AMPD2 is the liver isoform of AMPD that has been experimentally demonstrated in rabbit and mouse homologs to have harmful effects in regard to its hindering of AMPK function. AMPD1, which improves insulin sensitivity in metabolic syndrome through decreased uric acid production. AMPD2 has two additional isoforms AMPD3 and AMPD4 which are active in the skeletal muscle and brain respectively and are not related to fructose metabolism in the liver. While AMPD2 has been studied and characterized in various animal models, the full structure of the human protein is unknown, due to its rapid degradation during the purification process that often results in the truncation of the regulatory (N-terminal) domain. Based on previous experimentation that outlines the positive benefits of inhibiting AMPD2, this work looks to both characterize the structure of the regulatory domain of human AMPD2 and create bacmid for the AMPD3 and AMPD4 isoforms for insect cell expression. This will enable the creation of specific inhibitors that can isolate AMPD2 only, and these inhibitors can potentially be a treatment for diseases like metabolic syndrome.

Methods

Determining protein concentration using Lowery assay. N-term AMPD2 does not have any tryptophan residues for the absorption at 280nm. Using the Lowery method to measure the absorbance at 750nm of oxydized or peptide bonds, the concentration of protein was calculated using a standard curve from known BSA concentrations.

Crystal Analysis


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