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.
SHEEP LIVER PHOSPHOFRUCTOKINASE:

A COMPARISON OF THE PRIMARY STRUCTURE WITH THOSE OF OTHER MAMMALIAN ISOZYMES

A Thesis presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Biochemistry at MASSEY UNIVERSITY

KAY JENICE RUTHERFURD

1988
Phosphofructokinase (PFK) is the key regulatory enzyme of glycolysis, catalysing the synthesis of fructose 1,6-bisphosphate from fructose 6-phosphate and ATP.

Several PFK isozymes have been identified from different tissues, including muscle, liver and platelet. Each isozyme is under the control of a different structural locus in humans the muscle isozyme is carried on chromosome 1, liver on chromosome 21, and platelet on chromosome 10.

The tetramer of PFK is the lowest active form, and there are both species and tissue differences in the composition of the tetramer. Some, such as human muscle PFK consist of four identical subunits ($M_4$), whereas others, such as human erythrocyte PFK comprise a five membered isozyme system, made up of both muscle and liver subunits.

The activity of PFK is modulated by a variety of effectors such as; ATP, fructose 6-phosphate, ADP, AMP and fructose 2,6-bisphosphate, as well as covalent modification, and hormonal regulation. Each PFK isozyme exhibits its own characteristic kinetic properties in response to changes in effector concentrations. This project aims to compare the primary structures of two PFK isozymes; liver and muscle, from a single species (sheep), in order to explain their different kinetic properties in terms of differences in their primary structures.

A purification procedure for sheep liver PFK was developed, and a 48% yield was obtained, with a final specific activity of 100Units/mg of protein.

Sodium dodecyl sulphate-gel electrophoresis indicated a protomer molecular weight of 84 000 for sheep heart muscle PFK, and 81 000 for sheep liver PFK, indicating that liver PFK is approximately 30 residues shorter than the muscle enzyme.

The comparison of the amino acid compositions showed a significantly lower arginine and lysine content in liver PFK compared to muscle PFK. There was also a lower threonine content in liver PFK, accompanied by an increase in the number of serine residues, compared to muscle PFK. So overall, the number of residues with
hydroxy-alkyl sidechains remained the same. The tyrosine content of liver PFK was also higher than that of muscle PFK.

The comparison of HPLC and FPLC peptide maps of liver and muscle PFK provided by digestion with CNBr and trypsin, showed a number of interesting differences between the two isozymes. Generally, the CNBr peptides of liver PFK appeared to be smaller than those of muscle PFK, while the radioactively labelled cysteine containing peptides from the tryptic digest of muscle PFK, were more hydrophobic than those from liver PFK.

Sequence information was obtained from purified peptides produced by digestion of liver PFK with CNBr, trypsin, and trypsin following maleylation of the sheep liver PFK with maleic anhydride to block the lysine residues. 87% of the amino acid sequence of sheep liver PFK was determined, and aligned with the sequences from rabbit muscle and sheep heart PFK. Approximately 70% sequence identity was observed between the liver and muscle isozymes, particularly in the regions where contacts to substrates and effectors are thought to be made. There are two regions exhibiting major sequence changes between the muscle and liver isozymes, both are thought to lie on the exterior of the molecule, and therefore would not disrupt the tertiary structure. One of these regions however, is thought to contain the ATP inhibitory site; and the large variation in sequence at this site may explain the greater susceptibility of the liver enzyme to ATP inhibition. Other, more subtle changes in the sequence may account for the additional differences in the kinetic properties displayed by muscle and liver PFK.
ACKNOWLEDGEMENTS

I wish to thank my supervisors Dr C.H. Moore and Dr G.G. Midwinter for their invaluable advise, encouragement and assistance throughout the course of this study.

Thanks go also to Mr J.R. Reid for assistance with the amino acid analyses and running of the gas-phase sequencer, as well as Dr B.F. Anderson and Dr E.N. Baker for assistance with using the computer program FRODO and interpretation of some of the amino acid differences between the PFK isozymes.

I would also like to thank my husband Shane for his assistance in the preparation of this manuscript.
## LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADP</td>
<td>adenosine 5’-diphosphate</td>
</tr>
<tr>
<td>Am Bic</td>
<td>ammonium bicarbonate</td>
</tr>
<tr>
<td>AMP</td>
<td>adenosine 5’-monophosphate</td>
</tr>
<tr>
<td>ATP</td>
<td>adenosine 5’-triphosphate</td>
</tr>
<tr>
<td>βME</td>
<td>β-mercaptoethanol</td>
</tr>
<tr>
<td>Bs</td>
<td><em>Bacillus</em> <em>stearothermophilus</em></td>
</tr>
<tr>
<td>BSA</td>
<td>bovine serum albumin</td>
</tr>
<tr>
<td>Ca²⁺-CaM</td>
<td>Ca²⁺-calmodulin complex</td>
</tr>
<tr>
<td>CaM</td>
<td>calmodulin</td>
</tr>
<tr>
<td>cAMP</td>
<td>cyclic-AMP</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>DTNB</td>
<td>5,5’-dithiobis-2-nitrobenzoic acid</td>
</tr>
<tr>
<td>DTT</td>
<td>dithiothreitol</td>
</tr>
<tr>
<td>Ec</td>
<td><em>E. coli</em></td>
</tr>
<tr>
<td>EDTA</td>
<td>ethylenediamine tetraacetic acid</td>
</tr>
<tr>
<td>F6P</td>
<td>fructose 6-phosphate</td>
</tr>
<tr>
<td>F16BP</td>
<td>fructose 1,6-bisphosphate</td>
</tr>
</tbody>
</table>
F26BP  fructose 2,6-bisphosphate
FBPase  fructose bisphosphatase
F16BPase  fructose 1,6-bisphosphatase
F26BPase  fructose 2,6-bisphosphatase
FDNB  1-fluoro-2,4-dinitrobenzene
FPLC  fast protein liquid chromatography
α-GPD  α-glycerophosphate dehydrogenase
G1P  glucose 1-phosphate
G6P  glucose 6-phosphate
G16BP  glucose 1,6-bisphosphate
HPLC  high performance liquid chromatography
MLCK  myosin light chain kinase
NADH  nicotinamide adenine dinucleotide, reduced form
PEG  polyethylene glycol-6000
PEP  phosphoenolpyruvate
PFK  phosphofructokinase
PFK-2  phosphofructokinase-2
Pi  inorganic phosphate
PMSF  phenylmethylsulphonyl fluoride
POPOP 1,4-bis[2(5-phenyloxazolyl)]benzene

PPO 2,5-diphenyloxazole

RM rabbit muscle

SDS sodium dodecyl sulphate

SH sheep heart

SL sheep liver

SDS-PAGE sodium dodecyl sulphate-polyacrylamide gel electrophoresis

TCA tricarboxylic acid

TEMED N,N,N',N'-tetramethylethylenediamine

Tes N-tris[hydroxymethyl]methyl-2-aminoethanesulphonic acid

TPCK L-1-tosylamide-2-phenylethyl chloromethyl ketone

TPI triose phosphate isomerase

Tris tris (hydroxymethyl) aminomethane

The single letter amino acid code is used in accordance with the IUPAC recommendations.
# TABLE OF CONTENTS

**ABSTRACT**  

**ACKNOWLEDGEMENTS**  

**LIST OF ABBREVIATIONS**  

**TABLE OF CONTENTS**  

**LIST OF FIGURES**  

**LIST OF TABLES**

<table>
<thead>
<tr>
<th>CHAPTER 1</th>
<th>INTRODUCTION</th>
<th>page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>General Background</td>
<td>1</td>
</tr>
<tr>
<td>1.2</td>
<td>Structural Aspects of Prokaryote and Eukaryote Phosphofructokinases</td>
<td>2</td>
</tr>
<tr>
<td>1.3</td>
<td>Phosphofructokinase Isozymes In Mammals</td>
<td>3</td>
</tr>
<tr>
<td>1.3.1</td>
<td>Tissue Distribution of Phosphofructokinase Isozymes</td>
<td>3</td>
</tr>
<tr>
<td>1.3.2</td>
<td>Developmental Changes</td>
<td>5</td>
</tr>
<tr>
<td>1.3.3</td>
<td>Separation of Phosphofructokinase Isozymes</td>
<td>6</td>
</tr>
<tr>
<td>1.3.4</td>
<td>Immunology of Phosphofructokinase Isozymes</td>
<td>6</td>
</tr>
<tr>
<td>1.3.4.1</td>
<td>Cross-Reactivity With Homotetramers</td>
<td>7</td>
</tr>
<tr>
<td>1.3.4.2</td>
<td>Cross-Reactivity With Heterotetramers</td>
<td>7</td>
</tr>
<tr>
<td>1.3.5</td>
<td>Structural Properties of Phosphofructokinase Isozymes</td>
<td>8</td>
</tr>
<tr>
<td>1.3.5.1</td>
<td>Subunit Molecular Weight</td>
<td>8</td>
</tr>
<tr>
<td>1.3.5.2</td>
<td>Aggregation State</td>
<td>8</td>
</tr>
<tr>
<td>1.3.6</td>
<td>Physical Properties of Mammalian Phosphofructokinase</td>
<td>11</td>
</tr>
<tr>
<td>1.3.7</td>
<td>Stability of Phosphofructokinase</td>
<td>11</td>
</tr>
</tbody>
</table>
1.4 Kinetic Properties of Muscle and Liver Phosphofructokinase

1.4.1 Effects of ATP on Phosphofructokinase
1.4.1.1 Effects of pH on ATP Inhibition
1.4.1.2 Effect of F6P Concentration on ATP Inhibition

1.4.2 Effects of Adenine Nucleotides on Phosphofructokinase Activity

1.4.3 Effects of Fructose 1,6-Bisphosphate on Phosphofructokinase Activity

1.4.4 Activation of Phosphofructokinase By NH₄⁺ and K⁺

1.4.5 Inhibitors of Phosphofructokinase Activity
1.4.5.1 Tricarboxylic Acid Cycle Intermediates
1.4.5.2 Phosphate Esters

1.4.6 Fructose 2,6-Bisphosphate
1.4.6.1 Effect of Fructose 2,6-Bisphosphate on Phosphofructokinase Activity
1.4.6.2 Effects of Fasting on Fructose 2,6-Bisphosphate Levels
1.4.6.3 Changes in Phosphofructokinase Activity in Response to Glucagon Administration
1.4.6.4 Changes in Fructose 2,6-Bisphosphate Concentration in Response to Glucagon and Glucose Administration
1.4.6.5 Effect of Fructose 2,6-Bisphosphate on Fructose 1,6-Bisphosphatase Activity
1.4.6.6 Control of Glycolysis and Gluconeogenesis By Fructose 2,6-Bisphosphate

1.4.7 Covalent Modification of Phosphofructokinase By Phosphorylation
1.4.7.1 Extent of Phosphorylation of Phosphofructokinase
1.4.7.2 The Effects of Phosphorylation on Phosphofructokinase
1.4.7.3 Site of Phosphorylation
1.4.7.4 Factors Affecting Phosphorylation
1.4.7.4.1 Effects of Glucagon and Glucose on the Phosphorylation of Phosphofructokinase
1.4.7.5 Effect of Phosphorylation In Vivo
1.4.7.6 Significance of Phosphorylation
1.4.7.7 Phosphorylation and Actin
1.4.8 Interaction of Calmodulin With Phosphofructokinase
1.4.9 Effect of Hormones on Phosphofructokinase
1.4.10 Phosphofructokinase Association With Structural Elements of the Cell
1.5 pH and Protonation
1.5.1 Mechanism for the Inactivation of Phosphofructokinase By Decreasing pH
1.6 Glycolysis and Gluconeogenesis
1.7 Regulation of PFK Under Physiological Conditions
1.7.1 Regulation of Muscle Phosphofructokinase Under Physiological Conditions
1.7.2 Regulation of Liver Phosphofructokinase Under Physiological Conditions
1.8 Role of Specific Residues in Enzymic Activity
1.8.1 Thiol Groups
1.8.2 Identification of Reactive Cysteine Residues
1.8.3 Reactive Methionine Residues
1.8.4 Reactive Histidine Residues
1.9 Metabolite Binding Studies
1.10 The Evolution of Proteins
1.10.1 Evolution of Glycolysis and Phosphofructokinase
1.11 Aims of This Project

CHAPTER 2 MATERIALS

CHAPTER 3 METHODS, RESULTS AND DISCUSSION OF THE PURIFICATION OF SHEEP HEART AND SHEEP LIVER PFK
3.1 Development of a Purification Procedure for Sheep Liver Phosphofructokinase

3.1.1 Homogenization Buffer Trial

3.1.1.1 Procedure for the Homogenization Buffer Trial

3.1.2 Homogenization Experiment

3.1.3 Determination of the Effect of Fructose 2,6-Bisphosphate on the Thermal Stability of Liver Phosphofructokinase

3.1.3.1 Method, and Results for the Fructose 2,6-Bisphosphate Heat Stabilization Trial

3.1.3.2 Double Heat Step Trial

3.1.4 Ammonium Sulphate Precipitation Trial

3.1.5 Polyethylene Glycol Precipitation Trial

3.2 Purification of Sheep Liver Phosphofructokinase

3.3 Phosphofructokinase Enzyme Assays

3.4 Determination of Protein Levels

3.5 Preparation of the Cibacron Blue Column

3.6 Results and Discussion of the Purification of Sheep Liver Phosphofructokinase

3.6.1 DEAE-Cellulose Chromatography of Sheep Liver Phosphofructokinase

3.6.2 Cibacron Blue Chromatography of Sheep Liver Phosphofructokinase

3.6.3 Summary of the Purification of Sheep Liver Phosphofructokinase

3.7 Purification of Sheep Heart Phosphofructokinase

3.8 Results and Discussion of the Sheep Heart Phosphofructokinase Purification

CHAPTER 4 METHODS

4.1 SDS-Polyacrylamide Gel Electrophoresis

4.1.1 Preparation of Samples for SDS-Polyacrylamide Gel Electrophoresis

4.2 Carboxymethylation

4.3 Amino Acid Analysis
4.4 Tryptic Digestion of PFK
4.5 CNBr Digestion of PFK
4.6 Peptide Mapping of Sheep Heart and Liver PFK
  4.6.1 Mapping of Tryptic Peptides Using HPLC
  4.6.2 Mapping of CNBr Peptides Using HPLC
  4.6.3 Mapping of CNBr Peptides Using FPLC
4.7 Determination of Radioactivity
4.8 Preparation of PFK Peptides for Sequencing
  4.8.1 CNBr Peptides
  4.8.2 Tryptic Peptides
  4.8.3 Maleyl-Tryptic Peptides of Liver PFK
    4.8.3.1 Maleylation of Liver PFK
    4.8.3.2 Tryptic digest of Maleylated Liver PFK
    4.8.3.3 Removal of Maleyl Groups
    4.8.3.4 Separation of Acid-Soluble Maleyl-Tryptic Peptides
    4.8.3.5 Separation of Acid-Insoluble Maleyl-Tryptic Peptides
4.9 Sequencing of Peptides
4.10 FAB Mass Spectrometry
4.11 Separation of Phosphofructokinase Isozymes
  Using Non-Dissociating Conditions
4.12 Separation of Phosphofructokinase Isozymes
  Using Dissociating Conditions

CHAPTER 5 RESULTS
CHARACTERIZATION OF SHEEP LIVER PHOSPHOFRUCTOKINASE
5.1 SDS-PAGE of Purified Sheep Liver Phosphofructokinase
  5.1.1 Molecular Weight Determinations of Sheep Heart and Liver PFK
5.2 Separation of Phosphofructokinase Isozymes
  5.2.1 Separation of PKF Isozymes Using Non-Dissociating Conditions
  5.2.2 Separation of PKF Isozymes Using Dissociating Conditions
5.3 Amino Acid Compositions of Sheep Heart and Liver Phosphofructokinase 118
5.4 Peptide Mapping of CNBr Peptides of Muscle and Liver PFK on FPLC 121
5.5 Peptide Mapping of Tryptic Digests of Muscle and Liver PFK on HPLC 124
5.6 Peptide Mapping of Ammonium Bicarbonate-Insoluble Tryptic Peptides on HPLC 127
5.7 Peptide Mapping of CNBr Peptides of Muscle and Liver PFK on HPLC 127

CHAPTER 6 RESULTS
AMINO ACID SEQUENCE OF SHEEP LIVER PHOSPHOFRACTOKINASE
6.1 CNBr Peptides 132
6.1.1 Separation and Amino Acid Sequence of the Low Molecular Weight CNBr Peptides 134
6.1.2 Separation and Amino Acid Sequence of the High Molecular Weight CNBr Peptides 141
6.2 Tryptic Peptides 157
6.3 Maleyl-Tryptic Peptides 166

CHAPTER 7 DISCUSSION
7.1 General Discussion 195
7.2 Sequence and Structural Homology With Bacterial Phosphofructokinase 199
7.3 Sequence Homology Between Mammalian Phosphofructokinases 208
7.4 Comparison of the Subunit Interaction and Binding Sites 215
7.4.1 Comparison of the Residues Involved in Subunit Interactions 215
7.4.2 The Calmodulin Binding Sites 215
7.4.3 The Phosphorylation Site 217
7.4.4 The Active Site 219
7.4.4.1 The ATP Binding Site 219
7.4.4.2 Fructose 6-Phosphate Binding Site 222
7.4.5 The Fructose Bisphosphate Site 222
7.4.6 The ADP Binding Site 226
7.4.7 The Citrate Binding Site 231
7.4.8 The ATP Inhibitory Site 231
7.4.9 The Hinge 233
7.5 General Summary 235

APPENDIX 237

BIBLIOGRAPHY 238
<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Biosynthesis and degradation of fructose 2,6-bisphosphate in the liver.</td>
<td>27</td>
</tr>
<tr>
<td>2</td>
<td>Hypothesis of Calmodulin action in living muscle.</td>
<td>39</td>
</tr>
<tr>
<td>3</td>
<td>The effect of fructose 2,6-bisphosphate on the heat stabilization of sheep liver PFK.</td>
<td>71</td>
</tr>
<tr>
<td>4</td>
<td>Effect of a second heat step on the purification of sheep liver PFK.</td>
<td>73</td>
</tr>
<tr>
<td>5</td>
<td>Precipitation of sheep liver PFK by ammonium sulphate</td>
<td>75</td>
</tr>
<tr>
<td>6</td>
<td>Precipitation of PFK activity and protein by PEG.</td>
<td>78</td>
</tr>
<tr>
<td>7</td>
<td>Flow diagram of the purification procedure for sheep liver PFK.</td>
<td>81</td>
</tr>
<tr>
<td>8</td>
<td>The Phosphofructokinase enzyme assay.</td>
<td>82</td>
</tr>
<tr>
<td>9</td>
<td>Elution profile of sheep liver PFK from DEAE-cellulose.</td>
<td>84</td>
</tr>
<tr>
<td>10</td>
<td>Elution profile of sheep liver PFK from Cibacron Blue.</td>
<td>86</td>
</tr>
<tr>
<td>11</td>
<td>Flow diagram of the purification procedure for sheep heart muscle PFK.</td>
<td>92</td>
</tr>
<tr>
<td>12</td>
<td>Flow diagram of the preparation of the CNBr peptides for sequencing.</td>
<td>99</td>
</tr>
<tr>
<td>13</td>
<td>Flow diagram of the preparation of the tryptic peptides for sequencing.</td>
<td>101</td>
</tr>
<tr>
<td>14</td>
<td>Flow diagram of the preparation of the maleyl-tryptic peptides for sequencing.</td>
<td>104</td>
</tr>
</tbody>
</table>
15 SDS-7.5% polyacrylamide gel showing purified sheep liver PFK. 107
16 Electrophoretic mobilities of standard proteins calculated for the SDS-7.5% polyacrylamide gel shown in Fig. 15. 108
17 SDS-7.5% polyacrylamide gel of purified sheep liver PFK which had undergone proteolytic cleavage during purification. 110
18 Electrophoretic mobilities of standard proteins calculated for the SDS-7.5% polyacrylamide gel shown in Fig. 17. 111
19 Separation of sheep heart PFK on DEAE-cellulose using non-dissociating conditions. 114
20 Separation of sheep liver PFK on DEAE-cellulose using non-dissociating conditions. 115
21 SDS-7.5% Polyacrylamide gel of sheep liver PFK fractions separated on a DEAE-cellulose column using non-dissociating conditions. 117
22 FPLC peptide map of a CNBr digest of sheep heart PFK. 122
23 FPLC peptide map of a CNBr digest of sheep liver PFK. 123
24 HPLC peptide map of the ammonium bicarbonate-soluble peptides from a tryptic digest of sheep heart PFK. 125
25 HPLC peptide map of the ammonium bicarbonate-soluble peptides from a tryptic digest of sheep liver PFK. 126
26 HPLC peptide map of the ammonium bicarbonate-insoluble peptides from a tryptic digest of sheep heart PFK. 128
27 HPLC peptide map of the ammonium bicarbonate-insoluble peptides from a tryptic digest of sheep liver PFK. 129
28 HPLC peptide map of a CNBr digest of sheep heart PFK.
29 HPLC peptide map of a CNBr digest of sheep liver PFK.
30 FPLC elution profile of a CNBr digest of sheep liver PFK.
31 HPLC elution profile of fraction CNBr G chromatographed on a Resolve RC C-18 column.
32 HPLC elution profile of fraction CNBr H chromatographed on a Resolve RC C-18 column.
33 HPLC elution profile of fraction CNBr I chromatographed on a Resolve RC C-18 column.
34 HPLC elution profile of fraction CNBr B chromatographed on a Vydac C-4 column.
35 HPLC elution profile of fraction CNBr C chromatographed on a Vydac C-4 column.
36 HPLC elution profile of fraction CNBr D chromatographed on a Vydac C-4 column.
37 HPLC elution profile of fraction CNBr E chromatographed on a Vydac C-4 column.
38 HPLC elution profile of fraction CNBr F chromatographed on a Vydac C-4 column.
39 Sequence obtained from CNBr peptides.
40 HPLC elution profile of a tryptic digest of sheep liver PFK.
41 Sequence obtained from tryptic peptides.
42 HPLC elution profile of the acid-soluble maleyl-tryptic peptides chromatographed on a Mono-Q ion-exchange column.
<table>
<thead>
<tr>
<th>Page</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>43</td>
<td>HPLC elution profile of fraction MS 1 chromatographed on a Vydc C-18 column.</td>
</tr>
<tr>
<td>44</td>
<td>HPLC elution profile of fraction MS 2 chromatographed on a Vydc C-18 column.</td>
</tr>
<tr>
<td>45</td>
<td>HPLC elution profile of fraction MS 3 chromatographed on a Vydc C-18 column.</td>
</tr>
<tr>
<td>46</td>
<td>HPLC elution profile of fraction MS 4 chromatographed on a Vydc C-18 column.</td>
</tr>
<tr>
<td>47</td>
<td>HPLC elution profile of fraction MS 5 chromatographed on a Vydc C-18 column.</td>
</tr>
<tr>
<td>48</td>
<td>HPLC elution profile of the acid-insoluble maleyl-tryptic peptides chromatographed on a Mono-Q ion-exchange column.</td>
</tr>
<tr>
<td>49</td>
<td>HPLC elution profile of fraction MI 1 chromatographed on a Vydc C-18 column.</td>
</tr>
<tr>
<td>50</td>
<td>HPLC elution profile of fraction MI 2 chromatographed on a Vydc C-18 column.</td>
</tr>
<tr>
<td>51</td>
<td>HPLC elution profile of fraction MI 3 chromatographed on a Vydc C-18 column.</td>
</tr>
<tr>
<td>52</td>
<td>Sequence obtained from maleyl-tryptic peptides.</td>
</tr>
<tr>
<td>53</td>
<td>Amino acid sequence of sheep liver PFK obtained from the three digestion methods used.</td>
</tr>
<tr>
<td>54</td>
<td>Amino acid sequence of sheep liver PFK.</td>
</tr>
<tr>
<td>55</td>
<td>Schematic view of two subunits in the B₅-PFK tetramer, viewed along the x-axis.</td>
</tr>
</tbody>
</table>
56 Computer graphic view of two subunits of *E. coli* PFK viewed along the x-axis and y-axis, from the computer program FRODO. 202

57 Schematic diagram of the proposed tertiary structure of a mammalian PFK monomer. 206

58 Schematic diagram of the proposed mammalian PFK tetramer. 207

59 Computer graphic view of the ATP binding site of *E. coli* PFK from the computer program FRODO. 221

60 Conservation of the residues at the active site of mammalian PFK as compared to the *Bs* enzyme. 224

61a Computer graphic view of the ATP binding site of *E. coli* PFK from the computer program FRODO. 225

61b Computer graphic view of the F6P binding site of *E. coli* PFK from the computer program FRODO. 225

62 Residues at the proposed F26BP binding site. 227

63 Conservation of the residues at the ADP effector site of mammalian PFK as compared to the *Bs* enzyme. 230

64 Schematic diagram of the postulated ATP inhibitory site of mammalian PFK. 234
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Subunit molecular weights of the muscle, liver, and platelet isozymes of human, rat and rabbit phosphofructokinases.</td>
<td>9</td>
</tr>
<tr>
<td>II</td>
<td>Ki ATP values of rabbit muscle and liver phosphofructokinase.</td>
<td>14</td>
</tr>
<tr>
<td>III</td>
<td>Michaelis constants for F6P and ATP for rabbit muscle and liver phosphofructokinase.</td>
<td>15</td>
</tr>
<tr>
<td>IV</td>
<td>Activation of rabbit muscle and liver phosphofructokinase by adenine nucleotides.</td>
<td>17</td>
</tr>
<tr>
<td>V</td>
<td>Effect of NH₄⁺ and K⁺ on the activity of rabbit muscle and liver phosphofructokinase.</td>
<td>19</td>
</tr>
<tr>
<td>VI</td>
<td>Inhibition of rat liver phosphofructokinase by TCA cycle intermediates.</td>
<td>20</td>
</tr>
<tr>
<td>VII</td>
<td>Inhibition of rabbit muscle and liver phosphofructokinase by citrate.</td>
<td>20</td>
</tr>
<tr>
<td>VIII</td>
<td>Inhibition of rabbit muscle and liver phosphofructokinase by phosphate esters.</td>
<td>22</td>
</tr>
<tr>
<td>IX</td>
<td>Phosphate content in muscle.</td>
<td>30</td>
</tr>
<tr>
<td>X</td>
<td>Influence of metabolic state on the degree of phosphorylation of muscle PFK.</td>
<td>32</td>
</tr>
<tr>
<td>XI</td>
<td>Proposed number of metabolite binding sites in PFK.</td>
<td>53</td>
</tr>
<tr>
<td>XII</td>
<td>Homogenization buffers used for the purification of liver PFK.</td>
<td>64</td>
</tr>
</tbody>
</table>
XIII Components of the ten buffer systems used in the homogenization buffer trial. 65

XIV Results from the homogenization buffer trial. 67

XV Results from the homogenization experiment. 69

XVI Ammonium sulphate fractionations used in the purification of liver PFK from different species, and the resulting PFK yields. 76

XVII Purification of sheep liver PFK. 87

XVIII Distribution of PFK activity in rabbit tissues. 88

XIX Purification of liver PFK from different sources. 89

XX Purification of sheep heart PFK. 93

XXI Amino acid composition of sheep heart muscle and sheep liver PFK. 119

XXIIa Amino acid sequences of liver PFK peptides isolated from fraction CNBr G following HPLC. 135

XXIIb Alignment of sheep liver PFK peptides from fraction CNBr G with rabbit muscle and sheep heart PFK. 136

XXIIIa Amino acid sequences of liver PFK peptides isolated from fraction CNBr H following HPLC. 138

XXIIIb Alignment of sheep liver PFK peptides from fraction CNBr H with rabbit muscle and sheep heart PFK. 139

XXIVa Amino acid sequences of liver PFK peptides isolated from fraction CNBr I following HPLC. 140
XXVa Amino acid sequences of liver PFK peptides isolated from fraction CNBr B following HPLC. 142

XXVb Alignment of sheep liver PFK peptides from fraction CNBr B with rabbit muscle and sheep heart PFK. 143

XXVIa Amino acid sequences of liver PFK peptides isolated from fraction CNBr C following HPLC. 144

XXVIIa Amino acid sequences of liver PFK peptides isolated from fraction CNBr D following HPLC. 145

XXVIIb Alignment of sheep liver PFK peptides from fraction CNBr D with rabbit muscle and sheep heart PFK. 146

XXVIIIa Amino acid sequences of liver PFK peptides isolated from fraction CNBr E following HPLC. 147

XXVIIIb Alignment of sheep liver PFK peptides from fraction CNBr E with rabbit muscle and sheep heart PFK. 148

XXIXa Amino acid sequences of liver PFK peptides isolated from fraction CNBr F following HPLC. 150

XXIXb Alignment of sheep liver PFK peptides from fraction CNBr F with rabbit muscle and sheep heart PFK. 151

XXX Sheep heart muscle PFK CNBr peptides. 154

XXXI Changes in the position of methionine residues in sheep liver PFK compared to sheep muscle PFK 156

XXXIIa Amino acid sequences of tryptic peptides from sheep liver PFK following HPLC. 159

XXXIIb Alignment of the tryptic peptides from sheep liver PFK with rabbit muscle and sheep heart muscle PFK sequence. 161
XXXIII  Predicted [14C]-cysteine containing peptides from a tryptic
digest of sheep heart phosphofructokinase  164

XXXIVa  Amino acid sequences of acid-soluble maleyl-tryptic peptides
from sheep liver PFK isolated from fraction 1.  172

XXXIVb  Alignment of the MS 1 maleyl-tryptic peptides with
rabbit muscle and sheep heart muscle PFK.  174

XXXV a  Amino acid sequences of acid-soluble maleyl-tryptic peptides
from sheep liver PFK isolated from fraction 2.  177

XXXVb  Alignment of the sheep liver PFK MS 2 maleyl-tryptic peptides
with rabbit muscle and sheep heart muscle PFK.  178

XXXVI a  Amino acid sequences of acid-soluble maleyl-tryptic peptides
from sheep liver PFK isolated from fraction 3.  179

XXXVIIa  Amino acid sequences of acid-soluble maleyl-tryptic peptides
from sheep liver PFK isolated from fraction 4.  180

XXXVIIb  Alignment of the sheep liver PFK MS 4 maleyl-tryptic peptides
with rabbit muscle and sheep heart muscle PFK.  181

XXXVIIIa  Amino acid sequences of acid-soluble maleyl-tryptic peptides
from sheep liver PFK isolated from fraction 5.  182

XXXVIIIb  Alignment of the sheep liver PFK MS 5 maleyl-tryptic peptides
with rabbit muscle and sheep heart muscle PFK.  183

XXXIXa  Amino acid sequences of acid-insoluble maleyl-tryptic peptides
from sheep liver PFK isolated from fraction 1.  186

XL a  Amino acid sequences of acid-insoluble maleyl-tryptic peptides
from sheep liver PFK isolated from fraction 2.  187
<table>
<thead>
<tr>
<th>Section</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>XLIIa</td>
<td>Amino acid sequences of acid-insoluble maleyl-tryptic peptides from sheep liver PFK isolated from fraction 3.</td>
<td>188</td>
</tr>
<tr>
<td>XLIIb</td>
<td>Alignment of the sheep liver PFK MI 3 maleyl-tryptic peptides with rabbit muscle and sheep heart muscle PFK.</td>
<td>189</td>
</tr>
<tr>
<td>XLII</td>
<td>Number of each amino acid residue sequenced compared to the amino acid composition.</td>
<td>198</td>
</tr>
<tr>
<td>XLIII</td>
<td>Location of the inserted amino acid sequences in sheep liver PFK compared to Bs PFK.</td>
<td>204</td>
</tr>
<tr>
<td>XLIV</td>
<td>Sequence homology between the N and C-terminal halves of mammalian PFKs compared to Bs PFK.</td>
<td>209</td>
</tr>
<tr>
<td>XLV</td>
<td>Sequence homology between mammalian PFKs.</td>
<td>209</td>
</tr>
<tr>
<td>XLVI</td>
<td>Types of amino acid changes.</td>
<td>211</td>
</tr>
<tr>
<td>XLVII</td>
<td>Most frequently observed amino acid replacements between muscle PFKs and sheep liver PFK.</td>
<td>212</td>
</tr>
<tr>
<td>XLVIII</td>
<td>Residue changes involving major charge changes between sheep liver and muscle PFKs.</td>
<td>214</td>
</tr>
<tr>
<td>XLIX</td>
<td>Percentage of amino acid changes resulting from single and double base changes</td>
<td>214</td>
</tr>
<tr>
<td>L</td>
<td>Conservation of residues involved in subunit interactions compared to the Bs enzyme.</td>
<td>216</td>
</tr>
<tr>
<td>LI</td>
<td>Residues involved with the binding of ATP.</td>
<td>220</td>
</tr>
<tr>
<td>LII</td>
<td>Residues involved with the binding of fructose 6-phosphate.</td>
<td>223</td>
</tr>
<tr>
<td>LIII</td>
<td>Residues involved with the binding of ADP.</td>
<td>228</td>
</tr>
</tbody>
</table>