Using Structural Biology To Elucidate Differences In Kinetic Inhibition Data Between Different Isozymes Of PEPCK

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Our lab studies the phosphoenolpyruvate carboxykinase (PEPCK) family of enzymes. PEPCK enzymes catalyze the conversion of oxaloacetic acid to phosphoenolpyruvate as one of the key reactions of gluconeogenesis. These enzymes can be categorized into different subclasses according to the phosphoryl donor. Two of these subclasses, the ATP- and GTP-dependent isozymes, while having low sequence similarity, share a global structural homology with the same three mobile active site loops and key active site binding residues.

The majority of our kinetic and structural research has been done on the rat cytosolic enzyme (rcPEPCK), as a proxy for the GTP-dependent class of PEPCKs. A complete characterization of kinetic parameters and inhibition constants has been determined for the rat isozyme, as well as extensive collection of both wildtype and mutant structures with inhibitors and other ligands bound. While the E. coli ATP-dependent enzyme was the first PEPCK to have its structure determined, a similar body of comparable structural and functional data is lacking. Recently we have undertaken studies to rectify this knowledge gap for the E. coli/ATP-dependent enzyme class which has yielded several interesting and contradicting functional differences when comparing kinetic parameters between the two classes of nucleotide-dependent enzymes.

Surprisingly, despite the residues comprising the OAA/PEP and M1 metal binding sites being completely conserved between the ATP- and GTP-dependent enzymes, the ATP-dependent E. coli enzyme exhibits some significant differences in its functional properties as well as its inhibition by known substrate/intermediate analogues. One of the initial differences observed for the E. coli enzyme was the increase in catalytic activity, appearing to be significantly more dynamic than its GTP-dependent counterpart. In addition, the ability of the E. coli enzyme to carry out two alternative chemistries not catalyzed by the GTP-dependent enzyme is also observed. Based upon these functional differences, we present structural data to that are consistent with the idea that the functional differences between the two enzyme classes (as seen in the kinetic data) are due to differences in the energetic barriers for conformational changes that are required for chemical function rather than differences in enzyme ligand/substrate interactions. Using structural data in addition to enzyme kinetics can help characterize and compare minute differences between isozymes belonging to the same enzyme family.