2017 ACA Abstract Submission – Anion Inhibition of PEPCK

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Anion Inhibition of PEPCK Manifested as Substrate Inhibition; Using Crystallographic Methods to Determine Thermodynamic Data

Phosphoenolpyruvate carboxykinase (PEPCK) has traditionally been labelled as the enzyme responsible for the first committed step towards gluconeogenesis and is responsible for the reversible conversion of oxaloacetic acid (OAA) to phosphoenolpyruvate (PEP). Recently, this metabolic enzymes role has been greatly expanded as it has been implicated in cancer, *Mycobacterium tuberculosis* infection, glucose stimulated insulin secretion, aging, and general TCA cycle flux regulation. Many biochemical characterizations have been completed on various isozymes of PEPCK, and one kinetic phenomena has been identified as substrate inhibition. Our study has shown that this substrate inhibition is actually a manifestation of the kinetic assay conditions, in which bicarbonate (as a source of CO_2) is used. High concentrations of bicarbonate seemed to inhibit the enzyme. Upon further investigation, it was shown that the inhibition is a more general inhibition by anionic species. This information can give context regarding the preferred direction of catalysis *in vivo*, towards the production of PEP. Using macromolecular crystallographic techniques, we have determined inhibition constants of some anions that correspond with experimentally derived parameters.