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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  
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"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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## 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	Phosphofructokinase
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