Abstract

The role of biological reactions in the survival of organisms is prominent. Every organism takes care of its life in its own way but all need nucleotides that are the building blocks of nucleic acids for their growth and propagation. So nucleotide synthesis pathway is an important one which can be considered as a target for inhibiting the growth of pathogenic organisms. The progress of a biological reaction depends upon the action of the associated enzyme. OPRT is an enzyme that belongs to the group of ten PRTs that synthesize the nucleotides by adopting both salvage and *de novo* pathways. OPRT catalyzes the Mg^{2+} dependent reaction between nitrogenous base OA and the PRPP to form OMP and PP_i during the *de novo* synthesis of pyrimidines in several organisms.

OPRT action in different species has been experimentally investigated due to the fact that its deficiency causes different diseases of varying severity. Human OPRT exists as a complex along with another enzyme ODCase which dictates the decarboxylation of OMP. Orotic aciduria is one of the diseases caused due to the excretion of OA in urine. Also, it has been reported that human cancer cells need *de novo* pyrimidine synthesis to supply DNA and RNA precursors for maintaining rapid multiplication. One of the species that causes malaria in human beings is *P. falciparum*. Again for this organism, the need of *de novo* pathway has been reported. Pyrimidine nucleotide synthesis has been found to be an efficient target for developing antimalarial drugs. So the action of PfOPRT was needed to be understood. The study on the *T. gondii* OPRT restated the fact. Besides the action of OPRT in all these organisms, *M. tuberculosis* causing TB is important. The 2016 WHO report states TB as the highest death causing disease next to AIDS emphasizing the need of the study of OPRT action.

The experimental information of any study produces reliable observations which demand deeper understanding of the underlying phenomena. Theoretical studies can complement experimental observations to meet this purpose. Simulations at the molecular and electronic

levels have proved to be vital to gain insights into molecular systems. A good number of experimental studies and crystalline structures of OPRT from *S. cerevisiae* made us to carryout our study with *S. cerevisiae* as the source of OPRT. Experimentally, three mechanisms namely ping-pong mechanism, ordered mechanism and random mechanism have been reported for the OPRT action in different organisms. All these mechanisms differed in the order of binding of the substrates. This led to a disagreement on the mechanism of reaction. All these studies, however, unanimously stated the importance of the divalent metal ion for the reaction. Hence our study was devoted to determine the role of the divalent metal ion and its binding to OPRT and the substrates as well as the order binding of the substrates.

We have taken the PDB file with the code 2PS1 for the complex of Enz-Mg^{2+}-PRPP-OA from the *S. cerevisiae*. The missing hydrogens of the complex were added and the protein was solvated in a periodic water box to represent the biological environment present around the enzyme. The complex was equilibrated using an MD code NAMD followed by the QM/MM energetics evaluation. All the complexes were equilibrated in a similar manner. All the established pathways for Enz-Mg^{2+}-PRPP-OA complex formation were analyzed and found the feasible pathway that involved the binding of Mg^{2+}-PRPP to the Enz-OA complex. Hence the study presented in the Chapter 3 established the pathway for Enz-Mg^{2+}-PRPP-OA complex formation.

The role of Mg^{2+} was confirmed from the Chapter 3. Different biologically relevant divalent metal ions (Ca^{2+}, Mn^{2+}, Zn^{2+} and Co^{2+}) apart from Mg^{2+} were studied to reveal the inhibiting/activating characteristics. The mechanism of the reaction was unchanged by the change in the identity of divalent metal ion. Inhibiting character of Co^{2+} was explained on the basis of large Co^{2+}-PRPP binding and migration energies. Chapter 4 shows the trends obtained by our computational investigations of role of experimentally reported divalent metal ions.

A wealth of experimental kinetics information on OPRT in different organisms motivated us to develop a kinetic model by considering all the proposed mechanisms. We developed a kinetic model from the reaction steps of all the previously proposed mechanisms and the optimized parameters were obtained by using Levenberg-Marquardt algorithm. The model was validated using a dataset from other organisms. Hence we have developed a unified model which could describe the kinetics of OPRT action of any organism. This model is expected to reduce the experimental measurements needed for developing a mechanism.

OMP undergoes decarboxylation to produce UMP which is a nucleic acid precursor. So study of this reaction catalyzed by ODCase was also important. We have considered the experimental data available for yeast ODCase and human ODCase. The rate expressions developed for UMP synthesis were numerically solved along with regression of the parameters. The model could describe the growth and saturation regimes of the kinetics for yeast ODCase action and could predict it successfully for human ODCase. Hence this model is expected to describe the kinetics of the reaction from other organisms as well.

Our entire study investigated all the binding steps involved in the OPRT catalyzed reaction using computational methods. By providing the molecular reasonings behind the experimental observations, our study reported some observations which may not have been easy through experimentation. Developing models for both OMP and UMP synthesis reduced the experimental hurdles involved in establishing the mechanism. Hence the computational insights reported for OPRT mechanism were expected to guide in identifying the targets for developing drugs against the action of bacteria causing diseases like TB and malaria.