

Title page

Biocatalytic Conversion of Vanillin to 3-Carboxy Muconate

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Abstract

The organic chemical industry permeates our daily lives, giving us products such as pharmaceuticals, plastics, adhesives and paints. 90 to 95% by mass of all organic chemicals produced are derived from petroleum oil or natural gas [1]. To move towards a more sustainable chemical industry, processes which transform renewable feed stocks need to be discovered and developed.

Plant biomass is renewable, and typically contains 30% (w/w) lignin [2]. Lignin can be dismantled to yield a smaller and simpler compound, vanillin [3]. This project investigated the construction of a synthetic metabolic pathway to transform vanillin into the novel polymer building block 3-carboxy muconate (3CM).

The metabolic pathway which performs this transformation consists of three enzymes; 4-hydroxy benzaldehyde dehydrogenase (HBD), vanillate monooxygenase (VMO) and protocatechuate 3,4-dioxygenase (P34O). These enzymes were cloned from *Acinetobacter baylyi*, expressed in *Escherichia coli*, and purified. Characteristics pertinent to the behaviour of these enzymes acting in a pathway, such as kinetics, had been reported for only P34O. This information for HBD and VMO was gained by characterising these enzymes for kinetic behaviour, substrate specificity and stability.

The genes for all three enzymes were then combined within a single recombinant *E. coli* host. This whole cell biocatalyst transformed 1 mM vanillin into 1 mM 3CM. Evidence was gathered to show the rate limiting factor for the conversion was the expression level of VMO. *In vitro* characterisation had identified that this enzyme had markedly lower intrinsic activity than the other two enzymes, and required higher relative expression.

The usefulness of 3CM as a polymer building block was explored. The butadiene system of 3CM was chemically isomerised during conversion to a trimethyl ester form. This trimethyl ester was found to copolymerise with styrene. The composition of the copolymer could be varied by varying the concentrations of monomers in the feed.

This project increased the body of knowledge of the enzymology of the vanillin to 3CM pathway, demonstrated that 3CM can be made by biocatalytic transformation of the renewable compound vanillin, and that 3CM is a useful polymer building block.

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Declaration

This thesis does not contain material which has been accepted for the award of any other degree or diploma.

To the best of my knowledge, this thesis contains no material previously published or written by another person.

I was given technical assistance with the cloning described in Chapter 6 by Jane Fowler, and this contribution is acknowledged gratefully. The conception of that work, and the design of experiments was mine.

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List of Abbreviations

3CM	3-carboxy muconate
3CM-Me ₃	Trimethyl-3-carboxy muconate
A280	Ultra Violet absorbance at 280 nm
AIBN	Azo iso butyl nitrile
AmSO ₄	Ammonium sulphate
ATCC	American Type Culture Collection
ATP	Adenosine triphosphate
CoA	Coenzyme A
CoA-SH	Thiol form of coenzyme A
DNA	Deoxyribose nucleic acid
DTT	Dithiothreitol
ϵ	Molar extinction coefficient
EDTA	Ethylene diamine tetraacetic
FAD	Flavin adenine dinucleotide
FTIR	Fourier Transform Infra Red
GC-MS	Gas Chromatography – Mass Spectrometry
GPC	Gel permeation chromatography
HBD	4-hydroxy benzaldehyde dehydrogenase
HDA	(<i>E,E</i>) – hexadienedioic acid
HIC	Hydrophobic interaction chromatography
HPLC	High performance liquid chromatography
IEF	Isoelectric focus electrophoresis
IEX	Ion exchange chromatography
IMAC	Immobilised metal ion chromatography
IPTG	Isopropyl thio galactoside

k_{cat}^{app}	Apparent catalytic constant
K_m^{app}	Apparent Michaelis constant
k_{cat}	Catalytic constant
K_m	Michaelis constant
LB	Luria broth
LC MS	Liquid chromatography – mass spectroscopy
mRNA	Messenger ribonucleic acid
m/z	Mass to charge ratio
MW	Molecular weight
NAD(P) ⁺	Oxidised nicotinamide adenine dinucleotide (phosphate)
NAD(P)H	Reduced nicotinamide adenine dinucleotide (phosphate)
NMR	Nuclear magnetic resonance spectroscopy
OD ₆₀₀	Optical density at 600 nm
ORF	Open reading frame
P34O	Protocatechuate 3,4-dioxygenase
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PMSF	Phenyl methane sulphonyl fluoride
RBS	Ribosome binding site
RNA	Ribonucleic acid
SDS-PAGE	Sodium dodecyl sulphate polyacrylamide electrophoresis
SEC	Size exclusion chromatography
Tris-HCl	2-Amino-2-(hydroxymethyl)-1,3-propanediol hydrochloride
UF	Ultrafiltration
UV	Ultra violet

V_{\max}	Maximum velocity of enzyme reaction at constant enzyme concentration
VMO	Vanillate monooxygenase
v/v	Volume / volume
w/w	Weight / weight