Poster Presentation

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Truncated Human ATP-Specific Succinyl-CoA Synthetase

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Two isoforms of the heterodimeric enzyme succinyl-CoA synthetase (SCS) exist in the mitochondria of humans. One is specific for ATP, while the other is specific for GTP. Both catalyze the reversible reaction: succinate + CoA + NTP ⇌ succinyl-CoA + NDP + Pi, where N denotes adenosine or guanosine. SCS is best known as an enzyme of the citric acid cycle where the reaction generates NTP. In the reverse direction, SCS replenishes succinyl-CoA required for the catabolism of ketone bodies and for heme synthesis. Nucleotide-specific forms are thought to be required for SCS to serve its different metabolic roles. The nucleotide specificity lies in the β-subunit [1], and the β-subunit of human ATP-specific SCS has been shown to interact with the C-terminus of erythroid-specific aminolevulinic acid synthase (ALAS2) [2]. ALAS2 catalyzes the committed step in heme synthesis: succinyl-CoA + Gly ⇌ 5-aminolevulinate + CoA + CO_2. An interaction between SCS and ALAS2 makes biological sense, since this could provide channeling of succinyl-CoA from SCS to ALAS2. We hypothesize that the interaction is with the carboxy-terminus of the β-subunit of ATP-specific SCS because sequence comparisons show that the β-subunit of ATP-specific SCS has a carboxy-terminal extension when compared to other SCSs' β-subunits. To test this hypothesis, we added a carboxy-terminal His8-tag to the α-subunit of human ATP-specific SCS and mutated the codon for Thr 396β to a stop codon. This truncated version of human ATP-specific SCS has been produced in E. coli and purified. As well as testing to see if truncated human ATP-specific SCS interacts with ALAS2, we are using the truncated version in crystallization trials. Crystals of full-length human ATP-specific SCS diffract to only 3.2 Å and our goal is to obtain better-diffracting crystals of the complex of ATP with truncated human ATP-specific SCS.

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