Steady State and Transient State Enzyme Kinetics of Human Histidine Triad Nucleotide-Binding Protein 2 (HINT2)

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Histidine triad nucleotide binding proteins (Hints) are a particularly ancient class of enzyme that bind and hydrolyze phosphoramidate and acyl-phosphate pronucleotides. There is a growing research interest in this class of proteins because of their participation in nucleotide prodrug metabolism, including the multi-billion dollar prodrug, Sofosbuvir[1], as well as Hint's natural involvement in opioid tolerance[2]. Some important possible cellular roles have gradually emerged, however the natural substrates for this class of proteins are still unknown. Steady state kinetics and burst kinetics of the cytosolic isozyme Hint1 have been published, utilizing a fluorescence assay designed by the Wagner Lab. While this study and the rest of the literature that has formed around the isozyme HINT1 has helped reveal Hint1's enzymatic mechanism and some potential endogenous functions, much less attention has been given to the mitochondrial localized variant HINT2. With the aim of uncovering possible differences in the substrate specificity and thus the potential natural substrate of HINT2 from HINT1 (both of which still remain unknown), both steady state and transient burst phase analyses of HINT2 kinetics has been performed using a fluorescent substrate assay. Along the way, an interesting behavior within the amplitude of the HINT2 kinetic burst phase has been observed, leading to questions and some answers regarding a fundamental difference between HINT1 and HINT2.