X-RAY CRYSTALLOGRAPHIC ANALYSIS OF THE PYROPHOSPHATE-DEPENDENT PHOSPHOFRUCTOKINASE OF *SPIROCHAETA THERMOPHILUM*

A thesis presented in partial fulfillment of the requirements for the degree of Master of Science in Biochemistry at Massey University, New Zealand.

Andrew James Welham

2002

"I think, therefore I am"

René Descartes

ABSTRACT

The structure of a homodimeric, non-allosteric, PP_i-dependent phosphofructokinase from the thermophilic bacterium *Spirochaeta thermophilum* has been resolved by X-ray crystallography in two distinct conformations at 2.2 (R = 0.1991 [R_{free} = 0.2288]) and 1.85 Å (R = 0.1923 [R_{free} = 0.2035]) resolution. The 554 residue (M_r 61080 $g.mol^{-1}$) subunit, a homologue of the plant PP_i-PFK β-subunit exhibits an asymmetrical quaternary structure and shares both sequence and tertiary structure with the N- and C-terminal Rossmann-like domains of prokaryotic ATP-PFKs. *Spirochaeta thermophilum* PP_i-PFK exhibits three major inserts relative to the prokaryotic ATP-PFK of *E. coli*, an N-terminal insert, a C-terminal insert, and an insert within the PFK C-terminal domain which forms an autonomous α-helical domain. The active site is formed at the interface of the N and C domains. The 'open' and 'closed' subunit asymmetry of the *S. thermophilum* PP_i-PFK 1.85 Å atomic model mirrors that of the *B. burgdorferi* PP_i-PFK (1KZH [Moore et al.2002]) with the exception that the two unique β–hairpins (380-390 [α16-α17] and 485-495 [β14-β15]) of subunit A are not displaced into the active site. Both subunits of the *S. thermophilum* PP_i-PFK 2.2 Å atomic model adopt an 'open', apparently inactive conformation. The conformational change involves concomitant closure of the active site of both subunits via a rigid-body displacement of the C and α-helical domains, relative to the N domain. The N domain of one subunit and the C domain of the opposing subunit can be thought of as a rigid body, therefore closure of one active site dictates closure of the other. Rotation of the small domain forces Met251 of the MGR motif to adopt an active conformation and displacement of the α-helical domain, specifically the 380-390 β-hairpin into the active site 'folds' Arg253 (MG**R**) into an active conformation. Closure of the active site, which prevents wasteful hydrolysis, involves movement of the β14-β15 β-hairpin into the active site and simultaneous rearrangement of the PP_i-binding GGDD motif. The conformational change of the *S. thermophilum* PP_i-PFK is surprisingly complex and unique relative to prokaryotic ATP-PFKs and involves displacement of novel structural elements. These movements change the conformation of conserved motifs at the active site and therefore function to modulate PP_i-dependent activity.

ACKNOWLEDGEMENTS

This thesis is representative of the efforts of a number of people to whom I am indebted. Primarily I would like to thank my supervisor Dr. Stan Moore for his unbounded patience, encouragement, and support. Unforeseeably, giving me the opportunity to undertake this research also ceded a passion for science and research, a life long pursuit.

I would like to thank Dr. Ron Ronimus and Dr. Hugh Morgan from the Thermophile Research Unit, University of Waikato for the purified *Spirochaeta thermophilum* pyrophosphate-dependent phosphofructokinase.

I would like to thank Dr. Kathryn Stowell for her immense patience in listening to my ideas and the proof reading of this thesis.

I would like to thank past and present members of the Institute of Molecular Biosciences and the Institute of Fundamental Sciences for there tutorage, particularly Assoc. Prof. John Tweedie, Dr. Mark Patchet, Prof. Pat Sullivan, Dr. Gill Norris, Dr. Max Scott, Assoc. Prof. Geoff Jameson, Dr. Bryan Anderson, Dr. Emily Parker, and last but not least Dr. Simon Brown (an inspirational teacher).

I gratefully acknowledge financial support from the award of a Massey University Masterate Scholarship.

Thank you to my family: Mum, Uncle Howard, Aunt Beth, Gaylyn, Sylvia, Shaun, Martyn, Pieter, Jeannie and Michelle for your perpetual love, support, patience and faith in me. I will always be indebted.

TABLE OF CONTENTS

List of Figures

Chapter 1 Introduction and Literature Review

Chapter 3 Results: Data Quality and Atomic Model Statistics

Chapter 4 *Spirochaeta thermophilum* PP_i-PFK: Structure and Function

List of Tables

Abbreviations

$\|Fc\|$	Structure factor (calculated)
$\|F_o\|$	Structure factor (observed)
1,3-BPG	1,3-bisphosphoglycerate
2-PG	2-Phosphoglycerate
3-PG	3-Phosphoglycerate
Å	Angstrom (10^{-10}m)
ADP	Adenosine diphosphate
AMP	Adenosine monophosphate
APS	Ammonium peroxidisulphate
ATP	Adenosine triphosphate
CC	Correlation Coefficient
Da	Dalton
DHAP	Dihydroxyacetone phosphate
ED	Entner Doudoroff Pathway
F16bP	Fructose 1,6-bisphosphate
F26bP	Fructose 2,6-bisphosphate
F6P	Fructose 6-phosphate
FBPase	Fructose bisphosphatase
FOM	Figure of merit
G6P	Glucose 6-phosphate
GAP	Glyceraldehyde-3-phosphate
GAP:FdOR	Glyceraldehyde-3-phosphate: ferrodoxin oxidoreductase
GLK	Glucokinase
Hepes	N-(2-hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid)
M_r	Molecular mass (g. mol^{-1})
NAD	Nicotinamide adenine dinucleotide
NADPH	Nicotinamide adenine dinucleotide phosphate
PEG	Polyethylene Glycol
PEP	Phosphoenolpyruvate
PFK	Phosphofructokinase
PGI	Phosphoglucose isomerase
PP_i	Pyrophosphate
PPP	Pentose-Phosphate Pathway
RMS	Root mean squared
TCA	Tricarboxylic acid cycle
TEMED	N,N,N',N'-tetramethylethylenediamine
TIM	Triose phosphate isomerase
Tris	Tris(hydroxymetyl)aminomethane
$\Delta G^{o'}$	Free energy change

Amino Acids

Ala	A	Alanine
Arg	R	Arginine
Asn	N	Asparagine
Asp	D	Aspartic acid
Cys	C	Cystine
Gln	Q	Glutamine
Glu	E	Glutamic acid
Gly	G	Glycine
His	H	Histidine
Ile	I	Iso-leucine
Leu	L	Leucine
Lys	K	Lysine
Met	M	Methionine
Phe	F	Phenylalanine
Pro	P	Proline
Ser	S	Serine
Thr	T	Threonine
Trp	W	Tryptophan
Tyr	Y	Tyrosine
Val	V	Valine