The Properties of the Fatty Aldehyde Decarbonylase from *Synechocystis* PCC6803

Submitted by

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I certify that all materials in this thesis which is not my work has been identified and that no materials has been submitted and approved for the award of a degree by this or any other University.

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ABSTRACT

Alkanes dominate the constituents of gasoline, diesel, and jet fuel and are naturally produced by diverse species; saturated and unsaturated fatty acids are converted to alkanes and alkenes respectively by the enzyme aldehyde decarbonylase (AD). Here we describe the over-expression, purification, data collected and X-ray crystal structure solved for the AD protein from *Synechocystis* PCC6803.

This report describes the optimisation of over-expression, protein purification and characterization and crystallisation of the *Synechocystis* cyanobacterial AD enzyme (SynADC) has been carried out. The optimisation of protein expression has been carried out using the pET160, pET22b and pCold$^{TM\ II}$. Expression of soluble protein was obtained with all vectors. The initial Lumio^{TM} tag on pET160 prevented the protein from crystallising; the pCold$^{TM\ II}$ vector with a small His-tag was used for high soluble protein over-expression. The purification of the SynADC was optimized and the enzyme was characterised biochemically, SynADC was found to be a dimer of 29 kDa molecular weight. Metal contents were investigated using ICP-MS, SynADC protein was found to contain; Zn, Fe, Ni and Mn metals in a ratio (2.37, 1.16, 0.137, and 0.032) mg/l respectively.

The enzyme has been assayed using a series of ferredoxin assays of (C_8, C_{10}, C_{12}, C_{13}, C_{16} and C_{18}) and activity has been determined using C_{13} aldehyde and C_{18} aldehyde.

The enzyme has been successfully crystallised with four different ligands (valeric acid, Hexanoic acid, C_4 and C_8) using the microbatch method and metal soaking, this has allowed the X-ray structure to be determined. Based on this structure predication of electron transfer mechanism, a mutagenesis experiment has been carried out with the change of Asp143 to Asn, Leu and Ala. The enzyme has been assayed using PMS. Experiments to determine potential proteins, which could interact with SynADC, have been carried out. Positive results have been obtained using SDS-PAGE however, more protein is required for mass spectrometric determination.

This project was part of a larger study to clone and solve the structure of the *Synechocystis* Cyanobacterial AD in order to understand its substrate specificity and mechanism. Work carried out in collaboration with others is clearly mentioned in this thesis.

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ABBREVIATIONS

$^{\circ}C$	Degree centigrade
A	Amps
Å	Angstrom (10^{-10}m)
A_{280}	Absorbance at 280nm
A_{600}	Absorbance at 600 nm
APS	Ammonium persulphate
BLAST	Basic local alignment search tool
DMSO	dimethyl sulfoxide
EDTA	Ethylenediaminetetraacetic Acid
g	Acceleration due to gravity
g	grams
GF	Gel filtration
hr	Hour
IPTG	Isopropyl ß-D-galactopyranoside
K	Kelvin
kDa	Kilo Dalton
mg	milligrams
min	Minute
ml	milliliter
MW	Molecular weight
nm	nanometer
NMR	Nuclear Magnetic resonance
No.	Number
OD	Optical density
PAGE	Poly acrylamide gel electrophoresis
PCR	Polymerase chain reaction
PDB	Protein date bank
PMSF	phenylmethylsulfonyl fluoride
PI	Isoelectric point
ppt	Precipitate
rtm	Room Temperature
s	Second

SDS	Sodium dodecyl sulfate
SDS-PAGE	Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis
TEMED	N, N, N, N- tetramethylethylene diamide
Tris	Tris [hydroxymethyl] aminomethane
UV	Ultra violet
V	Volts
v/v	volume per volume
V_0	Initial velocity
V_{max}	Maximum velocity
Vol.	Volume
w/v	Weight to volume

Organism abbreviations

E. coli	*Escherichia coli*

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