Mammalian ADP-dependent glucokinase

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Abstract

The mammalian ADP-dependent glucokinase is the most recent mammalian glucokinase to have been discovered, and is unique in its ability to catalyse the phosphorylation of glucose to glucose-6-phosphate using ADP as the phosphoryl donor. Up until the discovery of this enzyme, the traditional biochemical view was that the first step of glycolysis was solely catalysed by ATP-dependent hexokinases, types I-IV.

The particular role played by ADP-GK in the mammalian cell and the significance of this role has not yet been determined, although it is hypothesised that the ADP-dependent glucokinase could be potentially significant in contributing to the survival of cells under low energy and hypoxic or ischemic conditions. By using ADP as the energy investment in phase one of the glycolytic cycle instead of ATP, it is predicted that glycolysis could be sustained for longer during lower energy conditions (conditions of high ADP:ATP ratios). Since the phosphorylation of glucose by ADP-GK results in the production of AMP, it may also be possible that this has a direct effect on the energy charge of the cell. The AMP produced could lead to the regulation of cellular metabolism during hypoxia and/or ischemia via the activation of the cell-energy regulator AMPK.

The study of mammalian ADP-dependent glucokinase is a very new area, and prior to this no investigation of the human ADP-GK enzyme had been undertaken. The main objective of this project was to clone, express and purify the recombinant ADP-GK so it could be kinetically characterised and directly compared with the recombinant mouse kinetic characteristics, the only other mammalian ADP-GK to have been studied. Unfortunately, due to complications in the expression and purification of soluble recombinant human ADP-GK, the project did not incorporate the kinetic characterisation of the enzyme. Acquiring data on the kinetic characteristics of the human ADP-GK will, in the long term, assist in the elucidation of the metabolic role of this enzyme, so the continuation of this project would be worthwhile.

Abbreviations

ADP-GK	ADP-dependent glucokinase
Amp	Ampicillin
APS	Ammonium persulfate
ATP	Adenosine triphosphate
bp	Base pairs (DNA)
BSA	Bovine serum albumin
CD	Circular dichroism
cDNA	Complimentary DNA
CHAPS	3[(3-Cholamidopropyl)dimethylammonio]-propanesulfonic acid
DMSO	Dimethyl sulfoxide
DNA	Deoxyribose nucleic acid
DNase I	Deoxyribonuclease I
dNTP	Deoxynucleoside triphosphate (dATP, dTTP, dGTP, dCTP)
DTT	Dithiothreitol
E. coli	*Escherichia coli*
EDTA	Ethylene diamine tetra-acetic acid
FPLC	Fast protein liquid chromatography
hADPGK	Human ADP-dependent glucokinase
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HRP	Horse radish peroxidase
IEF	Isoelectric focusing
IEX	Ion exchange chromatography
IPTG	Isopropyl β-D-thiogalactoside
KCl	Potassium chloride
kDa	Kilodaltons
LB	Luria Bertani bacteriological media
mAPDGK	Mouse ADP-dependent glucokinase
MGC	Mammalian gene collection
MOPS	3-(N-morpholino)propanesulfonic acid

mRNA	Messenger RNA
NaCl	Sodium chloride
NADH	Nicotinamide adenine dinucleotide
NADPH	Nicotinamide adenine dinucleotide phosphate
PAGE	Polyacrylamide gel electrophoresis
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
pI	Isoelectric point
PMSF	Phenylsulfonylmethyl fluoride
RNA	Ribonucleic acid
rpm	Revolutions per minute
SDS	Sodium dodecyl sulfate
SDS-PAGE	SDS-polyacrylamide gel electrophoresis
TAE	Tris acetate EDTA buffer
TBST	Tris-buffered saline-Tween 20
TEMED	N,N,N’,N’-Tetramethylethylenediamine
Tris	tris (hydroxymethyl)-aminomethane
UV	Ultra violet

List of Figures

Page number

Figure 1.1	**Schematic diagram of the glycolytic pathway**	**3**
Figure 1.2	**Phylogenetic tree of higher eukaryote ADP-GKs and archaeal ADP-GKs and ADP-PFKs**	**15**
Figure 3.1	**Schematic of the Invitrogen TOPO® Cloning technology**	**40**
Figure 3.2	**Primer sequences**	**41**
Figure 3.3	**PCR reaction to generate fragment for TOPO® cloning**	**43**
Figure 3.4	**Plasmid DNA isolated from a positive clone**	**44**
Figure 3.5	**PCR product produced from pET151/D-TOPO plasmid isolated from positive clones using T7 forward primer and RH2 reverse primer**	**46**
Figure 3.6	**Schematic representation of the restriction digest performed on pET151/D-TOPO containing the cloned cDNA sequence of the human ADP-GK gene**	**47**
Figure 3.7	**Digestion of pET151/D-TOPO plasmid containing the cDNA sequence of the human ADP-GK gene**	**48**
Figure 3.8	**Primer sequences**	**49**
Figure 3.9	**SDS-PAGE of whole cell lysates collected from 37°C BL21(DE3) trial expression cultures**	**53**
Figure 3.10	**SDS-PAGE of soluble and insoluble protein fractions produced From 37°C BL21(DE3) trial expression cultures**	**54**
Figure 3.11	**SDS-PAGE of soluble and insoluble protein fractions produced from 30°C Rosetta trial cultures**	**57**
Figure 3.12	**A schematic of the two-step expression protocol, adapted from (de Marco, 2007)**	**59**
Figure 3.13	**SDS-PAGE of whole cell lysates from 22°C Rosetta trial expression Cultures**	**61**
Figure 3.14	**SDS-PAGE of soluble and insoluble protein fractions produced from 22ºC Rosetta trial expression cultures**	**62**

Figure 3.15 SDS-PAGE of protein samples demonstrating the effect of changing lysis buffer components 66
Figure 3.16 Immunoblot of recombinant human ADP-GK fractionated lysis samples 68
Figure 4.1 SDS-PAGE of a trial purification using Ni-NTA resin 74
Figure 4.2 SDS-PAGE of a trial purification using Ni-NTA resin 75
Figure 4.3 SDS-PAGE of a trial purification using Ni-NTA resin 77
Figure 4.4 Denatured recombinant human ADP-GK isolated from inclusion bodies 81
Figure 4.5 Elution schematic and resulting SDS-PAGE for purification trial 88
Figure 4.6 Elution schematic and resulting SDS-PAGE for purification trial 91
Figure 4.7 Elution schematic and resulting SDS-PAGE for purification trial 94
Figure 4.8 Immunoblot of ADP-GK pre- and post- HisTrap column purification 96
Figure 5.1 Circular dichroism spectrum of recombinant human ADP-GK 102
Figure 5.2 Circular dichroism spectrum of recombinant mouse ADP-GK 103
Figure 5.3 Circular dichroism spectrum of recombinant mouse and human ADP-GK 104
Figure 5.4 Effect of varying pH on recombinant human ADP-GK activity 107
Figure 6.1 Immunoblot of cytoplasmic ADP-GK 110
Figure 6.2 ADP-GK detected on an array of total protein from adult and foetal tissue 112
Figure 6.3 ADP-GK detected on an array of total protein from adult normal and cancerous tissue 113

List of Tables

Page number

Table 2.1	**Constituents of the Champion™ pET Directional TOPO® cloning reaction**	**24**
Table 2.2	**Inclusion body wash buffer constituents**	**28**
Table 2.3	**Resolving and stacking gel solutions for SDS-PAGE**	**31**
Table 2.4	**Standard ADP-GK assay components**	**36**
Table 3.1	**PCR protocol for amplifying human ADP-GK coding sequence**	**42**
Table 3.2	**PCR protocol for amplifying ~1 680 bp product utilising T7 forward primer and RH2 reverse primer**	**45**
Table 4.1	**Detailed representation of the refolding screen undertaken using 57 wells of a 96 well plate**	**85**
Table 5.1	**K2D2 estimated percentages of secondary structure for the recombinant human ADP-GK**	**102**
Table 5.2	**K2D2 estimated percentages of secondary structure for the recombinant mouse ADP-GK**	**103**
Table 5.3	**pH dependent specific activity *(µmol/min/mg) values for recombinant human ADP-GK**	**106**

Table of Contents

		Page number
Acknowledgements		**i**
Abstract		**ii**
Abbreviations		**iii**
List of Figures		**v**
List of Tables		**vii**
Table of Contents		**viii**
Chapter 1:	**Introduction**	**1**
1.1	*Glycolysis*	*1*
1.2	*Isoenzymes of mammalian hexokinase*	*4*
1.3	*Warburg Effect*	*8*
1.4	*Hypoxia, cellular energetics and cancer*	*10*
1.5	*Hypoxia-inducible Factor 1*	*11*
1.6	*ADP-dependent glucokinase*	*12*
1.7	*The potential significance of ADP-dependent glucokinase*	*16*
1.8	*Research aims*	*17*
Chapter 2:	**Materials and methods**	**18**
2.1	*Materials*	*18*
2.2	*Methods*	*20*
2.2.1	*Agarose gel electrophoresis*	*20*
2.2.2	*Polymerase chain reaction*	*20*
2.2.3	*Elution of plasmid DNA from IsoCode® paper*	*22*
2.2.4	*Purification of PCR products*	*22*
2.2.5	*DNA quantification*	*22*
2.2.6	*Cloning PCR products into pET151/D-TOPO®*	*23*
2.2.7	*Transformation of Escherichia coli (E. coli) (XL-1, BL21(DE3), and Rosetta strains)*	*24*
2.2.8	*Isolation of plasmid DNA from E. coli*	*25*

2.2.9 Restriction endonuclease digests 26
2.2.10 DNA sequencing 27
2.2.11 Recombinant protein expression 27
2.2.12 Inclusion body expression of recombinant protein 28
2.2.13 Concentration of protein samples 29
2.2.14 Recombinant protein purification 29
2.2.15 Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) 30
2.2.16 Immunoblotting 33
2.2.17 Protein quantification 34
2.2.18 Circular dichroism 35
2.2.19 Enzyme activity assays 35
2.2.20 Extraction of total cellular protein from tissue 36

Chapter 3: Cloning and expression of recombinant human ADP-dependent Glucokinase 38
3.1 Introduction 38
3.2 Cloning strategy 39
3.2.1 Amplification of the human ADP-GK coding sequence by PCR 41
3.3.2 Cloning and transformation of Escherichia coli strains 44
3.3 Conformation of cloning strategy 45
3.3.1 Restriction endonuclease digests 47
3.3.2 DNA sequencing 49
3.4 Recombinant protein expression 50
3.5 Improving the solubility of expressed recombinant ADP -dependent glucokinase 55
3.5.1 Escherichia coli Rosetta host cell strain 56
3.5.2 Induction of molecular chaperone expression 58
3.5.3 A comparison of lysis buffers 64
3.6 Chapter summary 69

Chapter 4: Purification of recombinant human ADP-dependent glucokinase 70

4.1 Introduction 70

4.2 Initial purification trials and optimisation of Ni-NTA purification Conditions 71

4.3 Isolating recombinant protein from washed inclusion bodies 79

4.4 Refolding recombinant human ADP-GK protein 82

4.5 Purification of refolded recombinant human ADP-GK 86

4.6 Chapter summary 97

Chapter 5: Recombinant protein characterisation 99

5.1 Introduction 99

5.2 Analysis of refolded recombinant human ADP-GK secondary Structure 100

5.3 Enzyme activity relative to pH 105

5.4 Chapter summary 108

Chapter 6: Tissue screen for ADP-GK protein expression 109

6.1 Introduction 109

6.2 Immunoblotting for ADP-GK expression in porcine tissue 109

6.3 Immunoblotting for ADP-GK expression in human tissue 111

6.4 Chapter Summary 117

Chapter 7: Discussion and future research 119

7.1 Overview 119

7.2 Summary of results 120

7.3 Further research 124

References: 126

Appendix 1: 132

Appendix 2: 134

Appendix 3: 136

Appendix 4: **137**
Appendix 5: **141**