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X-RAY CRYSTALLOGRAPHIC
INVESTIGATIONS OF THE STRUCTURES
OF ENZYMES OF MEDICAL AND
BIOTECHNOLOGICAL IMPORTANCE

by

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ABSTRACT

This thesis is broadly in three parts. In the first, the problem of identifying conditions under which a protein will crystallize is considered. Then structural studies on two enzymes are reported, glucose-fructose oxidoreductase from the bacterium *Zymomonas mobilis*, and the human bile salt dependent lipase (carboxyl ester hydrolase).

The ability of protein crystals to diffract X-rays provides the experimental data required to determine their three dimensional structures at atomic resolution. However the crystallization of proteins is not always straightforward. A systematic procedure to search for protein crystallization conditions has been developed. This procedure is based on the use of orthogonal arrays (matrices whose columns possess certain balancing properties). The theoretical and practical background to the problem is discussed, and the relationship of the presented procedure to other published search methods is considered.

The anaerobic Gram-negative bacterium *Zymomonas mobilis* occurs naturally in sugar-rich growth media, and has attracted much interest because of its potential for industrial ethanol production. In this organism the periplasmic enzyme glucose-fructose oxidoreductase (GFOR) is involved in a protective mechanism to counter osmotic stress. The enzyme is unusual in that it contains tightly associated NADP which is not released during its catalytic cycle. The crystal structure of *Z. mobilis* GFOR has been determined by the method of multiple isomorphous replacement, and refined by restrained least squares methods using data extending to an effective resolution of 2.7 Å. The structure determination reveals that each subunit of the tetrameric protein is folded into two domains, one of which is the classical dinucleotide binding domain, or Rossmann fold. The C-terminal domain is a nine-stranded predominantly antiparallel β-sheet around which the tetramer is constructed. Preceding the Rossmann fold there is a 30 amino acid proline rich ‘arm’ which wraps around an adjacent subunit in the tetramer. The N-terminal arm buries the adenine ring of the NADP, and may also be involved in stabilization of the quaternary structure of the enzyme. The tight association of NADP is accounted for by the structure. An unsuspected structural relationship has been discovered between GFOR and the cytoplasmic enzyme glucose-6-phosphate dehydrogenase (G6PD). It is proposed that GFOR and G6PD derive from an common ancestral gene, and GFOR has evolved to allow it to function in the bacterial periplasm where it is required.

The human bile salt dependent lipase (BSDL) is secreted by the pancreas into the digestive tract, and by the lactating mammary gland into human milk, and is integral to the effective absorption of dietary lipids. It is markedly non-specific, and as its name implies is only active against water-insoluble substrates in the presence of primary bile salts. This differentiates the
enzyme from conventional lipases. Diffraction data has been collected from crystals of native BSDL (isolated from human milk), and from crystals of recombinant BSDL (including a truncated variant which lacks a C-terminal heavily glycosylated tandem repeat region found in the native enzyme). The structure of the truncated variant has been partially determined at 3.5 Å resolution, by the method of molecular replacement. The recent collection of a higher resolution (2.8 Å) data set should allow the completion of the structure. The current status of the crystallographic investigations of the human bile salt dependent lipase are reported.
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TABLE OF CONTENTS

ABSTRACT .................................................................................................................................................. i
ACKNOWLEDGEMENTS ............................................................................................................................... iii
TABLE OF CONTENTS .................................................................................................................................. iv
LIST OF FIGURES .......................................................................................................................................... vii
ABBREVIATIONS .......................................................................................................................................... ix
RELATED PUBLICATIONS ............................................................................................................................... xi

Chapter 1
PROTEIN CRYSTALLIZATION

1.1 INTRODUCTION ......................................................................................................................................... 1
  1.1.1 HISTORICAL BACKGROUND .................................................................................................................. 1
  1.1.2 THE EXPERIMENTAL PROBLEM TODAY ............................................................................................. 2
  1.1.3 PHYSICAL BACKGROUND .................................................................................................................... 2

1.2 SEARCH DESIGNS FOR PROTEIN CRYSTALLIZATION ....................................................................... 4
  1.2.1 TERMS ASSOCIATED WITH EXPERIMENTAL DESIGN ....................................................................... 4
  1.2.2 CURRENT APPROACHES TO SEARCHING FOR PROTEIN CRYSTALLIZATION CONDITIONS ............ 5
  1.2.3 GENERAL CRITERIA FOR INITIAL SEARCH EXPERIMENTS ............................................................... 6
  1.2.4 ORTHOGONAL ARRAYS ..................................................................................................................... 7
  1.2.5 UNDERLYING FACTORIAL STRUCTURE FOR SEARCH EXPERIMENTS ............................................. 13
  1.2.6 PRACTICAL IMPLEMENTATION OF ORTHOGONAL ARRAY-BASED SEARCH DESIGNS ................. 14
  1.2.7 EXPERIMENTAL CONSIDERATIONS ................................................................................................. 22

1.3 PRACTICAL APPLICATION TO SEVERAL PROBLEMS ......................................................................... 24
  1.3.1 BILE SALT DEPENDENT LIPASE ....................................................................................................... 25
  1.3.2 GLUCOSE-FRUCTOSE OXIDOREDUCTASE .......................................................................................... 27
  1.3.3 α2β2 EMBRYONIC HEMOGLOBIN ....................................................................................................... 28

1.4 RELATIONSHIP TO PUBLISHED SEARCH PROCEDURES .................................................................. 29

1.5 DISCUSSION AND CONCLUSION ........................................................................................................... 31
  1.5.1 ANALYSIS USING LINEAR MODELS .................................................................................................... 31
  1.5.2 DISTRIBUTION PROPERTIES OF ORTHOGONAL ARRAYS ............................................................... 32
  1.5.3 DYNAMIC LIGHT SCATTERING ........................................................................................................... 33
  1.5.4 CRYSTALLIZATION OF OTHER BIOLOGICAL MACROMOLECULES ............................................... 34
  1.5.5 CONCLUSION ..................................................................................................................................... 34
### TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.4.3</td>
<td>TIGHT ASSOCIATION WITH GFOR</td>
<td>85</td>
</tr>
<tr>
<td>3.4.4</td>
<td>EVOLUTIONARY IMPLICATIONS OF THE N-TERMINAL ARM</td>
<td>86</td>
</tr>
<tr>
<td>3.5</td>
<td>IMPLICATIONS FOR CATALYSIS</td>
<td>86</td>
</tr>
<tr>
<td>3.5.1</td>
<td>BACKGROUND</td>
<td>86</td>
</tr>
<tr>
<td>3.5.2</td>
<td>THE ACTIVE SITE OF GFOR</td>
<td>87</td>
</tr>
<tr>
<td>3.5.3</td>
<td>SEQUENCE AND STRUCTURAL SIMILARITIES</td>
<td>88</td>
</tr>
<tr>
<td>3.5.4</td>
<td>GENERAL DISCUSSION</td>
<td>90</td>
</tr>
<tr>
<td>3.6</td>
<td>GFOR AS A PERIPLASMIC ENZYME</td>
<td>91</td>
</tr>
<tr>
<td>3.7</td>
<td>CONCLUSION</td>
<td>92</td>
</tr>
</tbody>
</table>

## Chapter 4

**BILE SALT DEPENDENT LIPASE**

<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.1</td>
<td>INTRODUCTION</td>
<td>94</td>
</tr>
<tr>
<td>4.1.1</td>
<td>GENERAL BACKGROUND</td>
<td>94</td>
</tr>
<tr>
<td>4.1.2</td>
<td>LIPASES</td>
<td>94</td>
</tr>
<tr>
<td>4.1.3</td>
<td>BILE SALT DEPENDENT LIPASE</td>
<td>98</td>
</tr>
<tr>
<td>4.1.4</td>
<td>THE ROLE OF STRUCTURAL STUDIES</td>
<td>110</td>
</tr>
<tr>
<td>4.2</td>
<td>NATIVE BSDL</td>
<td>111</td>
</tr>
<tr>
<td>4.2.1</td>
<td>PROTEIN PURIFICATION AND CRYSTALLIZATION</td>
<td>111</td>
</tr>
<tr>
<td>4.2.2</td>
<td>CHARACTERIZATION OF THE CRYSTALS</td>
<td>111</td>
</tr>
<tr>
<td>4.2.3</td>
<td>ANISOTROPIC DIFFRACTION</td>
<td>114</td>
</tr>
<tr>
<td>4.2.4</td>
<td>DIFFUSE SCATTERING</td>
<td>116</td>
</tr>
<tr>
<td>4.2.5</td>
<td>ENZYMATIC DEGLYCOSYLATION</td>
<td>119</td>
</tr>
<tr>
<td>4.3</td>
<td>RECOMBINANT FULL LENGTH BSDL</td>
<td>122</td>
</tr>
<tr>
<td>4.3.1</td>
<td>EXPRESSION, PURIFICATION AND CRYSTALLIZATION</td>
<td>122</td>
</tr>
<tr>
<td>4.3.2</td>
<td>PRELIMINARY CRYSTALLOGRAPHIC INVESTIGATION</td>
<td>123</td>
</tr>
<tr>
<td>4.4</td>
<td>RECOMBINANT TRUNCATED BSDL</td>
<td>124</td>
</tr>
<tr>
<td>4.4.1</td>
<td>EXPRESSION AND PURIFICATION</td>
<td>124</td>
</tr>
<tr>
<td>4.4.2</td>
<td>CRYSTALLIZATION</td>
<td>125</td>
</tr>
<tr>
<td>4.4.3</td>
<td>DATA COLLECTION AND PROCESSING</td>
<td>127</td>
</tr>
<tr>
<td>4.4.4</td>
<td>STRUCTURE SOLUTION BY MOLECULAR REPLACEMENT</td>
<td>133</td>
</tr>
<tr>
<td>4.4.5</td>
<td>BUILDING AN INITIAL MODEL</td>
<td>136</td>
</tr>
<tr>
<td>4.4.6</td>
<td>REFINEMENT AT LOW RESOLUTION</td>
<td>138</td>
</tr>
<tr>
<td>4.4.7</td>
<td>DIFFICULTIES IN COMPLETION OF THE PARTIAL STRUCTURE</td>
<td>139</td>
</tr>
<tr>
<td>4.4.8</td>
<td>CURRENT STATUS OF THE STRUCTURE DETERMINATION</td>
<td>140</td>
</tr>
</tbody>
</table>

REFERENCES                                                                 | 143  |
LIST OF FIGURES

Chapter 1

FIGURE 1.1 GEOMETRIC REPRESENTATION OF THE 2 X 2 X 2 FACTORIAL AND SOME POSSIBLE SUBSETS ................................................................. 9
FIGURE 1.2 CRYSTAL OF NATIVE BSDL ................................................................. 26
FIGURE 1.3 CRYSTAL OF ZYMOMONAS MOBILIS GFOR .......................................... 28
FIGURE 1.4 GEOMETRIC REPRESENTATION OF TWO ORTHOGONAL ARRAYS, OA(8, 3, 2X2X4, 2) ..................................................................... 33

Chapter 2

FIGURE 2.1 STEREOGRAHIC PROJECTIONS OF THE SELF-ROTATION FUNCTIONS OF THE TWO CRYSTAL FORMS OF GFOR ...................................... 40
FIGURE 2.2 PATTERSON FUNCTION CALCULATED FROM THE FORM II DATA ......................... 41
FIGURE 2.3 THE RELATIONSHIP BETWEEN THE TWO CRYSTAL FORMS .................................. 42
FIGURE 2.4 DATA COLLECTION USING CRYSTALS MOUNTED IN LIQUID-FILLED CAPILLARIES .......... 44
FIGURE 2.5 CALCULATED AND OBSERVED ELECTRON DENSITY HISTOGRAMS ......................... 53
FIGURE 2.6 ELECTRON DENSITY MAPS FOR GFOR ....................................................... 56
FIGURE 2.7 STEREVIEW OF ELECTRON DENSITY MAPS CALCULATED FROM AN ATOMIC AND A 'GLOBIC' REPRESENTATION OF AN α-HELIX AT 3.0 Å RESOLUTION ......................... 63
FIGURE 2.8 STEREVIEW OF A DIFFERENCE FOURIER SYNTHESIS WITH RESIDUES IN THE REGION CONFLICTING WITH THE PUBLISHED SEQUENCE OMITTED ............................................ 66
FIGURE 2.9 RAMACHANDRAN PLOT FOR THE REFINED GFOR MONOMER ........................... 71
FIGURE 2.10 ELECTRON DENSITY MAP CALCULATED USING THE FORM II DATA ..................... 73

Chapter 3

FIGURE 3.1 Ca PLOT OF GFOR .............................................................................. 74
FIGURE 3.2 TOPOLOGY OF GFOR ........................................................................... 76
FIGURE 3.3 RIBBON DIAGRAMS OF GFOR AND G6PD ................................................... 78
FIGURE 3.4 QUATERNARY STRUCTURE OF GFOR .................................................. 81
FIGURE 3.5 CONFORMATION OF THE ENZYME-BOUND NADP ......................................... 83
FIGURE 3.6 HYDROGEN-BONDI NG INTERACTIONS BETWEEN GFOR AND NADP ................. 84
FIGURE 3.7 THE ACTIVE SITE OF GFOR ................................................................. 87
FIGURE 3.8 ALIGNMENT OF SEQUENCES WITH HOMOLOGY TO GFOR ......................... 89

Chapter 4

FIGURE 4.1 DIAGRAM SHOWING THE CONFORMATIONAL CHANGE ASSOCIATED WITH ACTIVATION IN CANDIDA RUGOSA LIPASE ......................................................... 96
FIGURE 4.2 SCHEMATIC DIAGRAM SHOWING THE CONFORMATIONAL CHANGE ASSOCIATED WITH INTERFACIAL ACTIVATION IN THE FUNGAL LIPASES .................. 97
FIGURE 4.3 ALIGNMENT OF KNOWN BSDL SEQUENCES ............................................. 102
FIGURE 4.4 TOPOLOGY DIAGRAM OF THE LIPASE/ESTERASE FAMILY FOLD ...................... 104
FIGURE 4.5 RIBBON DIAGRAM OF T. CALIFORNICA ACETYLCHOLINESTERASE ................. 105
FIGURE 4.6 BILE ACID STRUCTURE ......................................................................... 108
FIGURE 4.7 SPACE FILLING MODEL OF CHOLIC ACID ................................................. 109
FIGURE 4.8 CRYSTAL OF NATIVE BSDL ..................................................................... 111
FIGURE 4.9 DIFFUSE SCATTERING PATTERNS FROM NATIVE BSDL CRYSTALS (I) ............. 117
| FIGURE 4.10 | DIFFUSE SCATTERING PATTERNS FROM NATIVE BSDL CRYSTALS (II) | 118 |
| FIGURE 4.11 | ISOELECTRIC FOCUSING OF BSDL | 120 |
| FIGURE 4.12 | CRYSTALS OF DESIALIDATED BSDL | 121 |
| FIGURE 4.13 | CRYSTALS OF FULL-LENGTH RECOMBINANT BSDL | 123 |
| FIGURE 4.14 | CRYSTALS OF TRUNCATED RECOMBINANT BSDL | 125 |
| FIGURE 4.15 | GLASS-SLIDE MOUNTING DEVICE FOR CRYOCRYSTALLOGRAPHY | 131 |
| FIGURE 4.16 | BACKGROUND SCATTER AND ABSORPTION DUE TO THE SOLID-SURFACE MOUNT | 132 |
| FIGURE 4.17 | RESULTS OF PATTERSON CORRELATION REFINEMENT | 135 |
| FIGURE 4.18 | ELECTRON DENSITY FOR TRUNCATED RECOMBINANT BSDL | 141 |
ABBREVIATIONS

AChE  Acetylcholinesterase
AMPSO  3-[(1,1-Dimethyl-2-hydroxyethyl)amino]2-hydroxypropanesulfonic acid
BIS-TRIS PROPA NE  1,3-bis[tris(Hydroxymethyl)-methylamino]propane
BSDL  Bile salt dependent lipase
BSSL  Bile salt stimulated lipase
CDL  Colipase-dependent lipase
CHAPS  3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate
CRL  Candida rugosa lipase
dhPR  Dihydridipicolinate reductase
DNA  Deoxyribonucleic acid
EPPS  N-[2-Hydroxyethyl]piperazine-N'[3-propanesulfonic acid]
FAD  Flavin-adenine dinucleotide
FMN  Flavin mononucleotide
G6PD  Glucose-6-phosphate dehydrogenase
GAPDH  Glyceraldehyde-3-phosphate dehydrogenase
GCL  Geotrichum candidum lipase
GFOR  Glucose-fructose oxidoreductase
HEPES  N-[2-Hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid]
IEF  Isoelectric focussing
LDH  Lactate dehydrogenase
MDH  Malate dehydrogenase
MES  2-[N-Morpholino]ethanesulfonic acid
MIR  Multiple isomorphous replacement
MOPS  3-[N-Morpholino]propanesulfonic acid
NAD  Oxidized or reduced form of nicotinamide adenine dinucleotide
NADP  Oxidized or reduced form of nicotinamide adenine dinucleotide phosphate
NADP+  Oxidized form of nicotinamide adenine dinucleotide phosphate
NADPH  Reduced form of nicotinamide adenine dinucleotide phosphate
NAD(P)  NAD or NADP
NCBI  National Center for Biotechnology Information
NCS  Non-crystallographic symmetry
NIST  National Institute of Standards and Technology
PCR  Polymerase chain reaction
PEG  Polyethylene glycol
PEG-mme  Polyethylene glycol monomethyl ether
ABBREVIATIONS

**PIPS** 1,4-Piperazinediethanesulfonic acid
**PQQ** Pyrrolo-quinoline quinone
**RMS** Root mean square
**SEL** Sequential elimination of levels
**SIR** Single isomorphous replacement
**TAPS** N-tris[Hydroxymethyl]methyl-3-aminopropanesulfonic acid
**TcAChE** *Torpedo californica* acetylcholinesterase
**TRIS** Tris(hydroxymethyl)aminomethane
RELATED PUBLICATIONS

Some of the material presented in this thesis has already been published, or has been accepted for publication.
