Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.
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$_r$-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$_r$-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$_r$-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$_r$-PFK 1.85 Å atomic model mirrors that of the *B. burgdorferi* PP$_r$-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$_r$-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 (MGR) 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$_r$-binding GGDD motif. The conformational change of the *S. thermophilum* PP$_r$-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$_r$-dependent activity.
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.

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<tr>
<td>[Fc]</td>
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<tr>
<td>[Fo]</td>
<td>Structure factor (observed)</td>
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<td>2-PG</td>
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<tr>
<td>3-PG</td>
<td>3-Phosphoglycerate</td>
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<td>Å</td>
<td>Angstrom $\left(10^{\text{-}10}\text{m}\right)$</td>
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## Abbreviations

### Amino Acids

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