Structure and Mutational Analysis of *Trypanosoma Brucei* Prostaglandin $F_{2\alpha}$ Synthase

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Trypanosoma brucei prostaglandin (PG) $F_{2\alpha}$ synthase (TbPGFS), an aldo-keto reductase, catalyzes the NADPH-dependent reduction of the endoperoxide moiety of PGH_2 to $PGF_{2\alpha}$**.** The overproduction of $PGF_{2\alpha}$ during trypanosomasis causes miscarriage in infected subjects. Here we report the crystal structures of TbPGFS-$NADP^+$ bounds citrate or sulfate at 2.1 Å and 2.6 Å resolution respectively. TbPGFS adopts a parallel $(\alpha/\beta)_8$- barrel folds lacking the protrudent loops. The core active site structure is hydrophobic to bind hydrophobic substrates and contains tyrosine, lysine, histidine and aspartate known as a catalytic tetrad which is preserved in most of other aldo-keto reductases. These four residues are said to be indispensable for the reduction of PGH_2, but mutagenesis shows that Tyr52 and Asp47 are not involved in the enzyme reaction and identifies His110 and Lys77 work as catalytic dyad. His 110 acts as a general acid catalyst, while Lys 77 facilitates proton donation by His 110 through a water molecule and forms a salt-bridge to stabilize the Asp 47 that binds NADPH. By comparing the citrate and sulfate complex structure, we detected that Trp187 holds the nicotinamide ring of NADPH from tilting on the access of PGH_2. These findings reveal a novel catalytic mechanism for the biological reduction of the endoperoxide PGH_2 by an aldo-keto reductase. The structure should allow for rational design of specific inhibitors useful to investigate the physiological roles of TbPGFS in trypanosomes.

Keywords: aldo-keto reductase, prostaglandins, mutational analysis