

Kinetic Characterization of Two Human Acetyl-CoA Carboxylase Isoforms

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Acetyl-CoA Carboxylases (ACC) catalyze the ATP dependent carboxylation of acetyl-CoA to form malonyl-CoA, an intermediate metabolite that plays a central role in the regulation of fatty acid metabolism. Inhibition of human ACC2 and ACC1 are thus attractive targets for obesity, diabetes, dyslipidemia, and metabolic syndrome. A low volume fluorescent assay, monitoring ADP production, has been developed to identify novel ACC inhibitors, as well as to kinetically characterize both human isoforms. The supporting data presented in the accompanying poster details the characterization of a kinetic mechanism as determined through substrate kinetic and product inhibition analyses. This work details the determination of the kinetic parameters for the substrates acetyl-CoA, ATP, and bicarbonate, and for the reaction product inhibitor, malonyl-CoA. Malonyl-CoA exhibited competitive inhibition vs acetyl-CoA and mixed inhibition vs ATP, supporting a hybrid ping pong kinetic mechanism identical to other reported eukaryotic ACC's and biotin-dependent carboxylases. Optimized ACC1 and ACC2 kinetic assays provided the basis for identifying and characterizing selective and non-selective inhibitors, as well as a means to discriminate unique modes of inhibition for several different chemotypes. Compound A, recently identified from a high throughput screen, has been shown to be an ACC2 selective, ATP competitive inhibitor, whereas the reported non-selective rat ACC inhibitor, CP-640186, demonstrated mixed type inhibition versus each variable substrate. Double inhibitor titrations had been used to identify and differentiate different sites of inhibition. CP-640186 and malonyl-CoA were found to be mutually exclusive, whereas Compound A and malonyl-CoA were found to bind independently of one another. In summary, assays have thus been established to further identify and characterize newly discovered selective and non-selective inhibitors of the two human isoforms of ACC. The identification of site selective inhibitors has allowed for further exploration of the different catalytic activities of ACC. Data supporting these efforts will be presented.