**Poster Presentation**

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*Identification of Catalytic Residues in ATP-Citrate Lyase*

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An enzyme in the human body that regulates lipogenesis and cholesterolgenesis is ATP-citrate lyase (ACLY) [1]. ACLY synthesizes acetyl-CoA and oxaloacetate from citrate, CoA and ATP, with citryl-CoA as an intermediate [1]. The product acetyl-CoA is involved in cell growth as well as embryonic and brain development [1]. Studies in mice suggest that ACLY is important during brain development as homozygous Acly knockout mouse embryos died early in development [2]. Knowledge of the reaction mechanism is limited and will be of use in understanding the energy flow in cells. Our current insight into the mechanism by which citryl-CoA is cleaved to form acetyl-CoA and oxaloacetate is based on sequence similarity of ACLY to citrate synthase (CS). Both enzymes have histidine and aspartic acid residues at similar positions in their sequences. We hypothesize that citryl-CoA binds at this site in ACLY and is cleaved into acetyl-CoA and oxaloacetate using these residues. To test this hypothesis, we have mutated these residues to alanine in both human ACLY and ACLY from the bacterium, Chlorobium limicola. Enzymatic activities of the mutant proteins were tested using a coupled-enzyme assay with malate dehydrogenase. The inactive mutants are being used in crystallization trials with substrates, since complexes of the intermediate citryl-CoA can be trapped on the protein. To date, the crystal structure of full-length ACLY has not been published. The structure of only the amino-terminal two-thirds of the human enzyme has been determined [3]; however, the part of the protein that is similar to CS is the carboxy-terminal portion. This work identifies the catalytic residues of ACLY and complements the previous structure determination, increasing our currently limited knowledge about this enzyme.

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