Preliminary Studies on Glucose-6-Phosphate Dehydrogenase from Trypanosoma cruzi

Cecilia Ortiz,* Horacio Botti,* Nicole Larrieux,* Andrea Medeiros,* Alejandro Buschiazzo,* Marcelo A. Comini; *Group Redox Biology of Trypanosomes, †Unit of Protein Crystallography, Institut Pasteur de Montevideo, ‡Departamento de Bioquimica, Facultad de Medicina, Universidad de la República (Uruguay). E-mail: cortiz@pasteur.edu.uy

Trypanosoma cruzi is the causative agent of American trypanosomiasis (Chagas’ disease). Currently, there are no immunoprophylactic therapies available to combat a disease that affects 14 million people in Latino American countries. Moreover, treatment of the chronic and life-threatening stage of the disease relies on only two drugs that exhibit a high toxicity and poor efficacy. This highlights the urgent need to develop new antichagasic medicines with improved pharmacological properties. In this context, parasite’s proteins that play a key role in the parasite’s ability to survive are attractive targets for drug development. A short form of TcG6PD lacking an N-terminal sequence identity with its human orthologue. The goal of this work is to study TcG6PD at the biochemical and structural levels in order to hold promise as drug-target candidates. Glucose-6-phosphate dehydrogenase (G6PD) is the first enzyme of the pentose phosphate pathway, which produces important metabolites (e.g. NADPH, ribose-5-phosphate, ATP) and is essential for African trypanosomes [1]. The biological relevance as a virulence factor in the infective forms of Trypanosomes, P. falciparum and other Tec and Src protein kinases suggest significant differences and shape differences in the ligand binding site and suggest selectivity for Tec and Src protein kinases. The structural differences between BMX and Src protein kinases. The structural differences between BMX and other Tec family kinases suggest significant differences in protein conformation can exist while maintaining critical hinge binding interactions with the ligand. Conformational heterogeneity in the Glycine rich loop, alpha C helix and the DFG motif results in size and shape differences in the ligand binding site and suggest selectivity could be attained within the highly homologous ATP site.

Keywords: tyrosine kinase, ATP Inhibitor-binding, BMX

Structure analysis of inhibitor complex of OMP decarboxylase from P. falciparum

Yasuhide Takashima,* Eiichi Mizohata,* Keiji Tokuoka,* Yukiko Kusakari,* Sudararana R. Krungkrai,* Hiroyoshi Matsumura,* Jerapan Krungkrai,* Toshihiro Horii,* Tsuyoshi Inoue,* †Department of Applied Chemistry, Graduate School of Engineering, Osaka University, 2-1 Yamadaoka, Suita, Osaka 565-0871, †Department of Molecular Protozoology, Research Institute for Microbial Diseases,

ΔN_TcG6PD was crystallized, and its 3D structure solved with X ray diffraction techniques, in its apo form (2.85 Å resolution) as well as in complex with G6P (3.3 Å) and NADP (3.2 Å). Data was collected with our home source (Rigaku Micromax007-HF) and at the ALS synchrotron facility (Berkeley, USA), phasing was achieved using the human G6PD as a molecular replacement search probe. Overall, the structural analysis allowed the identification of particular features in the parasite’s enzyme, focusing our interest in the substrate-binding site and inter-subunit contacts. Slight conformational rearrangements were also detected as a result of ligand binding. We will report on the structural and biochemical features that distinguish ΔN_TcG6PD from its human counterpart. To the best of our knowledge, this represents the first report on the 3D structure of a NADPH dependent G6PD from a pathogenic microorganism.

Keywords: trypanosome, enzyme, drug

Poster Sessions
There are an estimated 300-500 million cases of malaria and up to 3 million people die from this disease annually [1]. The malarial parasite is totally dependent on de novo pyrimidine biosynthesis, which is consisted of six reaction steps [2], [3], [4]. In the final two steps, uridine 5'-monophosphate (UMP) requires the addition of a ribose phosphate moiety from 5-phosphoribosyl-1-pyrophosphate to orotate by orotate phosphoribosyltransferase (EC 2.4.2.10, ORPT) to form orotidine 5'-monophosphate (OMP) and pyrophosphate (PP), and the subsequently decarboxylation of OMP to form UMP, by OMP decarboxylase (EC 4.1.1.23, OMPDC). Inhibitors of the de novo pathway have strong antimalarial activity for in vitro P. falciparum growth [5], [6], [7], [8].

In usual in-silico technique, the chemical compound is chosen as a candidate in order of docking score. However, hit rate is low in vivo and vitro. Fukunishi and Nakamura developed Multiple Target Screening (MTS) and Docking Score Index (DSI), both of which use the matrix of JG containing several million compounds and several hundreds of protein structures to select the candidates that the most strongly with the target protein among the protein list in the matrix [9], [10].

On the other hand, the DSI method was developed as a method for screening similar compounds using by the matrix like MTS method. The DSI method need the three-dimensional structure of the target protein, however, the DSI method does not require the structure, but the matrix for screening of the similar compound. Generally, the similarity of the chemical compound is shown in the index of parameters in physical chemistry like hydrophobicity and molecular weight.

In this study, we reported a novel compound, 4-(2-hydroxy-4-methoxyphenyl)-4-oxobutanoic acid, exhibited a competitive physical chemistry like hydrophobicity and molecular weight. In vitro assay using SPR analyses and inhibition assay, furthermore the binding motif of the inhibitor was confirmed by the X-ray structural analysis.

Keywords: X-ray structure, OMP decarboxylase, inhibitor complex

MS16.P32

Functional characterization of UDP-glucose-4-epimerase from Aspergillus nidulans

Seán Dalrymple, John Ko, Inder Sheoran, David Sanders, Department of Chemistry, University of Saskatchewan, Saskatoon (Canada). E-mail: Sean.Dalrymple@gmail.com

Pathogenic fungi, namely Aspergillus spp., have gained notoriety in recent decades for posing an increased threat to human health[1]. Many challenges exist for treating fungal pathogens in human hosts due to the close evolutionary relationship between the eukaryotic systems[2]. In addition to the limited number of viable drug targets within fungi, most treatments are inhibited by either host toxicity or reduced potency due to the recent emergence of fungal drug resistance[3]. Antifungal drugs which block cell-wall synthesis are believed to be the most promising candidates for clinical treatment[4].

In this context, we are interested in the catalytic pathway of Aspergillus nidulans UDP-glucose-4-epimerase (GALE) which produces precursor building blocks for fungal lipopolysaccharide (LPS) biosynthesis. Specifically, GALE is responsible for reversibly inverting the 4'-hydroxyl configuration of UDP-glucose to form UDP-galactose. Given that GALE exhibits interspecies variation, targetable differences between GALE of the host and pathogen can be exploited for rational drug design. In order to identify such differences, detailed structural characterization of A. nidulans GALE is required. Ultimately, our goal is to elucidate the structure-function relationship responsible for galactose metabolism in A. nidulans GALE.

Crystals of A. nidulans GALE grown in the presence of UDP-Galactose via microbatch were diffracted to 2.4 Å resolution at the Canadian Light Source (CLS) 01HD-1 beamline. Examination of the data revealed non-merohedral twinning from which a single lattice was ultimately extracted for data processing. The final space group was C2 with unit-cell parameters a = 66.13Å, b = 119.15Å, c = 161.42Å, and β = 98.48°. The structure was solved by molecular replacement with human GALE (PDB code 1hjq) as the template model and subsequently refined through PHENIX. The electron density revealed the presence of NAD(H) co-factor and a UDP-sugar moiety bound within the active site. The structure is similar to human GALE and exhibits two distinct regions: an N-terminal domain characterized by a modified Rossmann fold of seven strands of parallel β-sheets flanked on either side by α-helices believed to be responsible for co-factor positioning and a C-terminal motif composed of six β-strands and five α-helices postulated for UDP-sugar binding. The characteristic XYYYY motif of the short chain dehydrogenase-reductase superfamily is contained within the N-terminal domain.

Inspection of the A. nidulans structure revealed nearly identical positioning of equivalent active-site residues which were shown to be responsible for NAD(H) anchoring and UDP-sugar binding in human GALE. Site-directed mutagenesis and kinetic studies on active-site residues indicate the A. nidulans mechanism is similar to that observed in the human form. Interestingly, while we have found A. nidulans GALE to be active with larger N-acetyl substrate derivatives, mutations which should inhibit the conversion of these substrates, based on previous biochemical studies of E. coli GALE, do not follow the expected trend.


Keywords: crystallography, enzymology, mutagenesis

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Structural study of the intertwined dimers of SH3 domains: biological implications of the domain swapping

Ana Camara-Artigas, Jose Manuel Martin-Garcia, Julio Bacarizo Roa, Montserrat Andujar-Sánchez. Departamento de Physical Chemistry, Biochemistry and Inorganic Chemistry Universidad de Almeria, Almeria (Spain). E-mail: acamara@ual.es