Crystallographic and Kinetic Analysis of Temperature Variant Isozymes

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Abstract

Biological catalysts have evolved to optimally work at a temperature regime at which their host organism thrives. The structural differences between homologous enzymes that work at temperature extremes can further our understanding of the underpinnings of enzyme function. In general, thermophilic enzymes have been described as "less-flexible" at room temperature, where their psychrophilic counterparts are "more flexible". The interplay between temperature, function, and dynamics is a relevant field of interest as it has impact upon the field of protein engineering. Our lab has a large host of structural and kinetic/functional data on our model enzyme, a mesophilic phosphoenolpyruvate carboxykinase (PEPCK), which must undergo a series of conformational changes to work optimally. Specifically, one dynamic element of PEPCK is a lid which transitions approximately 40Å (tip-to-tip) to orient and protect substrates during catalysis. This lid is proposed to be sensitive to temperature fluctuations, where at lower temperatures it would become more rigid (closed) and at high temperatures more flexible (open). Both non-optimal conditions would impact its function, highlighting a delicate balance of dynamic motions. We have successfully crystallized a psychrophilic PEPCK which can be used to further investigate its dynamic properties. Structural and kinetic comparison of these variants can show us how an enzyme thermally adapts highly dynamic elements to maintain functional harmony.