The Production, Purification, and Quantification of The Reduced Flavin-dependent Monooxygenase MsdD from *Pseudomonas Fluorescens* Pf0-1

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Background

- Sulfur is an essential element of life.
- Bacteria can directly intake sulfur from the environment or break down organosulfur compounds into a form that they can incorporate to their systems.
- The biological assimilation of dimethylsulfone can be achieved by a series of enzymatic reactions.
- The final step of the pathway is the conversion of methanesulfonate to inorganic sulfite catalyzed by the enzyme MsdD.

Biological assimilation of dimethylsulfone

Enzymatic reaction of methanesulfonate

Production of MsdD

- A culture of *Escherichia coli* BL21 with the MsdD plasmid was grown in lysogenic broth containing 0.05 mg/mL kanamycin sulfate.
- Isopropyl β-D-1-thiogalactopyranoside (IPTG) was added to final concentration of 50 μM to promote protein synthesis.

SDS-PAGE for MsdD

- From the left, there were protein ladder, pre-induction, post-induction, lysate, flow through, wash, pure MsdD, 10-fold diluted MsdD, and 50-fold diluted MsdD.
- The markers of MsdD were consistent with the reported molecular weight of 41765 Da.

Bradford assay

- Bovine serum albumin (BSA) was the standard protein for the calibration curve.
- The concentration of MsdD was calculated to be 0.78 mM.

Beer's law (absorption at 280 nm)

- The molar absorptivity of MsdD was 49515 M⁻¹·cm⁻¹.
- MsdD concentration determined by Beer's law was 0.72 mM.

Characterization of MsdD by mass spectrometry

- Purified protein was incubated in DTT, iodoacetic acid, ammonium bicarbonate solution (pH 8), and Pierce™ Trypsin Protease.
- Sample was submitted for 1D-MS/MS using an Orbitrap Fusion™ Lumos™ Tribrid™ Mass Spectrometer with the Easy-nLC 1200 with a 90-minute data-dependent CID/ETD decision tree method.

86.35% coverage

MDVFWFLPTHTGDGHYLGTTQGARPVTLNLYKQVAQAADSLGYHGVLP
TGRSCEDSWVIASALVPLTERLRYLVAIRPGIISPTVSARMAATLDRL
SNGRLINVTGGDPDENRGDSFLSHSERYEVTDFLKIWRRLVQGE
AVDFEGKHLKVNARALYPPVQKPYPPYFGSSDAADLAEEQVDVY
LTWGEPPAAVAEKLADVREAAHGRKVKFGIRLHVIVRETAEEAWKA
ADKLEHISDETI EAAQKSFSPDSEQRMAALHDGRDNL EAPNL
WAGVGLVRGSGTALVGDPQQAARIKEYADLGIESIFSGYPHLEEA
YRFAELVFLLPEPYASLAGRGVTNLGTFPGEMIANDVLPKANA

Selected sections signify peptide sequences without coverage

Future Research

- An endpoint assay will be run to verify if the MsdD enzyme is active.
- The kinetic parameters for MsdD can be determined with a colorimetric assay for sulfite concentration by its reaction with 5,5-dithio-bis-(2-nitrobenzoic acid) (DTNB).

References

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