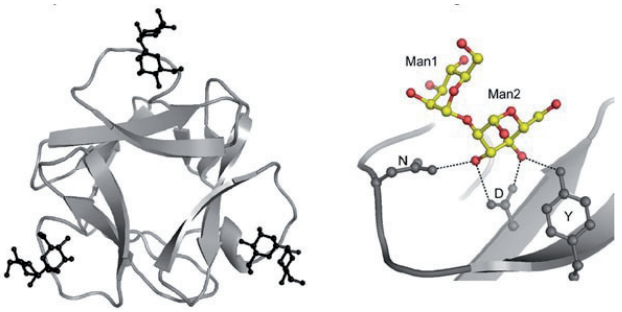

(*a*) (*b*)

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

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

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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

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X-Ray Crystal Structure of TEC Family Kinase BMX

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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

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

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