MSc Project

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Abstract

Rat brown adipose tissue (BAT) is recognized as a rich source of semi carbazide-sensitive amine oxidase (SSAO), yet no standardized methods for extracting SSAO from rat BAT have been published. This study aims to develop and optimize a robust methodology for SSAO extraction from rat BAT and to investigate its enzymatic kinetics using benzylamine as a substrate. The study involved comparing different extraction methods, quantifying the extracted protein using Bradford and BCA assays, and assessing SSAO activity through the Amplex® Red monoamine oxidase assay.

The kinetic parameters of SSAO were determined, with a Km value of 0.03193mM for benzylamine, closely aligning with literature under similar experimental conditions. Furthermore, the inhibitory effects of caffeine and simvastatin on SSAO activity were evaluated, although, the Ki values obtained were based on a single experiment, leading to wider error margins and lower confidence.

This research provides a detailed methodology for SSAO extraction from rat BAT, confirming that proteins can be successfully extracted and that SSAO activity remains quantifiable post-extraction. However, further studies with improved experimental controls are recommended to refine the inhibitory kinetics of caffeine and simvastatin on SSAO.

Abbreviations

BAT - Brown Adipose Tissue
BCA - Bicinchoninic Acid
BSA - Bovine Serum Albumin
CAF - Caffeine
Ki - Inhibition Constant
Km - Michaelis Menten Constant
SIM - Simvastatin
SSAO - Semi Carbazide Sensitive Amine Oxidase
V0 - Initial Velocity
VAP-1 - Vascular Adhesion Protein 1
Vmax - Maximum Velocity
HRP - Horseradish Peroxidase
EtOH - Ethanol
SEM - Standard Error in Mean

MAO A - Monoamine Oxidase A
MAO B - Monoamine Oxidase B
AOC - Amine Oxidase Copper-Containing
dH20 - Deionised Water
ReLi - Removal of Excess Lipids
CST - Cell Signalling Technologies – Commercial kit.
DMSO - Dimethyl Sulfoxide