The unsolved mystery of human ADP-dependent glucokinase

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Abstract

Human ADP-dependent glucokinase (hADP-GK) is the most recently discovered glycolytic enzyme that has been shown experimentally to phosphorylate glucose to glucose-6-phosphate. This reaction is catalysed using ADP as the phosphoryl donor, which is uniquely different from the traditional ATP-dependent hexokinase type I-IV enzymes that were initially shown to carry out the first step of glycolysis.

The functional role of hADP-GK within the cell and the significance of utilizing an ADP-dependent glucokinase have yet to be elucidated experimentally. It has been hypothesised that the unique characteristic of ADP utilisation may provide cellular advantages during times of limited energy and oxygen (hypoxia), as ATP is able to be conserved during anaerobic glycolysis. Cellular survival and sustainability may be increased during disease states as ADP is invested into the first step of glycolysis instead of ATP. AMP is also produced during this reaction, and may activate AMP-activated protein kinase (AMPK), an energy sensor within the cell, which may regulate energy metabolism during times of low energy and/or hypoxia.

Prior to this research no experimental work had been carried out on the regulation of hADP-GK at the transcriptional level. The main objectives of this project were to investigate the effect of glucose concentration on the expression of hADP-GK at the transcriptional level, and to investigate the promoter and potential transcription factors responsible for promoter activity. The cellular localisation of hADP-GK was also briefly investigated through microscopy. This experimental work has opened up a new path for future research, and therefore the continuation of this project would be important in understanding the role of hADP-GK.

Abbreviations

ADP-GK	ADP-dependent glucokinase
Amp	Ampicillin
AMP	Adenosine monophosphate
APS	Ammonium persulfate
ATP	Adenosine triphosphate
bp	Base pairs
BSA	Bovine serum albumin
cDNA	Complimentary DNA
CDTA	1,2-disminocyclohexane-N,N,N',N-tetraacetic acid
ChREBP	Carbohydrate response element-binding protein
CRE	cAMP response element
CREB	cAMP response element binding protein
cpm	counts per minute
Cq/Ct values	PCR crossing points
CV	Coefficient of variance
DAPI	4',6-diamidino-2-phenylindole
DEPC	Diethylpyrocarbonate
DMSO	Dimethyl sulfoxide
DNA	Deoxyribose nucleic acid
dNTP	Deoxynucleoside triphosphate (dATP, dCTP, dGTP, dTTP)
DTT	Dithiothreitol
E	Amplification efficiency
E. coli	*Escherichia coli*
EDTA	Ethylene diamine tetra-acetic acid
EMSA	Electrophoretic Mobility Shift Assay

ER	Endoplasmic reticulum
FCS	Fetal calf serum
FITC	Fluorescein-5-isothiocyanate
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
gDNA	Genomic DNA
GSB	Gel shift buffer
GR	Glucocorticoid receptor
hADP-GK	Human ADP-dependent glucokinase
HCl	Hydrochloric acid
HeLa	Human cervical cancer cell line
HEPES	N-[2-hydroxyethyl]piperazine-N'-[2-ethane sulfonic acid]
HRP	Horse radish peroxidase
IgG	Immunoglobulin G
IPTG	Isopropyl β-D-thiogalactoside
KCl	Potassium chloride
kDa	Kilodaltons
LB	Luria Bertani bacteriological media
mADP-GK	Mouse ADP-dependent glucokinase
mM	Milli Molar
µL	Micro Litre
µg	Micro Gram
mRNA	Messenger RNA
mt	Mutant
Mw	Molecular weight
NaCl	Sodium chloride
NADH	Nicotinamide adenine dinucleotide
ng	Nano Gram

NS	Non-specific
NTC	Non-template control
ONPG	o-nitrophenyl 1-β-D- galactopyranoside
PAGE	Polyacrylamide gel electrophoresis
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PDI	Protein Disulfide Isomerase
PM	Plasma membrane
RNA	Ribonucleic acid
RNase	Ribonuclease
rpm	revolutions per minute
RT-qPCR	Reverse transcription-quantification polymerase chain reaction
SDS	Sodium dodecyl sulfate
SDS-PAGE	SDS-polyacrylamide gel electrophoresis
Sp1	Specificity factor 1
TAE	Tris acetate EDTA buffer
TBE	Tris borate EDTA
TBST	Tris-buffered saline-Tween 20
TE	Tris-EDTA buffer
TEMED	N,N,N',N'-Tetramethylethylenediamin
Tm	Melting temperature
Tris	Tris (hydroxymethyl)-aminomethane
TRITC	Tetramethyl Rhodamine Isothiocyanate
TSP	Transcription start point
UV	Ultra violet
Wt	Wildtype
X-gal	5-bromo-4-chloro-3-indoyl-β-D-galactosidase

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