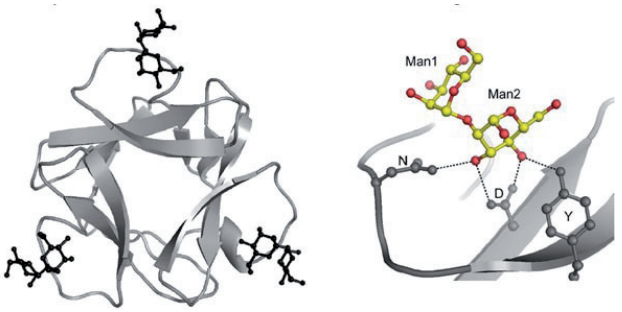

(*a*) (*b*)

Fig. 1. Actinohivin-mannobiose interaction, three mannobioses being bound symmetrically (*a*) and each recognized by the specific interactions (*b*).

[1] H. Tanaka *et al*., *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 15633-15638.

Keywords: X-ray structure, anti-HIV actinohivin, mannobiose binding

MS16.P29

Acta Cryst. (2011) A**67,** C297

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

Cecilia Ortíz,[a] Horacio Botti,[b] Nicole Larrieux,[a] Andrea Medeiros,[b,c] Alejandro Buschiazzo,[a] Marcelo A. Comini.[b] [a]*Group Redox Biology of Trypanosomes,* [b]*Unit of Protein Crystallography, Institut Pasteur de Montevideo,* [c]*Departamento de Bioquímica, 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 an indispensable cellular function and differ from the human counterpart 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 and glycolytic intermediates). The protein has been rated as a virulence factor in the infective forms of *T. cruzi* [2] and shown to be essential for African trypanosomes [1]. The biological relevance of TcG6PD lies on the critical need for intracellular redox homeostasis (e.g. antioxidant defense) and DNA synthesis (cell proliferation), both processes depending on the continuous supply of NADPH and ribose-5-phosphate, respectively. *T. cruzi* G6PD (TcG6PD) shares only 43 % sequence identity with its human orthologue. The goal of this work is to study TcG6PD at the biochemical and structural levels in order to determine whether the trypanosomal protein can be target of specific drug development. A short form of TcG6PD lacking an N-terminal stretch of 32 residues (ΔN_TcG6PD), absent in the human enzyme, was overexpressed in *Escherichia coli* and purified to homogeneity. In contrast to the human protein that undergoes rapid tetramer-dimer equilibrium, size exclusion chromatography of ΔN_TcG6PD showed that TcG6PD forms a stable non-covalently linked tetramer. High salt concentrations (1M NaCl), ligands (1mM G6P and NADP), a non-competitive inhibitor (dehydroepiandrosterone) as well as a reducing agent (10 mM DTT) did not disrupt the tetrameric structure. 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 Micormax007-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.

[1] Cordeiro et al. *Bioorganic. Med. Chem.* **2009.**

Keywords: trypanosome, enzyme, drug

MS16.P30

Acta Cryst. (2011) A**67,** C297

X-Ray Crystal Structure of TEC Family Kinase BMX

Jodi Muckelbauer,[a] John S. Sack,[a] Nazia Ahmed,[a] James Burke,[b] ChiehYing Chang,[a] Mian Gao,[a] Joseph Tino,[c] Dianlin Xie,[a] Andrew J. Tebben,[a] *Departments of* [a]*Chemical and Protein Technologies,* [b]*Discovery Biology,* [c]*Immunology Chemistry at Bristol-Myers Squibb Company, PO Box 4000, Princeton, NJ (USA) 08543-4000.* E-mail: jodi.muckelbauer@bms.com

Bone Marrow kinase gene on the X chromosome (BMX) is a member of the Tec family of tyrosine kinases that also includes BTK, ITK, TEC and TXK. Although the structures BTK and ITK are known, the X-ray crystal structures of other Tec family kinases have remained elusive. We have determined the X-ray crystal structure of BMX in complex with ATP binding site ligand. The BMX structure reveals a typical kinase protein fold with well ordered protein conformation that includes an open/extended activation loop conformation and a unique DFG motif conformation that renders the kinase in an inactive conformation. The ligand binds to BMX in the ATP binding pocket and it displays a similar binding mode to that observed in other Tec and Src protein kinases. The structural differences between BMX and other Tec and Src protein 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

MS16.P31

Acta Cryst. (2011) A**67,** C297-C298

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

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

Osaka University, 3-1 Yamadaoka, Suita, Osaka 565-0871, (Japan); [c]*Unit of Biochemistry, Department of Medical Science, Faculty of Science, Rangsit University, Patumthani 12000*, [d]*Department of Biochemistry, Faculty of Medicine, Chulalongkorn University, Rama IV Road, Bangkok 10330, Thailand*. E-mail: y_takashima@chem.eng.osaka-u.ac.jp

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, OPRT) to form orotidine 5'-monophosphate (OMP) and pyrophosphate (PP_i), 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 ΔG 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 by using the matrix like MTS method. The MTS 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 inhibition with an IC_{50} value of 170 μM, was found by *in silico* assay using multiple target screening MTS, DSI and MM-PBSA [11], in addition to *in vitro* assay using SPR analyses and inhibition assay, furthermore the binding motif of the inhibitor was confirmed by the X-ray structural analysis.

[1] Guerin *et al*., *Lancet Infect. Dis.* **2002**, *2*, 564–573. [2] Krungkrai *et al*., *Biochemistry* **1990**, *29*, 6270–6275. [3] M.E. Jones, *Annu. Rev. Biochem.* **1980**, *49*, 253–279. [4] G. Weber, *Cancer Res.* **1983**, *43*, 466–492. [5] Scott *et al*., *Mol. Biochem. Parasitol.* **1986**, *18*, 3–15. [6] Queen *et al*., *Antimicrob. Agents Chemother.* **1990**, *34*, 1393–1398 [7] Krungkrai *et al*., *Biochem.Pharmacol.* **1992**, *43*, 1295–1301 [8] Seymour *et al*., *Biochemistry.* **1994**, *33*, 5268–5274. [9] Fukunishi *et al*., *J. Mol. Graphics Modelling.* **2006**, *25* (1), 61-70. [10] Fukunishi *et al*., *J. Med. Chem.* **2006**, *49 (2)*, 523-533. [11] Srinivasan *et al*., *J. Am. Chem. Soc.* **1998**, *120 (37)*, 9401-9409.

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

MS16.P32

Acta Cryst. (2011) A**67,** C298

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

Sean Dalrymple, John Ko, Inder Sheoran, David Sanders, *Departament of Chemsitry, 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) 01ID-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 *C*2 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 1hzj) 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. Overall, 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 YXXXK 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.

[1] M.A. Pfaller, D.J. Diekema, *Clin. Microbiol. Rev.* **2007**, *20*, 133-163. [2] S.L. Baldauf, A.J. Roger, I. Wenk-Siefert, W.F. Doolittle, *Science* **2000**, *290*, 972-977. [3] F.C. Odds, A.J.P. Brown, N.A.R. Gow, *Trends Microbiol.* **2003,** *11*, 272-279. [4] D. Cappelletty, K. Eiselstein-McKitrick, *Pharmacotherapy* **2007**, *27*, 369-388.

Keywords: crystallography, enzymology, mutagenesis

MS16.P33

Acta Cryst. (2011) A**67,** C298-C299

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

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