Journal of Structural Biology 191 (2015) 22–31

Contents lists available at ScienceDirect

Journal of Structural Biology

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

Catalytic pathway, substrate binding and stability in SAICAR synthetase: A structure and molecular dynamics study

Kavyashree Manjunath [a], Jeyaraman Jeyakanthan [b], Kanagaraj Sekar [a,*]

[a] Laboratory for Structural Biology and Biocomputing, Supercomputer Education and Research Centre, Indian Institute of Science, Bangalore 560 012, India
[b] Department of Bioinformatics, Alagappa University, Karaikudi 630 003, Tamil Nadu, India

ARTICLE INFO

Article history:
Received 10 April 2015
Received in revised form 5 June 2015
Accepted 8 June 2015
Available online 10 June 2015

Keywords:
Purine *de novo* biosynthesis
Pyrococcus horikoshii OT3
SAICAR synthetase
ATP binding
Molecular dynamics
Enzyme mechanism

ABSTRACT

The *de novo* purine biosynthesis is one of the highly conserved pathways among all organisms and is essential for the cell viability. A clear understanding of the enzymes in this pathway would pave way for the development of antimicrobial and anticancer drugs. Phosphoribosylaminoimidazole-succinocarboxamide (SAICAR) synthetase is one of the enzymes in this pathway that catalyzes ATP dependent ligation of carboxyaminoimidazole ribotide (CAIR) with L-aspartate (ASP). Here, we describe eight crystal structures of this enzyme, in $C222_1$ and H3 space groups, bound to various substrates and substrate mimics from a hyperthermophilic archaea *Pyrococcus horikoshii* along with molecular dynamics simulations of the structures with substrates. Complexes exhibit minimal deviation from its apo structure. The CAIR binding site displays a preference for pyrimidine nucleotides. In the ADP·TMP·ASP complex, the ASP binds at a position equivalent to that found in *Saccharomyces cerevisiae* structure (PDB: 2CNU) and thus, clears the ambiguity regarding ASP's position. A possible mode for the inhibition of the enzyme by CTP and UTP, observed earlier in the yeast enzyme, is clearly illustrated in the structures bound to CMP and UMP. The ADP.Mg^{2+}·PO_4·CD/MP complex having a phosphate ion between the ATP and CAIR sites strengthens one of the two probable pathways (proposed in *Escherichia coli* study) of catalytic mechanism and suggests the possibility of a phosphorylation taking place before the ASP's attack on CAIR. Molecular dynamic simulations of this enzyme along with its substrates at 90 °C reveal the relative strengths of substrate binding, possible antagonism and the role of Mg^{2+} ions.

1. Introduction

Purine nucleotides are biosynthesized either by the *de novo* or the salvage pathway. Apart from a few archaea (Brown et al., 2011), which lack the enzymes in the *de novo* purine biosynthesis pathway, the chemical steps of this pathway are conserved across all three domains of life, except for one intermediate N^5-CAIR, which is bypassed in archaea and higher eukaryotes. Enzymes of this pathway are often the targets of anticancer, anti-inflammatory, and antimicrobial drugs (Christopherson et al., 2002; Cassera et al., 2011; Bertino et al., 2011). The enzyme SAICAR (phosphoribosylaminoimidazole-succinocarboxamide) synthetase catalyzes the seventh (in some archaea and higher eukaryotes) or eighth (bacteria and fungi) step of the *de novo* purine biosynthesis pathway, ligating L-aspartate (ASP) with 5-amino-1-(5-phospho-D-ribosyl)imidazole-4-carboxylate (CAIR) in the presence of ATP and Mg^{2+}, forming SAICAR. The synthesis of SAICAR was first suggested by Lukens and Buchanan (1959) way back in 1959. The first structure of SAICAR synthetase was reported from *Saccharomyces cerevisiae* in 1998 (Levdikov et al., 1998) and following this, several apo and ligand bound structures were reported from 12 different organisms. As on date, around 20 structures have been deposited in the PDB which includes monomeric, dimeric and bi-functional octameric forms. This structural variability occurs as a result of the underlying sequence differences. Most of the bacterial SAICAR synthetase structures exist as a dimer (Ginder et al., 2006; Manjunath et al., 2013; Wolf et al., 2014; Zhang et al., 2006), the structure from yeast and *Mycobacterium abscessus* is a monomer (Levdikov et al., 1998), and the bifunctional octameric forms are observed only in higher eukaryotes (Li et al., 2007; Taschner et al., 2013). Thus, the structural variations between microbial and human SAICAR synthetase

Abbreviations: SAICAR, phosphoribosylaminoimidazole-succinocarboxamide; CAIR, 4-carboxyaminoimidazole ribonucleotide; N^5-CAIR, N^5-carboxyaminoimidazole ribonucleotide; ATP, adenosine triphosphate; AMP, adenosine monophosphate; ASP, aspartate; AMP-PNP, adenosine 5′-(β,γ-imido)tri phosphate; SS, SAICAR synthetase; *Ph*, *Pyrococcus horikoshii*; *Sc*, *Saccharomyces cerevisiae*; *Ec*, *Escherichia coli*; *Sp*, *Streptococcus pneumoniae*.

* Corresponding author.
E-mail addresses: sekar@physics.iisc.ernet.in, sekar@serc.iisc.ernet.in (K. Sekar).

http://dx.doi.org/10.1016/j.jsb.2015.06.006
1047-8477/

may be efficiently exploited in deriving antimicrobials, provided a complete understanding of the enzyme exists. Till date, there have been no effective inhibitor against this enzyme except L-alanosine (Harasawa et al., 2002), which the enzyme catalyzes to an anti-metabolite that inhibits the next enzyme in the pathway.

The enzyme from *Pyrococcus horikoshii* shares a sequence identity of 41–51% with dimeric (bacterial or archaeal), ~29% with monomeric (yeast and *M. abscessus*) and ~27% with the octameric (higher eukaryotes) enzymes. Among the known structures, three are from hyperthermophilic organisms, *P. horikoshii*, *Methanocaldococcus jannaschii*, and *Thermotoga maritima*, and one is from a thermophilic organism, *Geobacillus kaustophilus*. Proteins from hyperthermophilic organisms generally have a higher oligomerization state compared to their mesophilic homologues (Vieille and Zeikus, 2001), however in case of SAICAR synthetase this scenario is not observed in the structures. Kinetic studies on the *Escherichia coli* enzyme have suggested a sequential random ter–ter mechanism (Nelson et al., 2005), in which the three substrates, ATP, CAIR, and ASP bind randomly to the active site followed by the release of the products, ADP, SAICAR, and PO_4. Based on these kinetic experiments, AMP-PNP, IMP, and maleate are found to be the best inhibitors with respect to ATP, CAIR, and ASP, respectively. This study found that CAIR binds more effectively to the apo enzyme than to the enzyme·ATP·ASP complex and the substrates exhibit mutual antagonism. Structural studies on the *E. coli* enzyme (Ginder et al., 2006) proposed two catalytic pathways based on the observations from a similar enzyme called adenylosuccinate synthetase. In first pathway, the γ-phosphate of ATP is transferred to the carboxylic group of CAIR forming a phosphoryl intermediate, which is eventually attacked by α-amino group of L-aspartate leading to the formation of SAICAR. In the second pathway, initially, a dioxyanion intermediate of ASP-CAIR adduct is formed which is then phosphorylated by ATP to form SAICAR. A recent study (Wolf et al., 2014) on *S. pneumoniae* enzyme suggested the possibility of the first pathway and formation of the phosphoryl intermediate through a relay mechanism involving the transfer of phosphate from ATP to CAIR via two conserved glutamate residues E178 and E89. However, structural evidences are unavailable to pin point to a specific pathway. In a related study, a group working on the yeast (Ostanin et al., 1989) enzyme had experimentally shown that GTP (guanosine triphosphate) and 2′-dATP can substitute for ATP in the reaction, while CTP (cytidine triphosphate) and UTP (uridine triphosphate) have inhibitory effect on the enzyme activity.

We had previously obtained the structure of apo form of *P. horikoshii* SAICAR synthetase (*Ph*SS) in $C222_1$ and H3 space groups (Manjunath et al., 2013). Here, we report the ligand bound structures of the same enzyme in $C222_1$ and H3 space groups. Structures reveal different aspects of the enzyme such as the binding site of ASP, possible pathway during the catalysis, preferences for various nucleotides and mode of inhibition. Due to the difficulties in performing kinetic studies at higher temperatures, molecular dynamic simulations are carried out on the enzyme along with its substrate at 363 K, as the enzyme is from a hyperthermophilic organism, for 25 ns (50 ns in one case) and analyzed in order to obtain deeper insights into the substrate binding/stability. Simulation studies show the binding stability of the substrates, possible existence of antagonism among them and role of magnesium ions, in addition to this, it substantiates the structural observations made regarding its enzyme mechanism.

2. Experimental procedure

2.1. Purification and crystallization

The gene of SAICAR synthetase PH0239 cloned in pET11a was over-expressed in *E. coli* BL21-CodonPlus (DE3)-RIL cells. Protein was extracted and purified as mentioned in our previous work (Manjunath et al., 2013). The purified protein was concentrated to 10–14 mg/ml for crystallization experiments in 20 mM Tris–HCl buffer at pH 8.0 with 200 mM NaCl. All ligands used for co-crystallization were purchased from Sigma except CAIR, which was synthesized according to a previously reported protocol (Srivastava et al., 1974; Meyer et al., 1992). Protein–ligand mixtures were incubated overnight before setting up crystallization. All protein–ligand complexes mentioned here were obtained by co-crystallization using microbatch under oil method. The crystallization conditions used for co-crystallization were same as those used for obtaining the apo-structures (in H3 and $C222_1$ space groups), described previously (Manjunath et al., 2010, 2013), and crystals were obtained within 10–15 days. The dimensions of the crystals were ~0.1–0.2 × 0.5–0.7 × 0.05 mm and ~0.2 × 0.2 × 0.1 mm in $C222_1$ and H3 space groups, respectively. Details of the concentrations of various ligands and ratios used during co-crystallization experiments are given in Table 1.

2.2. Data collection, structure solution and refinement

All data sets were collected at 100 K with Cu-Kα radiation from Bruker Microstar Ultra rotating anode X-ray generator (wavelength of 1.5418 Å) using MAR345 image plate detector at home source located at Molecular Biophysics Unit, Indian Institute of Science, Bangalore. All data sets were processed, scaled and merged using IMOSFLM (Steller et al., 1997) and SCALA (Evans, 2006, 2011), respectively. As the RCR (RMS correlation ratio) was greater than 1 in H3 data sets, the anomalous pairs were separated while

Table 1
Details of various ligands used in the co-crystallization.

PDBid	Ligands used for co-crystallization[a]	Major ligands	Description *(structure representation)*	RMSD[b] (Å)
4O7N	ATP (~2.5 mM), $MgCl_2$, soaked with CAIR	ADP; Mg^{2+}	ADP – ATP site; Mg^{2+} – bound to ADP *(ATP.Mg^{2+})*	0.29
4O7T	ADP, ASP, TMP (~7.5 mM each)	ADP; TMP; ASP	ADP – ATP site; TMP – CAIR site; ASP – putative ASP site *(ADP·ASP·TMP)*	0.29
4O7V	ATP, UTP (~5.0 mM each)	ADP; UDP; UMP	ADP – ATP site; UDP and UMP – CAIR site *(ADP·UD/MP)*	0.22
4O7 W	ATP, TTP (~7.5 mM each)	ATP; TDP	ATP – ATP site; TDP – CAIR site *(ATP·TDP)*	0.29
4O86	ATP, CTP (~7.5 mM each)	ADP; PO_4; CMP; CDP; Mg^{2+}	ADP – ATP site; CD/MP – CAIR site; Mg^{2+} bound to ADP; PO_4 – between ADP and CD/MP *(ADP.Mg^{2+}·PO_4·CD/MP)*	0.27
4O81	ATP (~10 mM)	AMP; ADP	Chain A: AMP – ATP site; AMP – CAIR site Chain B: ADP – ATP site; AMP – CAIR site *(AMP·AMP_ADP·AMP)*	0.28 0.26
4O82	ATP (~2.5 mM)	AMP; ADP	Chain A: ADP – ATP site; AMP – CAIR site Chain B: ADP – ATP site; AMP – CAIR site *(ADP·AMP_ADP·AMP)*	0.27 0.27
4O83	ATP (~5 mM)	AMP; ADP	Chain A: AMP – ATP site Chain B: ADP – ATP site *(AMP·ADP)*	0.22 0.22

[a] Drop ratio (in μl) for $C222_1$ – 2:2 of protein: condition; for H3 – 1.5:1.5:1.5 of protein: condition: additive.
[b] $C222_1$ structures are superposed on 3U55 and H3 structures are superposed on 3U54.