Snapshots Along the PEPCK Catalytic Pathway

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Phosphoenolpyruvate carboxykinase (PEPCK) catalyzes the reversible decarboxylation of oxaloacetic acid with the concomitant transfer of the γ-phosphate of GTP (or ITP) to form PEP and GDP (IDP) as the first committed step of gluconeogenesis. Recent kinetic, NMR and EPR studies have demonstrated that pH dependent changes occur with respect to the environment of the active site Mn^{2+}[1]. Structures of the Mn-, Mn-PEP- and Mn-PEP-GDP-PEPCK complexes presented here provide evidence for important changes that occur at the catalytic metal site along the catalytic pathway. The structures show an interesting Mn binding site that is composed of deprotonated lysine, histidine and aspartate residues. In addition, the involvement of a previously unrecognized cysteine sulfhydryl in the Mn-PEPCK complex is demonstrated. Upon formation of the PEPCK-Mn-PEP or PEPCK-Mn-GDP binary complexes cysteine 273 coordination is lost as the loop it resides in occupies a different conformation. The involvement of cysteine 273 in the coordination of the Mn^{2+} in the Mn--PEPCK complex provides the structural basis for previous observations that catalytic activity is stimulated by β-mercaptoethanol, and inhibited by Zn^{2+} and modification or ionization of cysteine 273. This suggests that stabilization of the cysteine coordinated metal complex traps the enzyme in a catalytically incompetent metal complex and may represent a mechanism of oxidative regulation. These structures of catalytically relevant complexes in conjunction with the previous kinetic data provide detailed insight into the mechanism of catalysis of this important metabolic enzyme.

[1] Holyoak T., Nowak T., *Biochemistry*, 2004, **43**, 7054.

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