Structural approach to inhibitor design for African trypanosome glycerol kinase

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Introduction

Human African Trypanosomiasis is caused by Trypanosoma brucei gambiense (Tbg) and T. b. rhodesiense. It is a threat to 60 million human lives. Currently available treatments have become unsatisfactory, hence the need for development of new chemotherapies. Although glycerol kinase (GK) of the parasites is a validated promising target of chemotherapy, an effective and selective parasite GK inhibitor is yet to be available. In this study we are utilizing structure-based approach to develop trypanosome GK-specific inhibitor(s). The enzyme was overexpressed in Escherichia coli, purified to homogeneity, and crystallized [1]. Crystals of unliganded (apo) and ligand-bound forms were obtained with precipitant solutions that was composed of 10 % sorbitol, 12 % isopropanol, and 0.1 M HEPES pH 7.0; and 11 % hexane-1,6-diol, 25 % PEG 400 in 0.1 M HEPES pH 7.5; respectively.

Results and discussion

Complete X-ray diffraction data sets were collected to 2.90, 2.40, 2.70, 1.90, and 2.0 Å resolutions respectively for Apo, glycerol, glycerol 3-phosphate, ADP, and ATP forms of TbgGK. While the Apo form crystals formed in space groups P2₁2₁2₁, the liganded belonged to P2₁. The structure of TbgGK revealed that the enzyme is a homodimer (Fig. 1), which is formed by a somewhat strong association of two monomer chains A and B where the dimer interface is made up of an anti-parallel β-sheet (β15) and three α-helices (α12, α14, and α19) that are contributed by each of the monomers. Contact surface area of each monomer is about 5700 Å², representing about 30 % of total surface area for each of them; Nature of interactions that forms the dimer interface is largely hydrophobic interactions. Each monomer of the enzyme is made up of two functional domains I and II, (Fig. 1). Domain I is made up of N-terminal residues 1-262, while domain II is composed of residues 269-512. They are linked by a hexapeptide loop made up of NMCFEK, which formed a turn between α11 and β12 of the respective domains on the monomer surface. In between these domains is the located a clearly carved out active site cleft/grove (Fig. 1). Each domain is made up of the secondary structure signature of the sugar kinase/Hsp70/actin super family that is the typical core ββαβββ structure. In total, each monomer is composed of 18 β-strands and 19 α-helices, with lengths ranging from 3-7 and 6-26 residues respectively.

Our findings have revealed some unique structural characteristics of TbgGK, which we have utilized for in silico screening (Fig. 2A). An encouraging number of inhibitors with novel scaffold, which have IC₅₀ in the nanomolar range have been identified. Shown in Figs 2B-2C are best docking poses of some of the inhibitors (Inh-1, Inh-2, and Inh-3, respectively), which may lead to design of potent drug candidates against the disease.