**A SPACE GROUP PUZZLE: E. COLI PORPHOBILINOGEN SYNTHASE COMPLEXED WITH PRODUCT**

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*E. coli* porphobilinogen synthase(PBGS) is an octameric enzyme that catalyzes the asymmetric condensation of two molecules of 5-aminolevulinic acid(ALA) to yield porphobilinogen, a precursor to pyrroles. Crystals of PBGS grown from a solution containing ALA and 2.2 M $MgSO_4$ at pH = 8 diffract to 2.4 Å in space group $P4_22_12$ with a = 130.3,c = 143.4Å. The addition of heavy metal ions causes the crystals to diffract to no better than 3 Å and to transform to $I4_22$. For the PBGS:ALA crystals, the reflections which make the cell primitive(h + k + l = odd) are weak relative to the (h + k + l = 2n) reflections. Molecular replacement for the primitive case gives two molecules related by a translation vector (1/2,1/2,1/2). Ignoring the (h + k + l = odd) data yields either $I4$ or $P4_22$ as the spacegroup. The structure has been refined in all three spacegroups with the worst refinement statistics going to $P4_22_12$ and the best to I4 with R = 0.175 and Rfree = 0.219. There is no evidence of twinning so 'Which is the correct answer?'. $P4_22_12$ is certainly the correct answer even though the statistics are better for the other choices. The final result is still to come.

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**Keywords: ENZYME STRUCTURAL CONFUSION SPACEGROUP AMBIGUITY**



**DEFINING THE TRPRS TRANSITION STATE ALONG A DYNAMIC DOMAIN REARRANGEMENT**

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A three-state structural reaction profile for Tryptophanyl-tRNA Synthetase (TrpRS) consists of open, closed pre-transition state, and closed products complexes whose different conformations correlate with the subsites on different domains that are linked by energy-rich phosphodiester bonds in the ligands. It matches each state in the TyrRS amino acid activation mechanism, confirming that the active site is assembled on ATP binding by a significant, unfavorable conformational change that costs ca. 9 Kcal/mole. ATP binding energy is stored in the unfavorable conformation and can therefore serve as the principal source of catalytic rate enhancement. The actual catalytic step occurs with a second, substantial domain rearrangement that simultaneously relocates the $PP_i$ leaving group by 1.2 Å and restores disrupted internal packing arrangements that destabilize the pre-transition state ground state. We propose that the transition state develops as ribose and $PP_i$ binding subsites move apart on going from pre-transition- to product- states. Domain motion driven in part by the effective strain stored in the protein on binding ATP favors a dissociative transition state, breaking the $PP_i$ - $\alpha PO_4$ bond, rather than providing static complementarity to a pentavalent α-phosphate transition-state. The structure of an analog of such a transition state, adenosine-5'tetraphosphate ($AP_4$) has been solved. The structure recruits new interactions from highly conserved residues to the γ- and δ-phosphates. These interactions, and the unusually high affinity of TrpRS for $AP_4$ suggest a dissociative transition state closer to the pre-transition state than to the product conformation.

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**Keywords**: **DOMAIN MOVEMENT, TRANSITION STATE STABILIATION**



**CLASSICAL ENZYMOLOGY AT ATOMIC RESOLUTION - SUBSTRATE ALIGNMENT & INDUCED FIT**

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Arginine Kinase, like its homologue Creatine Kinase, buffers cellular ATP levels and is a member of the most thoroughly investigated family of enzymes, it has been claimed. After 30 years of attempts, several structures of creatine kinase in its inactive substrate-free configuration have emerged. Our structures include the 42 kD arginine kinase as a transition state analog complex (the first for a bimolecular enzyme) now refined at 1.2 Å resolution with R = 12%.

Substrates are within a few degrees of their optimal reaction trajectories, suggesting that pre-alignment is a more important factor in catalysis than in the single-substrate enzymes visualized in prior transition state complexes. The structure is consistent only with molecular mechanics calculations that have a protonation state compatible with concerted proton/phosphoryl transfer. Mutants, alternative substrates, classical kinetics and structure determination have been used to probe the role of substrate alignment and other catalytic effects.

Recently, we have obtained the substrate-free structure, revealing the full extent of the induced-fit conformational changes. There is a 17° domain rotation near the guanidine substrate, a 10 Å closure around the nucleotide, and a 15 Å loop movement capping the active site. Unlike hexokinase and other small molecule kinases, both substrates induce change and configure the active site.

Such complicated movements may have evolved because both ATP and phosphoarginine are "high energy compounds" whose hydrolysis would be wasteful of the cell's energy. Thus, detailed structure analysis, combined with kinetics is beginning to cast new light on some classical enzymological questions.

**Keywords**: **ENZYME MECHANISM ATOMIC RESOLUTION**



**NEUTRON LAUE AND ATOMIC RESOLUTION X-RAY STUDIES OF ENDOTHIAPEPSIN: IMPLICATIONS FOR THE ASPARTIC PROTEINASE MECHANISM**

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The gaps in our knowledge of aspartic proteinase catalysis stem mainly from the inability of X-ray analyses at around 2 Å resolution to resolve hydrogen atom positions. Thus our objective with neutron diffraction was to locate crucial protons at the active site of an inhibitor complex since this would have major implications for detailed understanding of the mechanism of action. We have collected neutron Laue data on an inhibitor complex of the fungal aspartic proteinase endothiapepsin. The bound inhibitor H261 possesses a hydroxyethylene transition state analogue. The neutron data have been processed to a resolution of 2.1 Å and have been used to refine the structure of the complex to an R-factor of 23.5% and an R-free of 27.4%. The data provides convincing evidence that Asp215 is deuterated and that Asp32 is the negatively charged residue in the complex with the transition state analogue. The three dimensional structures of endothiapepsin bound to five transition state analogue inhibitors have also been solved using X-rays to atomic resolution. In one of the structures there is excellent electron density for the hydrogen on the inhibitory statine hydroxyl group, which forms a hydrogen bond with the inner oxygen of Asp32. The location of the proton is consistent with the neutron data. A number of short hydrogen bonds that may have a role in catalysis (2.6 Å) have been identified within the active site in of each structure; the length of these bonds has been confirmed using NMR techniques.

**Keywords**: **CATALYTIC MECHANISM`ATOMIC RESOLUTION `NEUTRON LAUE DIFFRACTION**