carry a proton to Cγ atom of the substrate.

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Keywords: three-dimensional protein structure, enzyme catalysis, conformational change

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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

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Crystal Structure of Prostaglandin $F_{2\alpha}$ Synthase from *Leshmania major*

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Leishmania major is the causative agent of leishmaniases. A gene encoding prostaglandin $F_{2\alpha}$ synthase, catalyzing NADPH-dependent reduction of 9,11-endoperoxide PGH_2 to $PGF_{2\alpha}$was identified from *L.major* (*Lm*PGFS)[1]. In the previous study, we have crystallized prostaglandin $F_{2\alpha}$ synthase from *Trypanosoma brucei* (*Tb*PGFS) using citrate as a precipitant and successfully solved the ternary structure of $NADP^+$/citrate/*Tb*PGFS[2]. We also attempted to solve the inhibitor complex of *Tb*PGFS, but a citrate molecule strongly binded to the active site.

In this study we have successfully crystallized *Lm*PGFS using PEG instead of citrate and determined the structure of *Lm*PGFS in the unliganded form by the molecular replacement method at 1.8 Å resolution. Comparing the structure of *Lm*PGFS with *Tb*PGFS, we found that the overall structure and the catalytic residues are almost the same without the extra α-helix between β4 and α4. The structural analysis of inhibitor complex is in progress to develop drug preventing a miscarriage

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Keywords: prostaglandins, X-ray crystal structure determination, parasites

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Structual Evidence of pH-induced Changes of the Reduction Potential of Mavicyanin from Zucchini

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Mavicyanin, a glycosylated protein isolated from *Cucurbita pepo medullosa* (zucchini) is a member of the phytocyanin subfamily containing one polypeptide chain of 109 amino residues and an unusual type-I Cu site in which the copper ligands are His^{45}, Cys^{86}, His^{91}, and Gln^{96}. The crystal structures of oxidized and reduced mavicyanin were determined at 1.6 and 1.9 Å resolution, respectively. Mavicyanin has a core structure of seven polypeptide β-strands arranged as a β-sandwich organized from two β-sheets, and the structure considerably resembles that with stellacyanin from cucumber (CST) or cucumber basic protein (CBP). A flexible region was not observed from superimposition of the oxidized and reduced mavicyanin structures. However, the residue of Thr^{15} rotated 60.0 degrees and O-γ 1-Thr^{15} moved from a distance of 4.78 to 2.58 Å toward the ligand Gln^{96} forming a new hydrogen bond between O-γ 1-Thr^{15} and ε-O-Gln^{96} upon reduction and changing, significantly altered the coordination structure containing Gln^{96}. It has been proposed that the reorganization of copper coordination geometry above pH 8 aroused reduction potential decreased [Battistuzzi *et al*. (2001) *J. Inorg. Biochem*.83, 223-227]. The rotation of Thr^{15} and the hydrogen bonding with the ligand Gln^{96} may provide structural evidence for the decrease of the reduction potential at high pH.

Keywords: crystal structure determination, blue copper proteins, structure comparison

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Crystal Structure of Rice Rubisco Complexed with NADPH

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Ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) catalyzes the carboxylation of ribulose 1,5-bisphosphate (RuBP) in the initial step of the photosynthesis. It can also catalyzes the oxygenation of RuBP in photorespiration. To exhibit carboxylation and oxygenation, Rubisco must be activated via the covalent carbamylation of a specific lysine residue on the active site and subsequent stabilization of the carbamate by Mg^{2+} coordination. This process is known to be relatively slow, and be modulating the activity of Rubisco. Evidence has been obtained for the role of several sugar phosphates as important regulators of the carbamylation of Rubisco. Both RuBP and 2-carboxyarabinitol 1-phosphate (CA1P) are reported to be potent inhibitors, whereas NADPH and 6-phosphogluconate accelerate the carbamylation of Rubisco [1]. To investigate the NADPH-accelerated carbamylation mechanism of Rubisco, we have crystallized the Rubisco from rice (Oryza sativa L.) under the condition similar to that in the stroma. Here, we report the 1.8 Å