the engineered protein would be able to deliver its cargo at very specific sites in the body.

We are also looking at re-designing the steroid-binding pocket of CBG so that it is able to bind other compounds with high affinities. Apart from that, it is also necessary to make the change in affinity between the S-state and the R-state much larger should the engineered protein ever be considered for use as a drug-delivery system. In order to study the binding site, and to understand in depth how the change in binding affinity is achieved by the S-to-R transition, it is necessary to obtain crystal structures of CBG in both forms from the same organism as the structures currently available for the native and cleaved forms of CBG are from different organisms [1], [2]. This makes it difficult to tell if the differences in the positions of various residues in the binding pocket are due to conformational changes or a lack of sequence identity.

These structural studies will be complemented with molecular modeling and biochemical experiments to determine how the residues lining the binding pocket contribute to ligand binding and to the mechanism that results in the change in ligand-binding affinity following proteolytic cleavage of the reactive centre loop.

Whilst studying other factors that can affect ligand binding, we also discovered that binding affinity decreases when temperature increases, even within the small range allowed physiologically. This observation was corroborated by a recent study by Cameron *et al* [3]. We think this is another property we can exploit because the local temperature in different parts of the body can vary with tissue type and disease state, and consequently may add another level of specificity for the release of potentially therapeutic compounds from the engineered protein.

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Structure-based design of anti-trypanosomal drugs

Matheus Pinto Pinheiro,[a] Josmar Rodrigues da Rocha,[b] Juliana Cheleski,[b] Helton José Wiggers,[b] Carlos Alberto Montanari,[b] Maria Cristina Nonato,[a] [a]*Laboratório de Cristalografia de Proteínas, Faculdade de Ciências Farmacêuticas de Ribeirão Preto - Universidade de São Paulo, FCFRP-USP, Brazil.* [b]*Grupo de Química Medicinal, Instituto de Química de São Carlos - Universidade de São Paulo, IQSC-USP, Brazil.* E-mail: mpp@fcfrp.usp.br

Dihydroorotate dehydrogenase (DHODH) catalyses the conversion of L-dihydroorote (DHO) to orotate, the fourth step and only redox reaction in the *de novo* pyrimidine biosynthetic pathway. The DHODHs can be divided into two major classes on the basis of their amino acid sequences and their cellular location. The enzyme dihydroorotate dehydrogenase (DHODH) has been considered a promising target for the design of trypanocidal agents. Interestingly, trypanosomatids and human DHODH have the different origins, belonging to families 1 and 2, respectively, and display structural differences that can be exploited for the development of selective drugs for the treatment of trypanosomal diseases.

It is our aim to contribute to the development of selective inhibitors for trypanosomal DHODHs. In order to achieve this goal, we have explored the human, *T. cruzi* and *L. major* enzymes, to guide the search for molecules that selectively bind to the trypanosomal enzymes, which is an important rationale behind the design of chemotherapeutic agents.

We report here the discovery of novel inhibitors of Trypanosomatids DHODH identified by virtual screening method and the crystal structure of these ligands in complex with DHODH from *L. major*. The crystal structure of DHODH from *L. major* was solved in the presence of different ligands. Monitoring of the enzymatic reaction in the presence of selected ligands together with structural information obtained from X-ray crystallography analysis have allowed the identification and validation of a novel site of interaction. Our results have provided important structural insights for the rational design of *T. cruzi* and *Leishmania major* DHODH inhibitors.

Keywords: Trypanosomatids, dihydroorotate dehydrogenase, drug design

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Biophysical and biochemical characterization of fumarases from *Leishmania major*

Patrícia Rosa Feliciano,[a] Marcelo Dias Baruffi,[b] Antonio Jose da Costa Filho,[c] Paul A. M. Michels,[d] Maria Cristina Nonato,[a] [a]*Laboratório de Cristalografia de Proteínas - FCFRP - USP, Ribeirão Preto - SP, (Brazil).* [b]*Departamento de Análises Clínicas, Toxicológicas e Bromatológicas - FCFRP – USP, Ribeirão Preto - SP, (Brazil);* [c]*Instituto de Física de São Carlos – USP, São Carlos - SP, (Brazil);* [d]*Research Unit for Tropical Diseases, de Duve Institute, UCL, Brussels, (Belgium).* E-mail: patricia.rf@usp.br

Leishmaniases, classified as neglected tropical diseases, is caused by the parasite *Leishmania* and affect 12 million people in 88 countries around the world.

Fumarate hydratases (FHs) catalyse the stereospecific reversible hydration of fumarate to malate. Eukaryotes express two isoforms of FH, the mitochondrial isoform which performs this reaction as part of the tricarboxylic acid cycle and as such is central to aerobic respiration and the cytosolic isoform which is thought to be involved in the metabolism of fumarate. As a first step aiming the validation of *Leishmania major* FH (LmFH) as target for drug design against leishmaniases, the recombinant enzymes have been used to perform kinetic, biophysical and structural characterization.

Circular dichroism studies have identified differences in secondary