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

by

Richard Lawrence Kingston

A dissertation submitted in partial satisfaction of the requirements for the degree of

Doctor of Philosophy

in the

Department of Biochemistry

at

MASSEY UNIVERSITY, NEW ZEALAND

November, 1996

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.

ACKNOWLEDGMENTS

I thank my principal supervisor, Professor Ted Baker for his friendship, his enthusiasm for science, and for allowing me much freedom to follow my ideas. I also thank Mrs. Heather Baker for her help and constant encouragement.

I thank my assistant supervisors, Dr. Bryan Anderson (for many discussions about crystallographic computing), and Professor Sylvia Rumball (who initiated a collaboration with Umeå University on bile salt dependent lipase).

I would like to specifically acknowledge the scientific contributions of Dr. Rick Faber, and Dr. Stanley Moore to the work presented in this thesis (when they weren't fly fishing, that is).

I would like to thank all the other friends I have made during my time at Massey. Andrew, Rosemary, Isobel, Shaun, Treena, Ross, Catherine, Alain and Anne-Gael, Mark, Michelle, Jakki and Paul, Neil and Liz, Phil, and Maria to name only some. Thanks.

The work on bile salt dependent lipase was carried out in collaboration with Professor Olle Hernell and Dr. Lars Bläckberg (Umeå University, Sweden); and Dr. Kerry Loomes (Auckland University, New Zealand). The work on glucose-fructose oxidoreductase was carried out in collaboration with Professor Robert Scopes (La Trobe University, Australia). I thank these people for their scientific contributions, and especially Kerry, for believing we could solve the structure and working so hard to overcome difficult technical problems.

For financial assistance while this work was completed I thank Massey University (through the award of a doctoral scholarship), and latterly Professor Ted Baker.

Thanks to the members of my family for encouragement and support. I owe much to my parents for supporting what I do, and helping to finance my study.

Finally I would like to thank Wendy, a very special friend, and someone whom it has been difficult to be separated from while this thesis was written

TABLE OF CONTENTS

Chapter 2
GFOR: STRUCTURE DETERMINATION

Chapter 3
GFOR: STRUCTURE AND FUNCTION

LIST OF FIGURES

Chapter 1

Chapter 2

Chapter 3

Chapter 4

ABBREVIATIONS

AChE Acetylcholinesterase
AMPSO 3-[(1,1-Dimethyl-2-hydroxyethyl)amino]2-hydroxypropanesulfonic acid
BIS-TRIS PROPANE 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 Dihydrodipicolinate 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

PIPES 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.

Kingston, R.L., Baker, H.M. & Baker, E.N. (1994) Search designs for protein crystallization based on orthogonal arrays. **Acta Crystallographica. D50**, 429-440.

Kingston, R.L., Scopes, R.K. & Baker, E.N. (1996) The structure of glucose fructose oxidoreductase from *Zymomonas mobilis*: an osmoprotective periplasmic enzyme containing non-dissociable NADP. **Structure**. in Press.