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**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 ( $R = 0.1991$  [ $R_{\text{free}} = 0.2288$ ]) and 1.85 Å ( $R = 0.1923$  [ $R_{\text{free}} = 0.2035$ ]) resolution. The 554 residue ( $M_r$  61080  $\text{g}\cdot\text{mol}^{-1}$ ) subunit, a homologue of the plant  $PP_i$ -PFK  $\beta$ -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  $\alpha$ -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  $\beta$ -hairpins (380-390 [ $\alpha 16$ - $\alpha 17$ ] and 485-495 [ $\beta 14$ - $\beta 15$ ]) 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  $\alpha$ -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  $\alpha$ -helical domain, specifically the 380-390  $\beta$ -hairpin into the active site 'folds' Arg253 (MGR) into an active conformation. Closure of the active site, which prevents wasteful hydrolysis, involves movement of the  $\beta 14$ - $\beta 15$   $\beta$ -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.

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# TABLE OF CONTENTS

<b>Abstract</b>	<b>i</b>
<b>Acknowledgements</b>	<b>ii</b>
<b>Table of Contents</b>	<b>iii</b>
<b>List of Figures</b>	<b>vii</b>
<b>List of Tables</b>	<b>x</b>
<b>Abbreviations</b>	<b>xii</b>
<b>Chapter 1 Introduction and Literature Review</b>	<b>1</b>
1.1 Metabolism	1
1.2 Glycolysis	1
1.3 Modified Embden-Meyerhof Pathways	4
1.3.1 Pyrophosphate-dependent Glycolysis	5
1.4 Kinases	6
1.4.1 Phosphoryl transfer	6
1.4.2 Dissociative S <sub>N1</sub>	7
1.4.3 Associative S <sub>N2</sub>	7
1.4.4 Ping-Pong	8
1.4.5 General Acid-Base Catalysis	9
1.4.6 Transition State Stabilization	9
1.4.7 Kinase Structural Folds	9
1.5 Structure: Prokaryotic ATP-PFKs	12
1.6 Active Site: Substrate Binding and Catalysis	15
1.6.1 F16bP Binding Site	15
1.6.2 ADP Binding Site	16
1.6.3 Mg <sup>2+</sup> Binding Site	19
1.7 Allosteric Site	19
1.7.1 R-T State Transition	24
1.8 Caveat: Failure of the Concerted Two-state Model	27



## Table of Contents

1.9 Evolution of the Phosphofructokinase A Family	27
1.9.1 PFK A Family	28
1.9.2 Group I ATP-PFKs	30
1.9.3 Group II PP <sub>i</sub> -PFKs	32
1.9.4 Evidence for a Latent Nucleotide Binding Site?	37
1.9.5 Group III PFKs	37
1.9.6 Summary: Evolution of PFK A PFKs	37
1.10 Aims of this Research	39
<b>Chapter 2 Materials and Methods</b>	<b>41</b>
2.1 Introduction	41
2.1.1 Experimental Strategy	41
2.2 Materials	42
2.3 Methods	43
2.3.1 Crystallization of <i>S. thermophilum</i> PP <sub>i</sub> -PFK	43
2.3.2 Determination of <i>S. thermophilum</i> PP <sub>i</sub> -PFK Concentration	43
2.3.3 SDS-PAGE Gel Electrophoresis	44
2.3.4 Equilibration	44
2.3.5 Commercial Crystallization Screens	45
2.3.6 X-ray Data Collection	45
2.3.6.1 Data set: 2.2 Å Resolution	45
2.3.6.2 Data set: 1.85 Å Resolution	46
2.3.7 Crystallographic Data Processing	46
2.3.8 Molecular Replacement	48
2.3.8.1 Rotation Function	49
2.3.8.2 Translation Function	50
2.3.9 AMORE	51
2.3.10 Restrained Crystallographic Refinement	52
2.3.10.1 Cross-validation	52
2.3.10.2 CNS Rigid-body Refinement	52

## Table of Contents

2.3.10.3	CNS Restrained Crystallographic Conjugate Gradient Minimization	52
2.3.10.4	Restrained Atomic Temperature Factor Refinement	53
2.3.10.5	Atomic Model Building and Electron Density Interpretation	53
<b>Chapter 3 Results: Data Quality and Atomic Model Statistics</b>		<b>55</b>
3.1	Introduction	55
3.2	Space Group and Unit Cell	55
3.3	AMORE: 2.2 Å Solution	58
3.3.1	Rotation Function	58
3.3.2	Translation Function	58
3.4	AMORE: 1.85 Å Solution	59
3.4.1	Rotation Function	60
3.4.2	Translation Function	60
3.5	Crystal Packing	61
3.6	Refinement of the <i>S. thermophilum</i> PP <sub>i</sub> -PFK Atomic Models	64
3.6.1	Model Building: 2.2 Å Model	65
3.6.2	Model Building 1.85 Å Model	66
3.7	Iterative Restrained Refinement	67
3.8	<i>S. thermophilum</i> PP <sub>i</sub> -PFK: Quality of the Final Atomic Models	67
3.8.1	Active Site	68
<b>Chapter 4 <i>Spirochaeta thermophilum</i> PP<sub>i</sub>-PFK: Structure and Function</b>		<b>79</b>
4.1	Quaternary Structure	79
4.2	Tertiary Structure	80
4.3	Secondary Structure	86
4.4	Insertions	88
4.5	Structural Asymmetry	90
4.6	Conformational Change	101



## Table of Contents

4.7 Dimer Interface	107
4.8 Active Site Geometry	112
4.9 F6P/F16bP Binding Site	114
4.9.1 Conformational Change: the MGR Motif	121
4.9.2 Arg146: Transition State Stabilization	127
4.10 Pyrophosphate Binding Site	129
4.10.1 Phosphoryl Donor Specificity	136
4.10.2 GGDD Motif	137
4.10.3 <i>B. burgdorferi</i> PP <sub>i</sub> -PFK: Pyrophosphate Binding Site	140
4.10.4 PKTIDGD: Lys203	143
4.11 GGPAPG Loop	146
4.12 Summary: <i>S. thermophilum</i> PP <sub>i</sub> -PFK	146
<b>Chapter 5 <i>Spirochaeta thermophilum</i> PP<sub>i</sub>-PFK: Synopsis</b>	<b>148</b>
5.1 Synopsis: Data Quality and Atomic Model Statistics	148
5.2 Synopsis: Structure and Function	148
5.2.1 F6P/F16bP Binding Site	150
5.2.2 Pyrophosphate Specificity	151
5.2.3 GGDD	152
5.2.4 Conformational Change: Substrate Analogues	152
5.3 Future Directions	154
5.3.1 Site-Directed Mutagenesis	154
5.3.2 Substrate/Product Binding	154
5.3.3 PP <sub>i</sub> -PFK Inhibition	155
5.3.4 Plant PP <sub>i</sub> -PFKs	155
5.4 Conclusion	155
<b>References</b>	<b>157</b>

## List of Figures

### Chapter 1 Introduction and Literature Review

Figure 1.1	Chair Conformation of Glucose	2
Figure 1.2	Embden-Meyerhof Pathway	3
Figure 1.3	Phosphoryl Transfer: Associative and Dissociative Mechanisms	8
Figure 1.4	The Rossmann-like Structural fold	10
Figure 1.5	Tertiary Structure of the Prokaryotic ATP-PFK of <i>E. coli</i>	13
Figure 1.6	Active Site of the Prokaryotic ATP-PFK of <i>E. coli</i>	17
Figure 1.7	Mechanism of the ATP inhibition of ATP-PFKs	20
Figure 1.8	Allosteric site of the Prokaryotic ATP-PFK of <i>E. coli</i>	22
Figure 1.9	R - T State Transition of the Prokaryotic ATP-PFK of <i>B. stearothermophilus</i>	25
Figure 1.10	Phylogenetic Tree of PFK A Family Members	31
Figure 1.11	Sequence Alignment of Group I PFKs	34
Figure 1.12	Sequence Alignment of Group II, Long Clade Family Members	
Figure 1.13	Sequence Alignment of Group I, III, and Group II, X Clade Family Members	36

### Chapter 3 Results: Data Quality and Atomic Model Statistics

Figure 3.1	Unit cells of the <i>S. thermophilus</i> PP <sub>i</sub> -PFK structures	61
Figure 3.2	Initial 2m F <sub>o</sub> - D F <sub>c</sub>   and m F <sub>o</sub> - D F <sub>c</sub>   Electron Density Maps of Met282B from the <i>S. thermophilus</i> PP <sub>i</sub> -PFK 2.2 Å Atomic Model	66
Figure 3.3	Ramachandran Plot of the <i>S. thermophilus</i> PP <sub>i</sub> -PFK 2.2 Å Atomic Model	72
Figure 3.4	Ramachandran Plot of the <i>S. thermophilus</i> PP <sub>i</sub> -PFK 1.85 Å Atomic Model	73

## List of Figures

Figure 3.5	Real Space Correlation Coefficient and Temperature Factor Plot of the <i>S. thermophilum</i> PP <sub>i</sub> -PFK 1.85 Å Atomic Models Subunits	75
Figure 3.6	Final 2m F <sub>o</sub>   - D F <sub>c</sub>   Electron Density Map of the <i>S. thermophilum</i> PP <sub>i</sub> -PFK 1.85 Å Atomic Models Subunit B N Domain β-Sheet	76
Figure 3.7	2m F <sub>o</sub>   - D F <sub>c</sub>   Electron Density Map of the <i>S. thermophilum</i> PP <sub>i</sub> -PFK 2.2 Å Atomic Models Subunit B N Domain β-Sheet	77

### **Chapter 4 *Spirochaeta thermophilum* PP<sub>i</sub>-PFK: Structure and Function**

Figure 4.1	Quaternary Structure of the PP <sub>i</sub> -PFK of <i>S. thermophilum</i>	79
Figure 4.2	Structure Based Sequence Alignment of Group I and Group II, Long Clade Family members	80
Figure 4.3	Superposition of the <i>S. thermophilum</i> PP <sub>i</sub> -PFK Subunit A with that of <i>E. coli</i> ATP-PFK	84
Figure 4.4	Hydrophobicity Plot of the <i>S. thermophilum</i> PP <sub>i</sub> -PFK	86
Figure 4.5	β-Sheet Topology of the <i>S. thermophilum</i> PP <sub>i</sub> -PFK	87
Figure 4.6	Tertiary Structure of the <i>S. thermophilum</i> PP <sub>i</sub> -PFK	88
Figure 4.7	Subunit RMS Coordinate Differences for the PP <sub>i</sub> -PFK Atomic Models	92
Figure 4.8	Subunit Asymmetry of the PP <sub>i</sub> -PFK Atomic Models	95
Figure 4.9	Cα - Cα Pseudo Torsion Angle Plot of the <i>S. thermophilum</i> PP <sub>i</sub> -PFK Atomic Models	100
Figure 4.10	Conformational Change of the <i>S. thermophilum</i> PP <sub>i</sub> -PFK	104
Figure 4.11	Conserved Orientation of the Large Domain Relative to the Small Domain of the Opposing Subunit	106
Figure 4.12	Superposition of the <i>S. thermophilum</i> PP <sub>i</sub> -PFK Dimer with the A:D dimer of <i>E. coli</i> ATP-PFK	108
Figure 4.13	Active Site of <i>S. thermophilum</i> PP <sub>i</sub> -PFK: Global View	113
Figure 4.14	F6P/F16bP Binding Site	114

## List of Figures

Figure 4.15	van der Waals Contact Between Leu420B and Tyr244B and Tyr426A of the <i>S. thermophilum</i> PP <sub>i</sub> -PFK 1.85 Å Atomic Model	117
Figure 4.16	Structure Based Sequence Alignment of helix α9 of Group I and Group II, Long Clade Family Members	119
Figure 4.17	MGR Motif of <i>B. burgdorferi</i> PP <sub>i</sub> -PFK	120
Figure 4.18	MGR Motif of the <i>S. thermophilum</i> PP <sub>i</sub> -PFK 2.2 Å Atomic Model	121
Figure 4.19	Relative Disorder of the MGR Motif of the <i>S. thermophilum</i> PP <sub>i</sub> -PFK 1.85 Å Atomic Model	124
Figure 4.20	Superposition of the MGR Motifs of the <i>S. thermophilum</i> PP <sub>i</sub> -PFK Atomic Models	124
Figure 4.21	Displacement of the Subunit A 380-390 β-Hairpin of the <i>S. thermophilum</i> PP <sub>i</sub> -PFK 1.85 Å Atomic Model	126
Figure 4.22	RMS Deviation of the Small Domain Between Subunits of the PP <sub>i</sub> -PFK Atomic Models	128
Figure 4.23	Structure based sequence alignment of the GGDD and PKTIDND motifs.	129
Figure 4.24	Pyrophosphate Binding Site of <i>S. thermophilum</i> PP <sub>i</sub> -PFK	130
Figure 4.25	Pyrophosphate Binding Site of <i>B. burgdorferi</i> PP <sub>i</sub> -PFK	133
Figure 4.26	Alternative conformations of the <i>S. thermophilum</i> <b>GGDD</b> motifs.	139
Figure 4.27	Pyrophosphate-binding site of <i>B. burgdorferi</i> PP <sub>i</sub> -PFK	141



## List of Tables

### Chapter 1 Introduction and Literature Review

Table 1.1	Conserved Motifs of PFK A Family Members	28
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### Chapter 3 Results: Data Quality and Atomic Model Statistics

Table 3.1	Space Group and Unit Cell Dimensions	55
Table 3.2	Data Refinement Statistics	57
Table 3.3	AMORE: Rotation Function Solutions for the 2.2 Å Data Set	58
Table 3.4	AMORE: Translation Function Solutions for the 2.2 Å Data Set	58
Table 3.5	AMORE: Rotation Function Solutions for the 1.85 Å Data Set	60
Table 3.6	AMORE: Translation Function Solutions for the 1.85 Å Data Set	60
Table 3.7	Crystal Contacts of the <i>S. thermophilum</i> PP <sub>i</sub> -PFK 2.2 Å Atomic Model	62
Table 3.8	Crystal Contacts of the <i>S. thermophilum</i> PP <sub>i</sub> -PFK 1.85 Å Atomic Model	63
Table 3.9	Non-Crystallographic Symmetry Groups	64
Table 3.10	Residues of the <i>S. thermophilum</i> PP <sub>i</sub> -PFK Atomic Models with Uninterpretable Electron Density	69
Table 3.11	Synopsis of Temperature Factors for the <i>S. thermophilum</i> PP <sub>i</sub> -PFK Atomic Models	70
Table 3.12	Synopsis of the Temperature Factor RMS Deviation for the <i>S. thermophilum</i> PP <sub>i</sub> -PFK Atomic Models	70
Table 3.13	Correlation Coefficients of the Initial and Final <i>S. thermophilum</i> PP <sub>i</sub> -PFK 1.85 Å Atomic Model	74
Table 3.14	Correlation Coefficients of the Initial and Final <i>S. thermophilum</i> PP <sub>i</sub> -PFK 2.2 Å Atomic Model	74

## List of Tables

Table 3.15	Synopsis of Refinement and Model Statistics of the <i>S. thermophilum</i> -PFK 1.85 Å Atomic Model	78
Table 3.16	Synopsis of Refinement and Model Statistics of the <i>S. thermophilum</i> -PFK 2.2 Å Atomic Model	78

### **Chapter 4 *Spirochaeta thermophilum* PP<sub>i</sub>-PFK: Structure and Function**

Table 4.1	Domain RMS Deviation Between <i>S. thermophilum</i> PP <sub>i</sub> -PFK Atomic Models	81
Table 4.2	Subunit Asymmetry of PP <sub>i</sub> -PFKs	91
Table 4.3	Domain Structure Comparison of the <i>S. thermophilum</i> PP <sub>i</sub> -PFK 1.85 Å and <i>B. burgdorferi</i> PP <sub>i</sub> -PFK Atomic Models	93
Table 4.4	Domain Structure Comparison of the <i>S. thermophilum</i> PP <sub>i</sub> -PFK Atomic Models	94
Table 4.5	Conserved Orientation of the N Domain of one subunit relative to the C Domain of the Opposing Subunit	102
Table 4.6	Inter-Subunit Contacts of the <i>S. thermophilum</i> PP <sub>i</sub> -PFK 1.85 Å Atomic Model	107
Table 4.7	Contacts Formed by Sulfate Ions Bound at the Active Site of the <i>S. thermophilum</i> PP <sub>i</sub> -PFK 1.85 Å Atomic Model	115
Table 4.8	Comparison of the MGR Motif Dihedral Angles	122
Table 4.9	Contacts formed by a Sulfate Ion Bound Beneath the Subunit A GGDD Motif of the <i>S. thermophilum</i> PP <sub>i</sub> -PFK 1.85 Å Atomic Model	130
Table 4.10	Disorder of Lys148B and Asp177B of the <i>B. burgdorferi</i> PP <sub>i</sub> -PFK Atomic Model	142



**Abbreviations**

F <sub>c</sub>	Structure factor (calculated)
F <sub>o</sub>	Structure factor (observed)
1,3-BPG	1,3-bisphosphoglycerate
2-PG	2-Phosphoglycerate
3-PG	3-Phosphoglycerate
Å	Angstrom (10 <sup>-10</sup> 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: ferredoxin oxidoreductase
GLK	Glucokinase
Hepes	N-(2-hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid)
M <sub>r</sub>	Molecular mass (g. mol <sup>-1</sup> )
NAD	Nicotinamide adenine dinucleotide
NADPH	Nicotinamide adenine dinucleotide phosphate
PEG	Polyethylene Glycol
PEP	Phosphoenolpyruvate
PFK	Phosphofruktokinase
PGI	Phosphoglucose isomerase
PP <sub>i</sub>	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(hydroxymethyl)aminomethane
ΔG <sup>o</sup>	Free energy change

## Abbreviations

### 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