Poster Presentation

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Pig GTP-specific succinyl-CoA synthetase in complex with succinate

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Succinyl-CoA synthetase (SCS) exists in the mitochondria of mammals as two different isoforms; one is ATP-specific and the other is GTP-specific. SCS is a heterodimer, and the two isoforms have a common α-subunit, but different β-subunits [1]. The β-subunit determines nucleotide specificity. Mutations in the α-subunit or the ATP-specific β-subunit can cause encephalomyopathy due to mitochondrial DNA depletion, along with lactic acidosis and methylmalonic aciduria (reviewed in [2]). The reaction catalyzed by SCS, succinyl-CoA+ NDP + Pi⇌succinate +CoA + NTP, is reversible, and the direction depends on the relative concentrations of substrates and products. Only after all substrate-binding sites are discovered can the catalytic mechanism of SCS be fully understood. Structures of SCS with ADP, GDP, GTP, Pi and CoA have been determined, but the succinate-binding site, or the binding site for the succinyl-portion of succinyl-CoA, is still unknown. Succinate is predicted to bind to the conserved sequence Gly-Gly-Ile-Val (327β-330β) located in a loop of the β-subunit of GTP-specific SCS. Crystals of other complexes with pig GTP-specific SCS have diffracted well, so we are crystallizing this enzyme in complex with succinate. Initially, plasmid containing the genes encoding pig GTP-specific SCS was transformed into E coli. After overproducing the desired protein with a 6-His tag on the C-terminus of the α-subunit, three different purification columns were used to obtain the GTP-SCS protein at high purity. Succinate was then co-crystallized with GTP-SCS under conditions containing polyethylene glycol 3350, magnesium formate and HEPES, pH 7.0.

[1] *J. D. Johnson, W. W. Muhonen, and D. O. Lambeth. The Journal of Biological Chemistry. 1998, 273, 27573-27579.,* ***[2]*** *A.W. El-Hattab and F. Scaglia. Neurotherapeutics. 2013, 10, 186-198.*

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