

Conformational Cycling of ATP-Grasp and Amidotransferase Domains in Carbamoyl Phosphate Synthetase

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Background: Carbamoyl phosphate synthetase (CPS) constitutes a model system for examining mechanisms of allosteric synchronization and regulation within the ATP-grasp and glutamine amidotransferase (GATase) families of biosynthetic enzymes, many of which are anticancer, antiparasitic, and/or antiviral targets. During the synthesis of carbamoyl phosphate, two ATP-grasp domains and one GATase domain allosterically coordinate three reactive intermediates through a series of tunnels spanning $>100\text{\AA}$. How domain motions specifically contribute to such synchronization has to this point been unknown.

Methods: The fluorescence properties of tryptophan are markedly sensitive to allosteric changes within proteins. Our strategy, consequently, is to genetically engineer tryptophan probes within parallel positions of the two ATP-grasp domains and monitor their steady-state and time-resolved fluorescence response to the binding of ligands.

Results: Protein variants bearing conservatively substituted tryptophans within each ATP-grasp domain are shown here to maintain catalytic activity and allosteric responsiveness. The impact of ATP binding on the probes' fluorescence intensity, lifetime, anisotropy, and exposure to quenching suggests a resultant burying of the fluorophores within the protein matrix.

Conclusions: Closure of ATP-grasp domains via association with the gamma-phosphate of ATP is speculated to contribute to the synchronizing, conformational cycling of reaction centers within this family of proteins. Here, we report direct evidence for and characterization of such dynamic motions functionally manifested within CPS.

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