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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 $P2_12_12_1$, the liganded belonged to $P2_1$. 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 Å^2, 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 ββ βαβαβ 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 IC_{50} 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.

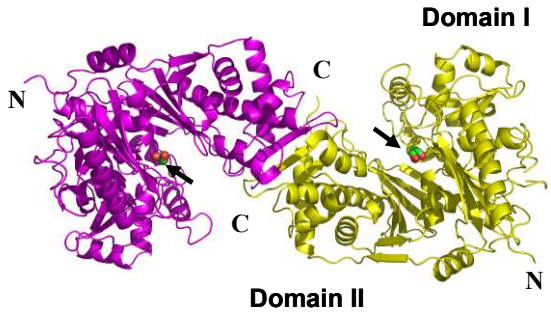

Fig. 1

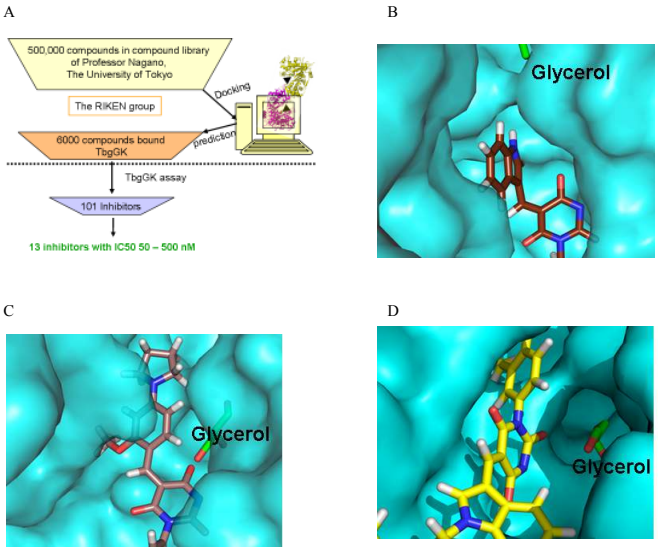

Fig. 2

[1] EO. Balogun *et al*., Acta Cryst. F 66, 304 (2010).

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