EXPRESSION, PURIFICATION AND MUTAGENESIS OF RECOMBINANT CLASS 1 ALDEHYDE DEHYDROGENASE

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ABSTRACT

Aldehyde dehydrogenase (AlDH) catalyses the conversion of aldehydes, for example acetaldehyde and retinal, to carboxylic acids in an NAD^+-dependent reaction involved in the detoxification of aldehydes and alcohols. There are several isoenzymes, class 1 being the cytosolic form.

Three over-expression and purification systems have been tested, in order to gain a high yield of active, pure class 1 aldehyde dehydrogenase. The traditional method, using T7 polymerase-driven expression in *E. coli* followed by ion-exchange and *p*-hydroxyacetophenone-affinity chromatography, gave 3 mg/L human aldehyde dehydrogenase with a high specific activity of 1.2 units/mg. Human Class 1 AlDH has been over-expressed and purified using the GST Gene Fusion System (Pharmacia Biotech), avoiding the need for AlDH-affinity chromatography and therefore allowing straight-forward purification of mutated enzymes. The GST fusion system produced 2.6 mg/L pure AlDH with a specific activity of 0.39 units/mg. The methylotrophic yeast *Pichia pastoris* was chosen for its high yields, in the region of grams/litre. Preparatory work was carried out with the construction of the expression plasmids and screening of the *Pichia* transformants.

A highly conserved lysine residue (Lys-272) may be involved in acid-base catalysis of aldehyde oxidation, as well as of the esterase reaction also catalysed by AlDH. Preliminary work has been carried out on the generation of the K272A, K272R, K272H and K272L altered enzymes. The resultant activity level, kinetic behaviour and active site structure of these modified enzymes should help to elucidate further the mechanism of action of AlDH.

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TABLE OF CONTENTS

ABSTRACT ii

ACKNOWLEDGEMENTS: iii

TABLE OF CONTENTS iv

LIST OF FIGURES ix

LIST OF TABLES x

1. INTRODUCTION AND LITERATURE REVIEW. 1

1.1 ALDEHYDE DEHYDROGENASE. 1

1.2 CLASSES OF ALDEHYDE DEHYDROGENASE. 3

1.2.1 Class 1 (Cytosolic) Aldehyde Dehydrogenase. 4

1.3 MECHANISM OF CATALYSIS. 4

1.3.1 Kinetic Experiments. 5
1.3.2 Chemical Modification. 6
1.3.3 Residue Conservation Analyses. 7
1.3.4 Mutagenesis Experiments. 7

1.4 OVER-EXPRESSION STUDIES. 9

1.4.1 The *E. coli* pT7-7 Expression System. 9
1.4.2 The *E. coli* GST-fusion Expression System. 11
1.4.3 The *Pichia pastoris* Yeast Expression System. 13

1.5 REQUIREMENTS FOR AN EFFECTIVE EXPRESSION SYSTEM. 15

1.6 AIMS FOR THIS THESIS. **16**

2. MATERIALS AND METHODS. **17**

2.1 MOLECULAR BIOLOGY AND CLONING TECHNIQUES. **17**

2.1.1 Buffers. 17
2.1.2 Agarose Gels. 17
2.1.3 Polymerase Chain Reaction (PCR). 17
2.1.4 Purification of DNA. 18
2.1.5 Precipitation of DNA. 18
2.1.6 Restriction Endonuclease Digests. 19
2.1.7 T4 DNA Polymerase. 19
2.1.8 Dephosphorylation of Vector. 20
2.1.9 Ligation. 20
2.1.10 Plasmid Transformation of *E. coli*. 21
2.1.11 Small-Scale Preparation of DNA. 21
2.1.12 Large-Scale Preparation of DNA. 22
2.1.13 DNA Sequencing. 22

2.2 EXPRESSION OF AlDH IN *E. COLI*. **24**

2.2.1 Solutions and Buffers. 24
2.2.2 Culture Growth and IPTG Induction. 24
2.2.3 Cell Lysis. 25
2.2.4 SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE). 25
2.2.5 Determination of Protein Concentration. 26
2.2.6 Activity Assay For AlDH. 26
2.2.7 Western Blotting. 26

2.3 YEAST MANIPULATION AND GROWTH. **28**

2.3.1 Growth Media for the Growth of *P. pastoris*. 28
2.3.2 *Pichia pastoris* KM71. 28
2.3.3 Characteristics of *Pichia pastoris* KM71. 29
2.3.4 Direct PCR From Yeast Cells. 29

2.3.5 G418 Screening. 29
2.3.6 Expression of shAlDH. 30

3. EXPRESSION OF hAlDH IN *E. coli.* 31

3.1 T7 RNA POLYMERASE / PROMOTER SYSTEM. 31

3.2 EXPRESSION AND PURIFICATION OF hAlDH USING THE T7 SYSTEM.31

3.2.1 Expression of hAlDH. 33
3.2.2 Purification of hAlDH. 34
3.2.3 Results and Comparisons. 35

3.3 GST GENE FUSION SYSTEM. 35

3.3.1 The Expression Vectors, pGEX. 36
3.3.2 Transformation of *E. coli* with pGEX-4T-3. 38

3.4 INITIAL EXPRESSION EXPERIMENTS. 38

3.4.1 Effect of A_{600} At Induction. 38
3.4.2 Effect of IPTG Concentration on Induction. 39

3.5 INITIAL PURIFICATION EXPERIMENTS - BATCH MODE. 41

3.5.1 Preparation Of The Cell Lysate. 41
3.5.2 Binding of the Fusion Protein to the Matrix. 43
3.5.3 Thrombin Cleavage While Matrix-Bound. 44
3.5.4 Glutathione Elution. 45
3.5.5 Thrombin Cleavage In Solution. 45

3.6 LARGE-SCALE EXPRESSION AND PURIFICATION. 46

3.6.1 Preparation Of Cell Lysate. 46
3.6.2 Purification by Cleavage on the Glutathione-Sepharose Column. 46
3.6.3 Secondary Purification - from the flow-through fraction. 49
3.6.4 Glutathione elution. 50
3.6.5 Replication of the 'cleavage on the column' experiment. 54

3.7 SUGGESTIONS FOR FUTURE WORK. **56**

4. *PICHIA PASTORIS* **61**

4.1 INTRODUCTION. **61**

4.2 THE *PICHIA PASTORIS* VECTORS, pPIC3K AND pPIC9K. **61**

4.3 CLONING OF SHEEP AlDH INTO pPIC3K AND pPIC9K. **64**

4.3.1 Preparation of the Secreted-Expression Construct, pPIC9K-shAlDH. 64

4.3.2 Preparation of the Intracellular-Expression Construct, pPIC3K-shAlDH. 70

4.4 TRANSFORMATION INTO *PICHIA PASTORIS*. **72**

4.4.1 Electroporation. 74

4.5 SCREENING FOR INTEGRATION AND MULTI-COPY INSERTION EVENTS. **75**

4.5.1 G418 Screening for Multi-copy Integration. 75

4.5.2 PCR Amplification of shAlDH From the Yeast Genome. 76

4.6 EXPRESSION OF THE RECOMBINANT *PICHIA* CLONES. **77**

4.6.1 Small-scale Cultures. 77

4.6.2 Large-scale Cultures. 79

4.7 SUGGESTIONS FOR FUTURE WORK. **81**

5. SITE DIRECTED MUTAGENESIS. **83**

5.1 INTRODUCTION. **83**

5.1.1 The Significance of Lys-272. 83

5.1.2 Methods for Site-Directed Mutagenesis. 85

5.2 THE 'UNIQUE SITE ELIMINATION' METHOD OF MUTAGENESIS 86

5.2.1 Design of the Selection and Mutagenic Primers. 86
5.2.2 Positive Control. 87
5.2.3 Methods and Results. 88
5.2.4 Changes Made to the Standard Protocol. 91

5.3 SUGGESTIONS FOR FUTURE WORK. 92

6. DISCUSSION 94

6.1 THE AIM FOR THIS THESIS. 94

6.2 WHY WAS THIS RESEARCH ATTEMPTED 94

6.2.1 Importance of AlDH as a Research Area 94
6.2.2 Progress Made So Far. 95
6.2.3 What Is Needed for Progression In the Area. 95
6.2.4 Approaches Used Previously to Investigate This Area. 96

6.3 THE APPROACHES I USED TO ACHIEVE THIS AIM. 97

6.3.1 Purification Using Ion-Exchange/Affinity Chromatography. 97
6.3.2 GST Fusion. 98
6.3.3 *Pichia pastoris*. 100

6.4 SITE-DIRECTED MUTAGENESIS OF LYS-272. 102

6.5 SUMMARY. 104

REFERENCES 106

LIST OF FIGURES

Figure 1-1: Schematic diagram of the oxidation catalysed by aldehyde dehydrogenase. 1
Figure 1-2: Model showing the reaction pathway for the aldehyde dehydrogenase catalysed oxidation of an aldehyde. 4
Figure 3-1: Schematic diagram of the induction process of the T7 polymerase expression system. 32
Figure 3-2: The structure of the glutathione-Sepharose 4B matrix. 36
Figure 3-3: Plasmid map of the pGEX-4T-3-hAlDH construct. 37
Figure 3-4: SDS-PAGE showing the expression of the GST-hAlDH protein after IPTG induction at different cell densities. 40
Figure 3-5: SDS-PAGE showing the expression of the GST-hAlDH protein after induction by different concentrations of IPTG. 42
Figure 3-6: SDS-PAGE of fractions collected in the purification using the cleavage on the column method. 48
Figure 3-7: SDS-PAGE showing the purification of hAlDH from the fusion protein, using glutathione elution followed by thrombin cleavage in solution. 52
Figure 3-8: SDS-PAGE showing fractions collected from purification using the cleavage on the column method. 55
Figure 4-1: Schematic diagram of pPIC9K (Invitrogen). 62
Figure 4-2: PCR amplification of the hAlDH cDNA from the pBluescript-hAlDH. 66
Figure 4-3: *Bgl*II Diagnostic digestion of the pPIC-hAlDH constructs compared to the original pPIC vectors. 68
Figure 4-4: Schematic diagram of the pPIC9k-shAlDH construct. 69
Figure 4-5: Schematic diagram of the shAlDH-pPIC3K construct. 72
Figure 4-6: A representative example of multi-copy screening. 78
Figure 4-7: PCR amplification of the hAlDH cDNA within the yeast genome. 78
Figure 4-8: SDS-PAGE of samples taken from the expression of the *Pichia* clone 9B. 80

LIST OF TABLES

Table 3.1: Purification of human cytosolic AlDH from the GST fusion protein. 57

Table 4.1: The number of transformants after ligation of shAlDH into pPIC9K. 67

Table 4.2: The number of transformants after ligation of shAlDH into pPIC3K. 71

Table 4.3: Results from the G418 screening. 76

Table 5.1: The number of colonies on each plate, resulting from the transformation into NM522 after primary digestion. 89

Table 5.2 The number of colonies on each plate, resulting from the transformation into XL-1 Blue after secondary digestion. 90