High Pressure X-ray Diffraction for Visualization of Transient Intermediates in the Tryptophan Synthase Mechanism

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Pyridoxal 5'-phosphate (PLP) dependent enzymes are involved in a vast number of catalytic reactions including hydrolases, isomerases, ligases, lyases, oxidoreductases, and transferases. Because of this catalytic diversity there is a vast interest in better understanding their reaction mechanism. Most PLP-dependent enzymes have a similar reaction mechanism involving a Schiff's base linkage to the aldehyde group on PLP. The internal aldimine form sequesters PLP to a conserved lysine residue in the β-active site and is easy to visualize with x-ray diffraction (XRD). An enzyme-specific unprotonated primary amino acid binds in the active site as a Michalis Complex which initiates bond rearrangement that passes through several transient steps culminating in a PLP-substrate external aldimine, a structure that can be visualized using inhibitors. In the first transient intermediate, PLP is bound as a gem-diamine to both the conserved lysine residue and unprotonated primary amino acid. The transient nature of the gem-diamine makes visualization challenging. Performing macromolecular x-ray crystallography at pressures of 2 to 5 kbar stabilizes transient intermediates while using real substrate. Tryptophan synthase (TS) is the prototypical Type-II PLP dependent enzyme that catalyzes the last two steps in the biosynthesis of tryptophan. TS is an αββα heterodimer in which the α-reaction converts indole-3-glycerole phosphate into indole and glyceraldehyde-3-phosphate and the β- reaction links indole arriving through an internal channel to a PLP activated serine. For our initial experiments, presented here, TS crystals were subjected to several different soaking conditions followed by XRD at various pressures. XRD (ambient pressure, cryo-trapped at 100K) of TS crystals soaked with K^+ or Na^+, and serine produced the internal aldimine Michalis complex, shown on the left. At 2-5 kbar, crystals of the same soaking conditions produced the gem-diamine transient intermediate, shown on the right. TS crystals measured at ambient pressure, soaked with serine, Na^+, and glycerol-3-phosphate, show ordering of the αL6 loop (Arg179-Thr193) near the α-active site with glycerol-3-phosphate in the α-active site and an internal aldimine in the β-active site. The same crystals under pressure (2 kbar) show disordering of the αL6 loop, no glycerol phosphate in the α-active site, and the gem-diamine intermediate in the β-active site. These results indicate the use of high-pressure XRD can be utilized to visualize and study transient reaction intermediates that are otherwise difficult.

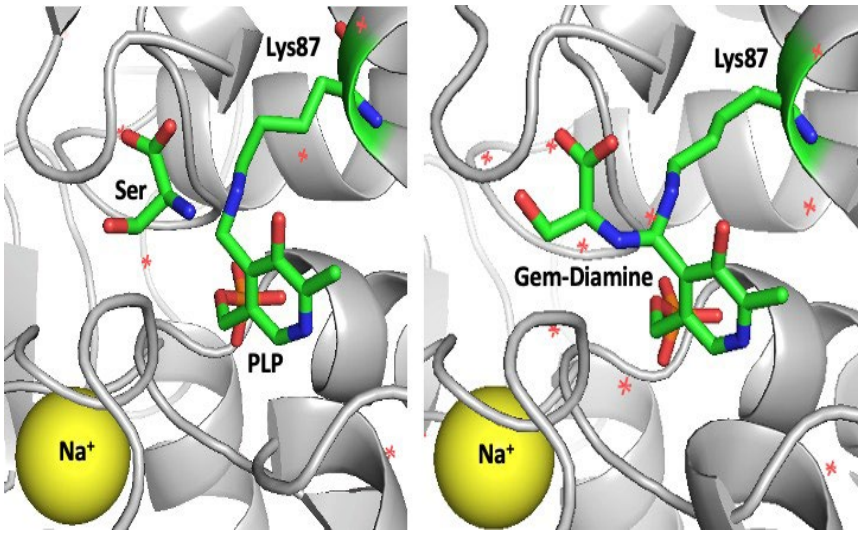

Figure 1