Abstract

The emerging view considers enzymes to be highly flexible and dynamic biomolecules, in contrast to the conventional view of enzymes as rigid structural scaffolds. The interplay of structure and dynamics with the enzyme function has recently garnered much attention. The thesis titled '**Biophysical Investigations of Adenylate Kinase (AK3L1) in the Crowded Milieu – Correlating Activity, Conformation, Structure and Dynamics**' is focused on understanding the correlation between activity, conformational flexibility, structure and dynamics of *Escherichia coli* adenylate kinase (AK3L1, UniProt Id: Q9UIJ7) under cell-mimicking crowded environments. The thesis entails a comprehensive investigation of the aspects that link enzyme catalysis to the dynamics at sub-nanosecond timescale and hence modulation of the overall energy landscape of the enzyme under different conditions, through fluorescence based spectroscopic studies.

Chapter 1 entitled '**Introduction**' includes a brief introduction to the biophysical view of the enzymes, emphasizing mainly on the conformational motions at different timescales affecting the enzyme catalytic turnover. The energetic coupling of the enzyme dynamics with solvent has been discussed from different perspectives. The implications of the complex cellular interior mimicking macromolecular crowders on enzyme kinetics, conformational changes and dynamics have been talked about. Later in this chapter, these aspects have been discussed with regards to the multidomain enzyme, adenylate kinase (AK3L1).

Chapter 2 entitled '**Materials and Methodologies**' describes chemical procurement, sample preparation along with different spectroscopic techniques used during the investigation. Specifically, UV-Vis spectroscopy, steady-state and time-resolved fluorescence, circular dichroism were used to carry out the characterization.

Chapter 3 entitled '**Expression, Purification, Mutations and Enzymatic Activity Assay of AK3L1**' describes recombinant expression and purification of recombinant E. Coli adenylate kinase (AK3L1). The expressed protein was subsequently characterized using spectroscopic techniques, like UV-Visible spectroscopy, fluorescence measurements and CD scans for secondary structure determination. The construction of (alanine to cysteine) three single point mutants using site directed mutagenesis has been discussed. Activity assay and analysis of kinetic parameters of AK3L1 as well as its mutants have also been described.

Chapter 4 entitled '**Understanding enzyme behavior in a crowded scenario through modulation in activity, conformation and dynamics'** addresses the different aspects by which

the crowded environment can affect the overall landscape of the multidomain enzyme AK3L1, through modulation in activity, structure and dynamics. Macromolecular crowders of different sizes and morphology have been used to mimic cellular conditions, namely, Ficoll 70, Dextran 40, Dextran 70 and PEG 8. The chapter focuses on the effect of crowders on the equilibrium properties of the enzyme, activity enhancement, domain movement monitored through FRET and local dynamics at sub-nanosecond timescale through solvation studies. Through this multipronged approach, a distinct correlation between activity, structure and dynamics has been observed, where crowders not only increased activity manifolds, but higher crowder concentration, mainly Ficoll 70, above 100 g/L, led to reduction in activity due to excess structural compaction and rigidity.

Chapter 5 entitled '**Correlating Local and Global Dynamics of an Enzyme in the Crowded Milieu**' describes an extensive study comparing the sub-nanosecond time-scale local dynamics with the global structural fluctuations of AK3L1 using solvation dynamics and quenching studies respectively, in the absence and presence of different concentrations of macromolecular crowders. Activation energy profiles of local dynamics were determined through temperature-dependent solvation studies at three different sites along the polypeptide backbone spanning different domains, while the activation energy for global dynamics was monitored using tryptophan quenching studies at different temperatures. In spite of the fact that solvation times increased as a function of crowding, the activation energies associated with the local dynamics undergoes a significant decrease as we increase the crowder concentrations. The crowded environment also aids in enhancing coupling between the local and global dynamics of multidomain enzyme. The study not only provides new insights into how crowding affects internal protein dynamics, but also mirrors the role of local motions as mechanical precursors in controlling the global motions when subjected to crowded conditions.

Chapter 6 entitled '**Towards the Energy Landscape of Adenylate Kinase in Crowded Milieu: Activity, Conformation, Structure and Dynamics in Sequence**' describes the activity, structure and dynamics of AK3L1 under crowded conditions as a function of urea induced chemical denaturation. The enzyme exhibited an initial increase in activity as a function of urea, in contrast to the expected decline in activity as a result of structural unfolding. The study presents interesting aspects, wherein a sequential trend of events was observed, with activity getting affected the first followed by structure, conformation and local dynamics. Higher urea concentration leads to appreciable alteration in structure (through CD) and conformation (through FRET). Surprisingly,

the increase in enzyme dynamics up to around 3-4 M urea shows that the enzyme gains structure around the local mutated site (A132C) even when it undergoes a significant decrease in global conformation. The results further prove that the observed protein dynamics are highly local in nature and are not necessarily coupled to the global structure of enzyme.

Chapter 7 entitled '**Conclusions and Future Perspectives**' encompasses the conclusions drawn from the overall investigations carried out and the future scope of the research work. In brief, the thesis presents an insightful experimental investigation of the modulation in activity, structure, conformation and dynamics of the enzyme under the effect of macromolecular crowders. The enhancement in enzyme activity, reduced activation energy barrier of local dynamics and overall enzyme landscape alterations are the observed effects of cell-mimicking crowders. The interplay of intrinsic factors of proteins along with the extrinsic solvent factors have also been highlighted. The overall findings of this work provide a solid groundwork for a number of future directions, such as similar investigations in mixed macromolecular crowders, *in vivo* (cell based) studies probing conformational changes using FLIM and FRET approaches, single molecule studies using FCS and smFRET and much more.