Crystallography

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X-ray crystal structure analysis of glycerol kinase from African human trypanosomes for anti-trypanosomal drug design

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Introduction

Trypanosoma brucei rhodesiense and *T. b. gambiense* are parasitic protozoa that cause the human African trypanosomiasis (HAT), a highly fatal disease that is a threat to public health. In the blood stream forms (BSFs) of these parasites, glycerol kinase (GK) is one of the nine glycosomally-compartmentalized enzymes that play vital roles in their energy metabolism. In sharp contrast to mammalian GK, the trypanosomal enzyme is able to catalyze the conversion of glycerol-3-phosphate (g3p) and ADP to glycerol and ATP. This major distinction and ability portrays trypanosome GK as an attractive potential chemotherapeutic target. In this study, *T. b. gambiense* GK (TbgGK) was overexpressed in *Escherichia coli*, purified to homogeneity, and crystallized by the sitting-drop vapor diffusion method using PEG400 as a precipitant [1]. For the first time, the structure of an essential eukaryotic GK was determined.

Results and discussion

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.

Glycerol-bound crystals are formed by 2 dimers, although the physiologic form is a dimer. The glycerol molecules are coordinated to the active site portion of domain I for each monomer, by hydrogen bond interactions involving their O1, O2, and O3 of hydroxyl groups and side chains of Arg84 (N and Nη2), Glu85 (Oε1), Asp254 (Oδ1 and Oδ2), Gln255 (Nε2); and hydrophobic stacking by Trp104 and Phe279 rings (Fig. 2). The reverse substrate, g3p, is bound to the enzyme by Thr11 and Thr276 in addition to all the glycerol binding residues. O2P, O3P, and O4P phosphate groups of g3p forms hydrogen bond interactions with Nε2, Oγ1, Oγ1, and Oδ1 of Gln255, Thr11, Thr276 and Asp254, respectively. So far, our findings have revealed some unique structural characteristics of TbgGK, which may lead to design of potent drug candidates against the disease.

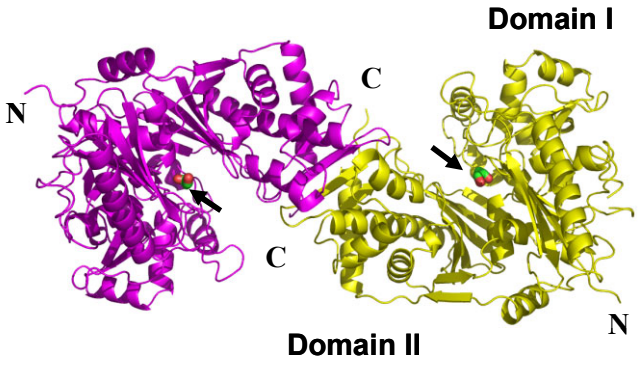

Fig. 1

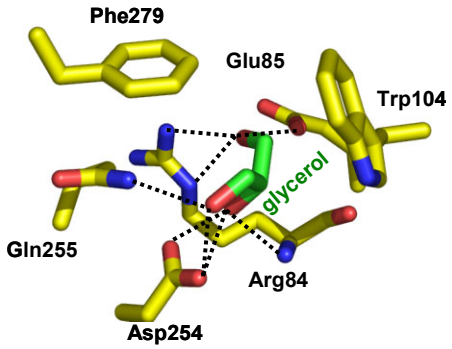

Fig. 2

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

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