Structural And Functional Divergence of A "New" Class of Phoshoenolpyruvate Carboxykinase - Insights into Allosteric Regulation Via Oligomeric Changes

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Phosphoenolpyruvate carboxykinases (PEPCK) are metabolic enzymes controlling the TCA- cycle. They have been implicated as potential targets in treating diabetes, cancer, and Mycobacterium tuberculosis infections, and have a role in aging and longevity. These enzymes interconvert oxaloacetic acid (OAA) to form phosphoenolpyruvate (PEP). PEPCKs are widely distributed across life and occur in three classes depending on the nature of phosphoryl donor used for their catalyzed reactions. Of the three classes, the most understudied are PPi-dependent PEPCKs, which are structurally and functionally distinct from the nucleotide-using classes (GTP and ATP). PPi-dependent PEPCKs have a conserved core (~60 kDa) that comprises the general fold of both nucleotide-dependent classes.

However, their structure has significant additions (~70 kDa) that form allosteric sites and oligomeric interfaces, and that have likely led to divergent functional properties. Here we have used size-exclusion chromatography, enzyme kinetics, crystallography and small- angle x-ray scattering to understand the structural and functional aspects of three PPi- dependent PEPCK isozymes. Actinomyces israelii PPi-dependent PEPCK is found as a constitutive dimer with significantly reduced activity. Propionibacterium freudenreichii (Pf- PPi-PEPCK) differs from its nucleotide counterparts in its enzyme-catalyzed reaction, metal- dependencies, and allosterically induced activity-regulation via monomer-dimer transition. The third isozyme is from the human parasite Entamoeba histolytica (Eh-PPi-PEPCK). Eh-PPi-PEPCK occurs in three paralogs with different sequences, and these paralogs can form a novel heterotrimeric state not previously observed with the other two studied variants. The exceptional diversity makes this “new” class of PEPCKs an excellent model for understanding how evolution can iteratively change a common core scaffold to innovate new functions.