Journal of Structural Biology 191 (2015) 365–375

Contents lists available at ScienceDirect

Journal of Structural Biology

journal homepage: www.elsevier.com/locate/yjsbi

High resolution structures of *Plasmodium falciparum* GST complexes provide novel insights into the dimer–tetramer transition and a novel ligand-binding site

Markus Perbandt [a,c,e,*,1], Raphael Eberle [a,1], Lena Fischer-Riepe [b], Huaixing Cang [d], Eva Liebau [b], Christian Betzel [a,e]

[a] Institute of Biochemistry and Molecular Biology, University of Hamburg, Laboratory of Structural Biology of Infection and Inflammation, c/o DESY, Notkestr. 85, Build. 22a, D-22603 Hamburg, Germany
[b] Department of Molecular Physiology, University of Münster, Schlossplatz 8, D-48143 Münster, Germany
[c] Institute of Medical Microbiology, Virology and Hygiene, University Medical Center Hamburg-Eppendorf (UKE), D-20246 Hamburg, Germany
[d] Institute of Biophysics, Chinese Academy of Sciences, Beijing, China
[e] The Hamburg Centre for Ultrafast Imaging, Luruper Chaussee 149, D-22761 Hamburg, Germany

ARTICLE INFO

Article history:
Received 4 March 2015
Received in revised form 8 June 2015
Accepted 9 June 2015
Available online 10 June 2015

Keywords:
Malaria
Glutathione S-transferase
GST
Tetramerization
Ligandin
Non-substrate binding site

ABSTRACT

Protection from oxidative stress and efficient redox regulation are essential for malarial parasites which have to grow and multiply rapidly in pro-oxidant rich environments. Therefore, redox active proteins currently belong to the most attractive antimalarial drug targets. The glutathione S-transferase from *Plasmodium falciparum* (*Pf*GST) is a redox active protein displaying a peculiar dimer–tetramer transition that causes full enzyme-inactivation. This distinct structural feature is absent in mammalian GST isoenzyme counterparts. A flexible loop between residues 113–119 has been reported to be necessary for this tetramerization process. However, here we present structural data of a modified *Pf*GST lacking loop 113–119 at 1.9 Å resolution. Our results clearly show that this loop is not essential for the formation of stable tetramers. Moreover we present for the first time the structures of both, the inactive and tetrameric state at 1.7 Å and the active dimeric state in complex with reduced glutathione at 2.4 Å resolution. Surprisingly, the structure of the inactive tetrameric state reveals a novel non-substrate binding-site occupied by a 2-(N-morpholino) ethane sulfonic acid (MES) molecule in each monomer. Although it is known that the *Pf*GST has the ability to bind lipophilic anionic ligands, the location of the *Pf*GST ligand-binding site remained unclear up to now.

1. Introduction

Malaria is among the most prevalent infectious diseases in the developing world, imposing a vast burden of mortality and perpetuating cycles of poverty (WHO, 2010). The control of malaria is challenged by drug resistance and new antimalarial drugs are urgently needed (Perez et al., 2012). The parasite *Plasmodium falciparum* causes malaria tropica, the most prevailing parasitic disease worldwide. The emergence of strains resistant to drugs used for prophylaxis and treatment and no vaccine available makes the characterization of alternative drug targets an urgent requirement (Greenwood and Mutabingwa, 2002; Olliaro, 2001).

Sophisticated defense strategies have evolved in parasitic organisms that enable them to deal with a broad range of foreign and endogenously toxic compounds. *P. falciparum* parasites utilize host proteins as food source during their erythrocytic stage and large quantities of hemoglobin are digested, confronting the parasite with high amounts of toxic hemin. Thus, the discovery of compounds selectively inhibiting the detoxification of hemin is of high importance for the development of novel antimalarial drugs.

Glutathione S-transferases (GSTs) [EC 2.5.1.18] play a key role in detoxification processes. On the basis of amino acid sequence homology, immunological cross reactivity, substrate specificity and variation of active site residues, GSTs can be grouped into

Abbreviations: *Pf*GST, *Plasmodium falciparum* glutathione S-transferase; GSH, glutathione; G-site, glutathione-binding site; H-site, xenobiotic substrate-binding site; L-site, ligand-binding site; MES, 2-(N-morpholino) ethanesulfonic acid.

* Corresponding author at: Institute of Biochemistry and Molecular Biology, University of Hamburg, Laboratory of Structural Biology of Infection and Inflammation, c/o DESY, Notkestr. 85, Build. 22a, D-22603 Hamburg, Germany.
E-mail address: markus.perbandt@uni-hamburg.de (M. Perbandt).

[1] Both authors contributed equally to the work.

http://dx.doi.org/10.1016/j.jsb.2015.06.008
1047-8477/

different classes (Frova, 2006). They catalyze the nucleophilic addition of the tripeptide glutathione (GSH) to a large variety of nonpolar or toxic compounds and thereby enhance the solubility and excretion of the resulting products. GSTs are functional and stable dimers and the active site is composed of two neighboring substrate-binding sites. The so called G-site binds reduced glutathione and the hydrophobic H-site can accommodate a variety of electrophilic co-substrates (Hayes et al., 2005; Mannervik and Danielson, 1988). Several crystal structures of the wild-type GST from *P. falciparum* (*Pf*GST) are already known (Fritz-Wolf et al., 2003; Hiller et al., 2006; Perbandt et al., 2004; Tripathi et al., 2007). Although the overall three-dimensional structure is homologue to other known GST isoforms, the *Pf*GST could not be clearly assigned to any of the specified GST classes.

Previous studies revealed that the *Pf*GST is an important intracellular ligandin and is able to efficiently bind large lipophilic molecules like bilirubin, hemin and certain drugs (Axarli et al., 2004; Deponte and Becker, 2005; Harwaldt et al., 2002). Distinct ligand-binding sites have been described for GSTs from different organisms (Ji et al., 1996; McTigue et al., 1995; Oakley et al., 1999; Prade et al., 1997; Reinemer et al., 1996). While the localization of the *Pf*GST ligand-binding site (L-site) has not been structurally described so far, it was proposed to be part of the H-site or the inter-subunit cleft (Liebau et al., 2002., 2005).

Two properties of the *Pf*GST never observed in other members of the GST superfamily are of particular interest and underline the outstanding significance of this enzyme. Firstly, the enzyme shows a strong positive homotropic behavior that modulates the hemin affinity of the two subunits (Liebau et al., 2005). Secondly, in the absence of GSH the *Pf*GST gets inactivated in a relative short period of time and loses its ability to bind hemin (Liebau et al., 2005).

This inactivation process is known to be related to a dimer–tetramer transition of the protein (Fritz-Wolf et al., 2003; Hiller et al., 2006; Tripathi et al., 2007). The dimer–tetramer transition has not been observed for any other GST (Liebau et al., 2009). In the absence of reduced GSH or other ligands, two active dimers form a tetramer. The homodimers are interlocked with each other by loop 113–119 which occupy the H-site of the other monomer and vice versa. Loop 113–119 connects two conserved α-helices and bears the TNLFKQN sequence. The PfGST is the only enzyme of the GST superfamily, which is present as a tetramer rather than a dimer. It was already reported that truncation, increased rigidity or even a simple point mutation of this loop causes a dramatic decrease in the tetramerization kinetics (Liebau et al., 2009). It was also proposed that an interaction between Asn112 and Lys117 plays a key role in keeping the tetramer structure stable (Quesada-Soriano et al., 2014).

Here we report for the first time the high resolution structure of *Pf*GST in the active dimeric state in complex with GSH. Furthermore, based on the structural analysis of three different crystal structures of *Pf*GST in both, the active and inactive state, our obtained results extend the knowledge concerning the requirements for the dimer–tetramer-transition and provide novel insights with respect to the ability of the enzyme to bind lipophilic and toxic substances, thereby securing the inactivation and immobilization of these compounds.

2. Material and methods

2.1. Recombinant expression and purification of the PfGST

*Pf*GST was cloned, expressed and purified as reported previously (Burmeister et al., 2003; Liebau et al., 2002; Perbandt et al., 2004). After 4 h growth at 37 °C, bacterial cells were harvested by centrifugation; the cell pellet was dissolved in 100 mM Tris/HCl buffer pH 8.5 and sonicated. The cell lysate was centrifuged at 16.000×*g* and 4 °C for 1 h. The supernatant was mixed with Superglu glutathione superflow resin (Generon, Berkshire, UK) and incubated for 1 h at 4 °C. Unspecific bound proteins were removed by washing the matrix with 10× bed volumes of 100 mM Tris/HCl buffer, pH 8.5 and the recombinant protein was eluted with reduced GSH. Cloning, recombinant expression and purification of the mutant *Pf*GST$^{\Delta 114–118}$ was carried out as previously described (Liebau et al., 2009).

2.2. Crystallization

Crystallization of the wild type *Pf*GST in the tetrameric state (*Pf*GST^{apo}) was performed using the hanging-drop vapor-diffusion method. Hanging droplets were prepared by mixing 2 µl of the protein solution at a concentration of 18 mg/ml together with 2 µl of precipitating solution (2.1 M ammonium sulfate, 100 mM MES pH 6.0) and 0.5 µl of a seed stock (1:1000 diluted), containing micro-seed crystals. The preparation of the seed stock is described below.

The setups to grow the active form in complex with GSH (*Pf*GST^{GSH}) were performed and stored under an oxygen free environment using phosphate buffer instead of MES buffer (2.1 M ammonium sulfate, 100 mM phosphate buffer pH 6.5). Crystals appeared after 1 week at 20 °C with maximum seize of 500 µm.

Crystallization of the *Pf*GST$^{\Delta 114–118}$ was performed using the same method with the following modifications: Hanging droplets were made by mixing 3 µl of the protein solution with a concentration of 10 mg/ml with 3 µl of precipitating solution (2.3 M ammonium sulfate, 100 mM Tris/HCl pH 8.5). Droplets were equilibrated against 1 ml of precipitating solution. The crystallization trials were kept for about 1 week at 20 °C.

2.3. Preparation of a micro-seed-stock

Microgravity experiments were performed in terms of the DLR SIMBOX experiment (Preu and Braun, 2014) onboard of the chinese space mission Shenzhou8 (30.10.2011–17.11.2011). The SIMBOX counter diffusion crystallization experiments were coordinated by the Institute of Biophysics of the Chinese Academy of Sciences, Beijing. Prior to the microgravity experiments, crystallization conditions applying the compact counter diffusion flight hardware were extensively optimized under laboratory conditions, including transport simulations. The final protein solutions were analyzed by dynamic light scattering and showed a stable monodisperse peak for several days. The capillary length of the SIMBOX crystallization hardware was 10 mm with an inner diameter of 1 mm. The capillary was filled with a *Pf*GST solution using a concentration of 18 mg/ml and 2 % (w/v) low melting point agarose (Serva) was used to seal the capillaries. Subsequently, the capillaries were inserted carefully into a plastic casing, containing four separated sections filled with foam material and sealed with a metal lid. The foam was saturated with approximately 300 µl of precipitant solution (2.3 M ammonium sulfate, 100 mM Tris/HCl pH 8.5). For the microgravity experiment 4 capillaries were positioned in the DLR SIMBOX. The microgravity experiment was performed at a stable temperature of 20 °C for 17 days (30.10.2011–17.11.2011) onboard the Shenzou8 space mission. Following the space mission the capillaries were inspected directly after arrival in Beijing. The capillaries were opened at one end and the agarose was carefully removed. The obtained micro-crystals were used to prepare seed-stock solutions following the manufacturer's instructions of Jena BioScience Beads-for-Seeds.