Biochimie 93 (2011) 570–582

Contents lists available at ScienceDirect

Biochimie

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

Research paper

Structure of *Leishmania major* methionyl-tRNA synthetase in complex with intermediate products methionyladenylate and pyrophosphate

Eric T. Larson [a,b], Jessica E. Kim [a,b], Frank H. Zucker [a,b], Angela Kelley [b,c], Natascha Mueller [b,c], Alberto J. Napuli [b,c], Christophe L.M.J. Verlinde [a,b], Erkang Fan [a,b], Frederick S. Buckner [b,c], Wesley C. Van Voorhis [b,c], Ethan A. Merritt [a,b], Wim G.J. Hol [a,b,*]

[a] Department of Biochemistry, University of Washington, Seattle, WA 98195-7742, USA
[b] Medical Structural Genomics of Pathogenic Protozoa (MSGPP[1]), USA
[c] Department of Medicine, University of Washington, Seattle, WA 98195-7185, USA

ARTICLE INFO

Article history:
Received 25 October 2010
Accepted 29 November 2010
Available online 7 December 2010

Keywords:
Aminoacyl-tRNA synthetase
Protozoa
Drug target
Leishmaniasis
Trypanosomiasis
Enzyme product complex

ABSTRACT

Leishmania parasites cause two million new cases of leishmaniasis each year with several hundreds of millions of people at risk. Due to the paucity and shortcomings of available drugs, we have undertaken the crystal structure determination of a key enzyme from *Leishmania major* in hopes of creating a platform for the rational design of new therapeutics. Crystals of the catalytic core of methionyl-tRNA synthetase from *L. major* (LmMetRS) were obtained with the substrates MgATP and methionine present in the crystallization medium. These crystals yielded the 2.0 Å resolution structure of LmMetRS in complex with two products, methionyladenylate and pyrophosphate, along with a Mg^{2+} ion that bridges them. This is the first class I aminoacyl-tRNA synthetase (aaRS) structure with pyrophosphate bound. The residues of the class I aaRS signature sequence motifs, KISKS and HIGH, make numerous contacts with the pyrophosphate. Substantial differences between the LmMetRS structure and previously reported complexes of *Escherichia coli* MetRS (EcMetRS) with analogs of the methionyladenylate intermediate product are observed, even though one of these analogs only differs by one atom from the intermediate. The source of these structural differences is attributed to the presence of the product pyrophosphate in LmMetRS. Analysis of the LmMetRS structure in light of the *Aquifex aeolicus* MetRS–$tRNA^{Met}$ complex shows that major rearrangements of multiple structural elements of enzyme and/or tRNA are required to allow the CCA acceptor triplet to reach the methionyladenylate intermediate in the active site. Comparison with sequences of human cytosolic and mitochondrial MetRS reveals interesting differences near the ATP- and methionine-binding regions of LmMetRS, suggesting that it should be possible to obtain compounds that selectively inhibit the parasite enzyme.

1. Introduction

A spectrum of diseases called leishmaniasis, with outcomes ranging from debilitating disfiguration to death, is caused by multiple species of *Leishmania* parasites. These unicellular eukaryotic organisms, belonging to the order of Kinetoplastida and family of Trypanosomatidae, are transmitted by certain species of sand fly. It is estimated that there are two million new cases each year in the tropics and subtropics with over 350 million people at risk worldwide [1,2]. Current anti-leishmania drugs have serious shortcomings as evidenced by miltefosine, the only oral drug against visceral leishmaniasis, which is teratogenic [3]. Moreover, resistance against existing drugs is increasing and there are no effective vaccines [4]. The need for novel effective drugs for treatment of leishmaniasis is therefore urgent. The same can be said for diseases caused by other

Abbreviations: aaRS, aminoacyl-tRNA synthetase; AQP, adenosine-5′-tetraphosphate; CP1, connective peptide insertion 1; CP2, connective peptide insertion 2; AspRS, aspartyl-tRNA synthetase; IleRS, isoleucyl-tRNA synthetase; LysRS, lysyl-tRNA synthetase; MetRS, methionyl-tRNA synthetase; EcMetRS, *E. coli* MetRS; LmMetRS, *L. major* MetRS; EG, ethylene glycol; MetAMP, L-methionyladenylate; Metol-AMP, L-methioninyl adenylate; MetSA, 5′-*O*-[*N*-(L-methionyl)-sulfamoyl] adenosine; MSGPP, Medical Structural Genomics of Pathogenic Protozoa; PDB, Protein Data Bank; PDBID, PDB Identifier; RMSD, root mean square deviation; SSRL, Stanford Synchrotron Radiation Lightsource; TrpRS, tryptophanyl-tRNA synthetase; SCF, stem-contact-fold; TCEP, tris(2-carboxyethyl)phosphine; TLS, translation/libration/screw; TLSMD, translation/libration/screw motion determination.

* Corresponding author. Department of Biochemistry, University of Washington, Box 357742, Seattle, WA 98195-7742, USA. Tel.: +1 206 685 7044.
E-mail address: wghol@u.washington.edu (W.G.J. Hol).

[1] www.msgpp.org.

0300-9084/$ – see front matter © 2010 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.biochi.2010.11.015

closely related trypanosomatid species such as *Trypanosoma brucei*, the causative agent of sleeping sickness, and *Trypanosoma cruzi*, responsible for Chagas disease [5–7], and it is hoped that information obtained about key proteins from any of these parasites will benefit drug design efforts against the other species.

A major goal of the Medical Structural Genomics of Pathogenic Protozoa collaboration is to identify and characterize potential drug targets in several eukaryotic pathogens, including the kinetoplastids, to provide a platform for anti-protozoan drug development [8]. In this pursuit, we selected the aminoacyl-tRNA synthetases (aaRS) for structural and biochemical characterization [9–12]. AaRS are essential to the core biological process of translating nucleotide-encoded gene sequences into proteins and are thus present in all organisms spanning all three domains of life. They are grouped into two classes based on structural and sequence features of the catalytic core [13–19]. Both classes are further divided into subclasses based on additional commonalities of sequence motifs and structural elements [18]. Very generally speaking, each aaRS recognizes a single amino acid and attaches it to a tRNA whose anticodon matches a codon for that amino acid, but exceptions to these rules are prevalent and many aaRS additionally rely on various proofreading or editing mechanisms to ensure faithful translation of the genetic code. To charge a tRNA, the aaRS must catalyze at least two reactions. The amino acid is first activated with ATP to form an aminoacyl-adenylate intermediate and then is transferred onto the terminal adenosine of the tRNA [18]. Interference with any step throughout this process will inhibit the formation of properly charged tRNA leading to disruption of normal protein chain elongation during translation.

Consistent with the crucial role of aaRS in translation, studies in various organisms have shown these enzymes to be essential. Most eukaryotes carry two genes for each aaRS (cytoplasmic and mitochondrial) but these cannot compensate for each other when one is knocked down. Surprisingly, trypanosomatids appear to harbor only one aaRS per amino acid except for AspRS, LysRS and TrpRS [5–7]. RNA interference experiments have shown that each of the two *T. brucei* TrpRS enzymes are essential [20] and that knockdown of the single *T. brucei* HisRS [10], IleRS (unpublished), or MetRS (manuscript in preparation) leads to complete growth arrest in the bloodstream forms of this protozoan. As a general rule, these enzymes are required for cell survival and consequently are excellent drug targets from a functional perspective. However, because of their conservation in all living organisms, the ability to selectively inhibit the pathogen enzyme over the host enzyme remains a challenge. This underscores the utility of incorporating structural knowledge of the enzyme into the drug design process.

Despite this challenge, aaRS have been successfully targeted by selective inhibitors. A prime example is the drug mupirocin, targeting prokaryotic IleRS [21]. It is used topically to treat bacterial infections including methicillin-resistant *Staphylococcus aureus* (MRSA). Amazingly, selectivity over host homologs has been attributed to differences in only two residues at the active site [22]. Also, a lead compound (SB-425076) targeting *S. aureus* MetRS [23–26] was further developed into an analog (REP8839) that shows antibacterial activity against several Gram-positive bacteria and reached clinical trials [27–30]. Another related MetRS-specific diaryldiamine compound (REP3123) has shown promising results in preclinical studies against *Clostridium difficile* [31–33]. This series of MetRS inhibitors, which are methionine- but not ATP-competitive, shows that selective inhibition of pathogen MetRS is achievable.

We report here the 2.0 Å crystal structure of MetRS from *Leishmania major* (LmMetRS) in complex with two products, L-methionyladenylate (MetAMP) and Mg–pyrophosphate. MetRS belongs to aaRS subclass Ia, which contains a catalytic core comprised of a Rossmann fold with a connective peptide insertion divided into two contiguous parts named CP1 and CP2, the two characteristic Class I aaRS sequence motifs HIGH and KMSKS (KISKS in *L. major*), the stem-contact-fold (SCF), and a helical bundle (Fig. 1). The present structure is the first class I aaRS to be captured as a complex with the two intermediate products methionyladenylate (MetAMP) and pyrophosphate, along with a bridging Mg^{2+}, in the active site. It has the most compact active site observed in MetRS structures to date, displaying large conformational changes compared to other MetRS structures, particularly in the KISKS loop and in CP1, that are attributed to the presence of the pyrophosphate. Importantly, there are several amino acid differences near the methionine and adenosine binding pockets of LmMetRS compared to even the closest human homolog, the mitochondrial enzyme. This holds promise for obtaining inhibitors with higher affinity for the parasite than the human enzymes.

2. Materials and methods

2.1. Protein expression and purification

The catalytic core (residues 206–747) of the 747 residue MetRS from *L. major* (GeneDB identifier LmjF21.0810) was PCR amplified from genomic DNA of *L. major* strain Friedlin and cloned into *Escherichia coli* expression vector AVA0421. This catalytically active N-terminal truncation mutant was pursued for structural studies after significant effort was expended attempting to crystallize the full-length protein to no avail. Protein was purified by a Ni-NTA affinity column followed by size-exclusion chromatography (SEC) on an XK 26/60 Superdex 75 column (Amersham Pharmacia Biotech) using SGPP standard buffer (20 mM HEPES, 0.5 M NaCl, 1 mM tris(2-carboxyethyl)phosphine (TCEP), 5% glycerol, 10 μM $ZnCl_2$, 0.025% NaN_3 at pH 7.0) [34]. The peak SEC fractions containing the target protein were pooled and spiked with 10 mM Mg–ATP, 10 mM L-methionine and 10 μM $ZnCl_2$. The protein sample was concentrated to 24 mg/ml using an Amicon concentrator. Purified protein retained a 22 amino acid residue expression tag composed of an N-terminal His_6 tag and a 3C protease cleavage site.

2.2. Protein crystallization

Purified LmMetRS protein was screened by sitting drop vapor diffusion for crystallization leads using a Phoenix crystallization robot (Art Robbins Instruments) and the JCSG$^+$ Suite sparse matrix

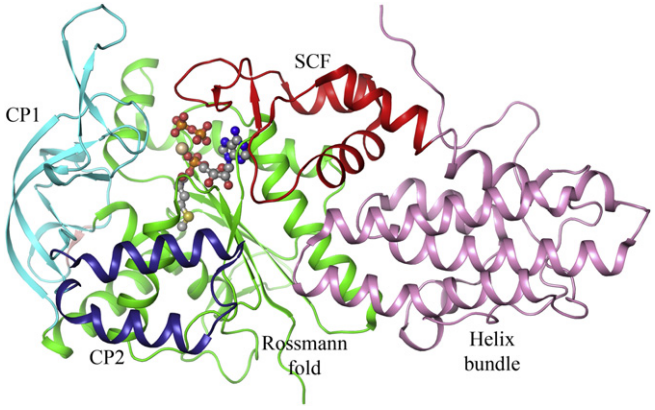

Fig. 1. Crystal structure of the catalytic core of *L. major* methionyl-tRNA synthetase. Green, the Rossmann fold; cyan and dark blue, the CP1 and CP2 connective peptide insertions, respectively; salmon, the MetRS-specific β-strand insertion; red, the stem-contact-fold (SCF); pink, the α-helix bundle. The intermediate products, methionyladenylate (MetAMP) and pyrophosphate are shown as ball-and-sticks, and the Mg^{2+} ion is shown as a gold sphere. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).