Molecular & Biochemical Parasitology 189 (2013) 26–32

Contents lists available at SciVerse ScienceDirect

Molecular & Biochemical Parasitology

Crystal structures of *Plasmodium falciparum* cytosolic tryptophanyl-tRNA synthetase and its potential as a target for structure-guided drug design☆

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ARTICLE INFO

Article history:
Received 17 February 2013
Received in revised form 26 April 2013
Accepted 29 April 2013
Available online 7 May 2013

Keywords:
Plasmodium falciparum
Malaria
Tryptophanyl-tRNA synthetase
Crystal structure
Conformational changes
Drug design

ABSTRACT

Malaria, most commonly caused by the parasite *Plasmodium falciparum*, is a devastating disease that remains a large global health burden. Lack of vaccines and drug resistance necessitate the continual development of new drugs and exploration of new drug targets. Due to their essential role in protein synthesis, aminoacyl-tRNA synthetases are potential anti-malaria drug targets. Here we report the crystal structures of *P. falciparum* cytosolic tryptophanyl-tRNA synthetase (*Pf*-cTrpRS) in its ligand-free state and tryptophanyl-adenylate (WAMP)-bound state at 2.34 Å and 2.40 Å resolutions, respectively. Large conformational changes are observed when the ligand-free protein is bound to WAMP. Multiple residues, completely surrounding the active site pocket, collapse onto WAMP. Comparison of the structures to those of human cytosolic TrpRS (*Hs*-cTrpRS) provides information about the possibility of targeting *Pf*-cTrpRS for inhibitor development. There is a high degree of similarity between *Pf*-cTrpRS and *Hs*-cTrpRS within the active site. However, the large motion that *Pf*-cTrpRS undergoes during transitions between different functional states avails an opportunity to arrive at compounds which selectively perturb the motion, and may provide a starting point for the development of new anti-malaria therapeutics.

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1. Introduction

Malaria is one of the most serious diseases in the world, causing around 1.2 million of deaths in the year 2010 [1]. The most common and serious human malaria parasite is *Plasmodium falciparum*. Currently, the near term prospects of arriving at a highly effective vaccines appear to be challenging [2], while drug resistant parasites remain one of the biggest threats in malaria control [3]. Therefore, it is important that new drugs are developed to fill the pipeline of anti-plasmodium therapeutics.

The aminoacyl tRNA synthetases (aaRSs) form a group of ubiquitous enzymes that perform the essential function of charging amino acids to their cognate tRNAs during protein synthesis [4]. In general, the reaction is completed in two steps:

(1) amino acid + ATP → aminoacyl-AMP + PP_i
(2) aminoacyl-AMP + tRNA → aminoacyl-tRNA + AMP

During this reaction, large conformational changes in aaRSs are often needed. Different conformations of the enzyme are required for binding of substrates, arranging the active site for catalysis and releasing products. Depending on the substrate bound, several structural states are observed in crystal structures of tRNA synthetases. Focusing here on tryptophanyl-tRNA synthetases (TrpRSs), extensive structural data are available on both the bacterial and the eukaryotic (cytoplasmic) TrpRS enzymes [5–10]. For bacterial TrpRS, at least three conformational states have been reported. In the open, ligand-free state (F-state), either Trp or ATP can bind to the enzyme without major changes in conformation. However, the simultaneous binding of Trp and ATP in the pre-transition state requires a compact active site where a conserved KMSKS loop closes onto the active site to interact with ATP, and its anticodon binding C-terminal domain moves toward the active site-containing Rossmann-fold domain. After the intermediate tryptophanyl-adenylate (WAMP) is formed, both the KMSKS loop and the C-terminal domain move slightly away from the catalytic core to allow turnover in the partially closed product-state (P-state) [5–7]. In contrast, in eukaryotic (cytoplasmic) TrpRS, the binding of Trp is accompanied by a change from an open F-state to a closed to Trp-bound state. Only the closed conformation has a Trp-binding pocket that complements the amino acid well. Structural elements that ‘close’ onto the active site are mainly the N-terminus and a conserved AIDQ motif, but the domain motion observed in

☆ Coordinates and structure factors for *Pf*-cTrpRS F- and P-states are deposited in the Protein Data Bank under accession codes 4J76 and 4J75, respectively.

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http://dx.doi.org/10.1016/j.molbiopara.2013.04.007

bacterial TrpRS does not occur. Subtle movements in the KMSKS loop subsequently assist ATP binding, amino acid activation and product release, but the overall closed conformation is maintained in the P-state [8]. The N-terminus then needs to be displaced while the rest of the enzyme stays in the closed conformation to allow the acceptor arm of the $tRNA^{Trp}$ to access the active site, resulting in another conformational state [9]. Hence TrpRS, like many other aaRSs, undergo a series of complex conformational changes to perform their function.

Owing to its vital role in protein synthesis, this group of enzymes is in principle an important target for the development of anti-parasitic agents [11]. Recently, there have been an increasing number of reports targeting aaRSs to treat infectious diseases [12–18]. Inhibitors of aaRSs typically bind to the active site or the editing site of these enzymes, preventing the binding or release of amino acid, ATP or tRNA. In order to be effective, these substrate binding site inhibitors need to have a high affinity for the pathogen but not for the homologous enzymes of the human host. High resolution three-dimensional structures should be helpful in identifying differences in the substrate binding sites and also for exploring the nature of conformational changes. Such changes might be prevented by small molecules, ultimately leading to new drugs.

As part of our ongoing efforts to explore aaRSs as anti-parasitic drug targets [14,19–25], we have determined the crystal structure of one of the two tryptophanyl-tRNA synthetases from *P. falciparum*. Like many other higher eukaryotes, *P. falciparum* has two genes encoding for TrpRS. The two gene products are predicted to be localized in the cytosol (PlasmoDB PF3D7_1336900) and in the apicoplast (PlasmoDB PF3D7_1251700) [26]. Here we name these two proteins *Pf*-cTrpRS and *Pf*-aTrpRS, respectively, to denote their likely localization compartments. The structures reported here are of *Pf*-cTrpRS. Based on sequence comparison, *Pf*-cTrpRS is more closely related to human cytosolic TrpRS (*Hs*-cTrpRS, ~44% identity) than to human mitochondrial TrpRS (~16% identity). In addition to the Rossmann-fold and the C-terminal anticodon-binding α-helical domains common to all Class I aaRS, *Pf*-cTrpRS has an N-terminal extension of 300 residues. The first 228 residues appear to be apicomplexan-specific, with weak homology to the editing domain of archaeal and eukaryotic AlaRS, although in *P. falciparum* the function of these residues is unknown. The next 72 residues of the N-terminal extension can be aligned with the eukaryote-specific extension (ESE) of *Hs*-cTrpRS (Fig. 1).

We crystallized *Pf*-cTrpRS in its F-state and solved its structure to 2.34 Å resolution. Subsequently, tryptophan (Trp), ATP and Mg^{2+} were soaked into crystals which resulted in the 2.40 Å structure of the WAMP-bound P-state of this malaria parasite enzyme. Based on these two structures, which reveal considerable conformational differences, we examine the potential of this enzyme as a drug target.

2. Materials and methods

2.1. Protein expression and purification

Attempts to express soluble protein from the full-length *Pf*-cTrpRS sequence (PlasmoDB PF3D7_1336900) were unsuccessful. However, soluble expression of a protein from a construct represent residues 229–632 of the protein was achieved. The sequence represents the complete ESE, Rossmann-fold domain and C-terminal helical domain (Fig. 1A). While not tested for *Pf*-cTrpRS, the deletion of the first part of N-terminal extension did not affect the aminoacylation activity of the homologous apicomplexan *Cryptosporidium parvum* TrpRS [25]. The construct was cloned into the AVA0421 vector for expression in *E. coli* [27]. Protein was purified by a Ni-NTA affinity column followed by overnight cleavage of the N-terminal hexa-histidine tag using N-terminally histidine tagged 3C protease at 4 °C. Cleaved protein was purified by a second Ni-NTA step and then size-exclusion chromatography on a Superdex 75 column (Amersham Pharmacia Biotech) using a buffer containing 25 mM HEPES, 500 mM NaCl, 2 mM DTT, 5% glycerol, and 0.025% NaN_3 at pH 7.0. Purified protein retained five residues of the 3C protease cleavage site (GPGSM) at the N-terminus.

2.2. Protein crystallization and treatment

Crystals of *Pf*-cTrpRS were obtained by vapor diffusion using sitting drops equilibrated at room temperature against 50 μL of a reservoir containing 0.2 M sodium citrate and 20% PEG 3350. The drop consisted of 0.15 μL protein at 21.8 mg/mL plus 0.15 μL of the reservoir solution. Crystals grew in 1–2 days at room temperature. Crystals were cryo-protected with 20% of glycerol before freezing under liquid nitrogen for data collection. In order to determine the intermediate product WAMP-bound structure, the crystals were soaked in the cryo-protecting solution in the presence of 5 mM L-Trp, ATP and $MgCl_2$ for 5 min before freezing.

2.3. Data collection and structure determination

Data for the ligand-free structure was collected from a single crystal using a wavelength of 0.98 Å at synchrotron beamline 5.0.2 of the Advanced Light Source in Berkeley, CA. Data was processed with HKL2000 [28] (Table 1). The coordinates of TrpRS from *C. parvum* were used as search model for phase determination by molecular replacement using the program Phaser [29]. The solution was fed to the model building software ARP/wARP [30] for automatic model rebuilding. Subsequent iterated manual building/rebuilding and refinement of models were performed using Coot [31] and Refmac5 [32], respectively. In the final cycles of refinement, protein structures were refined with translational/libration/screw (TLS) groups identified by the TLS motion determination server [33] before restrained refinement in Refmac5. The structure validation server MolProbity [34] was used throughout the process to monitor the progress of structure determination. The final crystallographic refinement statistics are given in the Table 1. Figures were created and rendered with Pymol [35]. Superposition of structures were also carried out with Pymol, which first aligns proteins by sequence followed by structural alignment using five cycles of refinement to improve the fit by discarding pairs with high relative variability [35].

Data for the WAMP-bound structure were collected from a single crystal soaked for 5 min with 5 mM of L-Trp, ATP and $MgCl_2$ using a MicroMax-007 HF rotating anode (Rigaku) equipped with VariMax HF (Osmic) monochromator and a Saturn 994 (Rigaku) CCD detector at a wavelength of 1.54 Å. Data were integrated using XDS [36] and scaled with Scala [37]. Coordinates of the ligand-free structure of *Pf*-cTrpRS were used as search model for molecular replacement in Phaser [29]. Subsequent model building and validation process are the same as described above for the ligand-free structure.

3. Results and discussion

3.1. Overall structure and domain architecture

The construct used for the structure determination contains the ESE (Ser229 to His300), the Rossmann-fold (Lys301 to Thr520 and Thr611 to Met632) and the C-terminal helical domain (Asp521 to Leu610) (Fig. 1). *Pf*-cTrpRS crystallized in space group $P2_12_12$ with a dimer in the asymmetric unit (Fig. 2A). The structure of the ligand-free F-state was refined to 2.34 Å with good statistics (Table 1). Part

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