

**STRUCTURE/FUNCTION STUDIES OF THE PHOSPHORYL-
TRANSFER ENZYME: PHOSPHOENOLPYRUVATE
CARBOXYKINASE**

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Phosphoenolpyruvate carboxykinase (PCK) catalyzes the conversion of oxaloacetate to phosphoenolpyruvate and carbon dioxide with the subsequent conversion of nucleoside triphosphate to nucleoside diphosphate. This reaction is the first committed step of gluconeogenesis in all organisms. A divalent metal cation, such as Mg²⁺ or Mn²⁺, is also required for catalysis. Both metal ions are required for optimal catalysis. *E. coli* causes >90% of urinary tract infections developed outside of hospitals. Glucose limits *E. coli* growth in urine and PCK activity is believed necessary for this bacterial infection.

In humans and other mammals, PCK is a central enzyme in carbohydrate metabolism. In mammals, PCK helps regulate blood glucose levels. Non-insulin-dependent diabetes mellitus (NIDDM), is a complex disease characterized mainly by insulin resistance and impaired pancreatic β -cell function/insulin secretion. Increased gluconeogenesis is responsible for NIDDM patients' high blood glucose level after an overnight fast. Transgenic mice overexpressing PCK showed unsuppressed liver glucose production and developed NIDDM; PCK has therefore been suggested as a potential drug target in the treatment of this disease.

The structure of *Escherichia coli* phosphoenolpyruvate carboxykinase (PCK) was determined at 1.9 Å resolution with data collected at Brookhaven National Laboratory, New York [1]. Each PCK molecule consists of a 275-residue N-terminal domain and a 265-residue C-terminal domain with the active site located in a cleft between these domains. Each domain has an α/β topology and the overall structure represents a new protein fold. Furthermore PCK has a unique mononucleotide-binding fold. The structure of the complex of ATP-Mg²⁺-oxalate with PCK was elucidated at 1.8 Å resolution with data collected on beamline BL18B at the Photon Factory in Tsukuba, Japan [2]. The structure of this complex revealed a twenty degree hinge-like rotation of the N- and C-terminal domains, which closed the active site cleft. The ATP was found in the unusual *syn* conformation as a result of binding to the enzyme. PCK represents a new structural family under the Structural Classification of Proteins scheme [3]. As expected, the ATP-dependent *Trypanosoma cruzi* PCK structure [4] showed these features. The recently determined GTP-dependent human PCK structure [5] also confirmed the novel features of *E. coli* PCK; however, the GTP was bound to the enzyme in the usual *anti* conformation. The bacterial HPr kinase/phosphorylase illustrated a similar active site for phosphotransfer and

seems to be evolutionarily related to PCK [6, 7] and is also a member of this new family of phosphotransferases [8, 9].

Many phospho-transfer enzymes selectively utilize magnesium and manganese in NTP binding and catalysis and PCKs from all sources are among these enzymes. Kinetically, both ATP- and GTP-dependent PCKs illustrate that a combination of Mg^{2+} and Mn^{2+} induce a synergistic effect on enzyme activity. *E. coli* PCK can also employ Ca^{2+} in place of Mn^{2+} , to induce optimal catalytic activity. Mg^{2+} and Mn^{2+} are preferentially partitioned between two proximal sites, with Mg^{2+} forming the metal-nucleotide complex and Mn^{2+} binding to the active site of the enzyme. Mg^{2+} appears to activate the γ -phosphate of ATP for nucleophilic attack. This cation polarizes one of the P-O bonds in the γ -phosphoryl group, making the P γ atom more electrophilic and thus more susceptible to nucleophilic attack by the enolate of pyruvate, a proposed intermediate in PCK's conversion of OAA to PEP. Also, along with the side chain of Lys254, Mg^{2+} neutralizes charges on the P β and P γ oxygen atoms and stabilizes an extended, eclipsed conformation of the P β and P γ phosphoryl groups. The sterically-strained high energy conformation likely lowers the free energy of activation for phosphoryl transfer. Additionally, the γ -phosphoryl group becomes oriented in-line with the appropriate enolate oxygen atom, which strongly supports a direct S_N2 -type displacement of this γ -phosphoryl group by the enolate anion. The 1.8 Å resolution structure of the complex of PCK with Mg^{2+} /ATP/ Ca^{2+} /pyruvate has also been solved [10]. In this latter structure, one oxygen atom of pyruvate and six other ligands coordinate the Ca^{2+} ion. The Ca^{2+} serves to activate the PCK catalytic reaction by directly bridging and activating ATP and the enolate anion of pyruvate.

A debate exists over whether the mechanism of phosphoryl transfer by enzymes such as PCK is associative or dissociative. The associative transition state is characterized by a trigonal bipyramidal structure where the axial ligands are the nucleophile of the attacking substrate and the bridging oxygen atom between the β - and γ -phosphoryl groups of ATP; this transition state is dominated by bond formation and has an overall charge distribution of -3. The dissociative transition state has a planar trigonal metaphosphate with an overall charge of -1; this transition state is dominated by bond breaking, as it lacks coordination to the apical oxygen atoms. Two complexes of PCK were crystallized, one (I) with AlF_3 and ADP- Mg^{2+} and the other (II) with AlF_3 , ADP- Mg^{2+} and pyruvate. Data were collected on (I) to 2.0 Å resolution at the Photon Factory and 2.0 Å data were collected on (II) at the Advanced Photon Source at Argonne National Laboratories (U.S.A.). The structures revealed the ADP- Mg^{2+} and AlF_3 bound to the active site of PCK and these results strongly suggest that phosphoryl transfer by PCK occurs via a direct displacement mechanism with associative qualities [11].

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