

REAL-TIME NMR AND PROGRESS CURVE ANALYSIS TO EXAMINE THE KINETICS OF ACETYLCHOLINESTERASE ENZYME ACTIVITY

Thesis Defense Presented by
Candido Breceda, Jr.
Krish Lab

Friday, September 29, 2023
Noon to 1:50 p.m., Ag Mechanics, Room 102

Abstract

A new simplified method has been developed to investigate the kinetics of the well-known enzyme acetylcholinesterase (AChE) using its natural substrate acetylcholine (ACh), which is not used as often as acetylthiocholine (ATCh) in previous studies. Spectroscopic methods have been used to study AChE kinetics, but they almost exclusively use ATCh as the substrate. This study used progress curve analysis and ^1H NMR to demonstrate the application of ACh as the natural substrate for studying AChE kinetics to calculate Michaelis-Menten constants (K_M) and V_{\max} . The activity of AChE has been the focus of research in cases of dementia where cognitive impairment is treated using AChE inhibitors. An additional study was performed using a neutral oxime to investigate the direct interaction of cationic oximes with the catalytic amino acid residue Serine-203. The results showed that the K_M values systematically increased by introducing a neutral oxime (thiophene-2-carbaldoxime) to the AChE-substrate system, indicative of competitive inhibition. However, the V_{\max} values were minimally affected. These results demonstrate that NMR-based analysis could potentially study the enzyme and inhibition kinetics of AChE.