

Abstract

Understanding the structural details of a biological macromolecule is highly essential for a clear interpretation of its functional roles. Structural biology evolved over a period of time has been able to explain various cellular processes which has consequently lead to the discovery of several effective drugs. X-ray crystallography is one of the powerful tools in structural biology, contributing to $\sim 88\%$ of the three-dimensional structures deposited in the Protein Data Bank (PDB). A single experiment can however provide only one of the numerous conformational states a macromolecule can exhibit. This setback is overcome by classical mechanics based molecular dynamics simulation technique developed based on various force fields that can generate many possible conformational states a macromolecule can take up. This thesis describes the use of both X-ray crystallography and molecular dynamics simulation techniques to elucidate the structural and dynamic aspects of a thermostable enzyme from a hyperthermophilic organism focusing on its thermostability and catalytic mechanism.

Nucleotides have significantly numerous roles in the cell, forming the basis of an organism's genome, providing cellular energy, assisting as coenzymes in many enzyme catalyzed reactions, acting as secondary messengers etc. These biologically important chemical entities are classified into purines and pyrimidines. Biochemical synthesis of either purine or pyrimidine nucleotides are accomplished either by *de novo* or salvage pathway. SAICAR synthetase is one of the enzymes involved in *de novo* purine biosynthesis pathway. It catalyzes the seventh (in humans and higher eukaryotes) or eighth step (in bacteria and fungi) of *de novo* purine biosynthesis pathway. The enzyme is an ATP dependent ligase which forms C-N bond between 5-Amino-1-(5-phospho-D-ribose)imidazole-4-carboxylate (CAIR) and L-aspartate (ASP), in the presence of magnesium, resulting in the formation of 5-Amino-4-imidazole-N-succinocarboxamide ribonucleotide (SAICAR). An overview of the structural and biochemical aspects of SAICAR synthetase along with an introduction to the structural and dynamic basis of protein thermostability is described in chapter-1.

The work described in this thesis starts with the clone of SAICAR synthetase. A series of procedures involving protein expression and its subsequent purification results in a pure protein which is crystallized by underoil microbatch crystallization method using appropriate conditions. X-ray diffraction data of the protein crystals are collected using

Cu-K α radiation (1.5417 Å) as a source on MAR Research image plate detectors. Diffraction data are processed using IMOSFLM, scaled using SCALA. Molecular replacement method is used for solving the structures using PHASER. Refinement of the structures are performed using REFMAC. Model building of the structures are carried out using COOT. Structures are validated using RCSB validation tools. Simulations of the structures are carried out using a popular open source program, GROMACS. A detailed account of all the programs and tools used to carry out the work is described in chapter-2.

Chapter-3 elaborates on the structure of the enzyme SAICAR synthetase from *Pyrococcus horikoshii* along with the details on its thermal stability in comparison with the structures from other sources. Crystals in two space groups - H3 (Type-1) and C222₁ (Type-2) are obtained. These are the first apo structures of SAICAR synthetase from a hyperthermophilic archaea reported. Some of the structural deviations between two forms are due to the presence of Cd²⁺ ion used in the crystallization condition. Structurally they closely resembled the enzyme from other organisms. Probing the amino acid compositions of mesophilic, thermophilic and hyperthermophilic SAICAR synthetase structures reveal the prevalence of certain amino acids in each temperature class. Certain type of non-bonded interactions are prominent in hyperthermophilic structures.

As an extension of structural studies, simulations using molecular dynamics of SAICAR synthetase structures, revealed yet another facet of thermostability of proteins. The observations made from the study are in consensus with the Somero's corresponding states hypothesis according to which the proteins show similar degrees of flexibility at their respective growth temperatures. Helices are observed to be relatively more susceptible to unfolding among the secondary structures. Increased flexibility of the mesophilic proteins at higher temperatures manifests by forming more number of short lived contacts. Further, hyperthermophilic proteins tend to reduce the hydrogen bonding interactions with water at higher temperatures compared to the mesophilic proteins. Of many non-bonded interactions in the protein, salt-bridge and hydrophobic interactions play a key role in providing thermostability to the hyperthermophilic proteins. The finer points are presented in chapter-4.

Co-crystallization experiments and simulations of the ligand bound SAICAR synthetase structures from *Pyrococcus horikoshii* are elucidated in chapter-5. All eight crystal structures described in this chapter contain an adenine nucleotide. With no major structural deviations detected in the ligand bound structures compared to their apo forms, these structures however confirmed the position of one of the substrates - aspartate. Out of other two substrates binding sites, CAIR site harbors any nucleotide, while, ATP site binds only to an adenosine nucleotide. One of the structures, explains the possibility of a phosphorylation occurring prior to aspartate attack on CAIR. Simulation studies are able to demonstrate the structural roles of the magnesium ions. The stability and antagonism among the substrates are also expounded in the chapter.

Few other crystal structures of nucleotide complexes with SAICAR synthetase are described in chapter-6. Six structures provide answers to the mode of inhibition of some of the nucleotides observed in yeast SAICAR synthetase. One of the structures also provides a possible path taken for the entry of the nucleotide into the CAIR site. However, further experiments are being planned to explore the activity of SAICAR synthetase in detail.