Using the anomalous scattering of iodide to elucidate the mechanism of anionic inhibition of PEPCK

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Phosphoenolpyruvate carboxykinase (PEPCK) is known to be a thermodynamically reversible enzyme *in vitro*. In contrast to this thermodynamic reversibility, in eukaryotes, PEPCK is observed to primarily catalyze the forward direction, converting oxaloacetic acid to phosphoenolpyruvate as one of the principle steps in gluconeogenesis. Based upon these seemingly conflicting pieces of data, we hypothesize that there must be an underlying mechanism regulating the reversibility of the catalyzed reaction *in vivo*.

To this end, we present structural and kinetic evidence for the presence of a putative allosteric site in PEPCK, which we propose binds negatively charged ions such as chloride which is found in abundance *in vivo*. This allosteric regulation serves to inhibit catalysis in the reverse direction and contribute to the observed unidirectionality in the direction of PEP synthesis *in vivo*.

As supporting evidence for this hypothesis, we have collected anomalous diffraction data on several PEPCK crystals that have been exposed to a range of iodide concentrations. From the corresponding anomalous signals at each concentration, we were able to analyze anomalous peak heights and generate binding isotherms for various anion binding sites on PEPCK. Importantly, one of these anion binding sites was observed to titrate with increasing iodide concentration, yielding an apparent binding constant similar to which was obtained from our kinetic inhibition studies. This was in contrast to the other iodide sites which were shown to not titrate over the same range of iodide concentrations.

The anomalous data in combination with kinetic data was essential in assigning the allosteric mechanism of regulation as the origin of the observed inhibition. This result was in contrast to preliminary anomalous maps calculated at saturating concentrations of iodide which suggested anions binding to the active site in a purely competitive fashion. This mechanism of inhibition was further validated through additional anomalous diffraction and kinetic studies on three mutant enzyme variants, giving strong support for the proposed mechanism of regulation, an effect that has interesting biological ramifications for the *in vivo* functioning of PEPCK.