Meet the Family: Structural and Kinetic Comparisons of Representative PEPCKs

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Phosphoenolpyruvate carboxykinases (PEPCK), are a family of enzymes primarily known for their role in gluconeogenesis which can be categorized into three different enzyme classes based upon the identity of the phosphoryl donor/acceptor used in the chemical reaction. The best studied members use adenosine and guanosine nucleotides as the phosphoryl donor/acceptor and are classified as ATP- and GTP-dependent PEPCKs respectively. The former subfamily is found in many bacteria and some eukaryotes, while the latter is broadly distributed through archaea, bacteria and higher eukaryotic species. The third PEPCK subfamily is populated by the enzymes formerly known as phosphoenolpyruvate carboxytransphosphorylases (PEPCTP) which have been recently re-classified as pyrophosphate-dependent PEPCKs (PPi-PEPCK), and they are predominantly found in bacteria.

Each of these classes of enzyme catalyze the same basic chemical reaction while only sharing a low sequence homology ranging from 9 to 20% amino acid identity between them. Despite this, representative enzymes across the three distinct classes show the same global fold and the conservation of a core structure containing the active site. Even more importantly, outside of the few specific residues involving the binding of the different phosphoryl donor/acceptors (ATP, GTP and PPi), all of the remaining catalytic residues found at the active site are also conserved. To date, our lab has collected extensive data on GTP-PEPCKs, and more recently on representative enzymes from the ATP- and PPi-dependent classes as well for comparison. As expected, due to the conserved core structure, shared catalytic similarities and binding affinities were observed, however there were several notable and surprising differences as well. This research will continue to highlight structure-function relationships and provide insight as to how these similarities could have evolved while emphasizing the differences observed among the diverse members of the PEPCK family.