Structure and function of the eukaryotic ADP-dependent glucokinase

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Abstract

The ADP-dependent glucokinase enzymes (ADPGK) are the first new glycolytic enzymes to be discovered in over 40 years. This class of enzymes was first described in thermophilic archaea in 1994. A decade later, an ADPGK from a eukaryote was also identified and characterised. The ADPGK enzymes catalyse a phosphorylation reaction converting glucose and ADP to glucose-6-phosphate and AMP. The enzyme is well studied in extremophilic archaea, where ADPGK is part of a set of glycolytic enzymes that use ADP instead of ATP for the phosphorylation of various sugars. However, ADPGK has also been found in the genomes of mesophilic species and higher eukaryotes, suggesting that the enzyme is not necessarily an adaption to high temperatures. In eukaryotes, ADPGK has been linked to a modified glycolysis pathway that is required for T-cell activation. While crystal structures of the archaeal ADPGKs are known, no structure of a eukaryotic ADPGK had been solved before the work undertaken in this thesis. In this thesis, the kinetic analysis of a recombinant form of *Homo sapiens* ADPGK and the crystal structure of a truncated form of *Mus musculus* ADPGK are presented. Both enzymes were expressed recombinantly in *E. coli* and purified in soluble form. The kinetic parameters determined for *H. sapiens* ADPGK proved to be comparable to the mouse enzyme, which had been published earlier. In addition, the phosphoryl acceptor specificity of *H. sapiens* ADPGK was extensively tested by ^{31}P-NMR, where the enzyme proved to be highly specific for D-glucose. Residues important for catalysis have been modified by site-directed mutagenesis and the variants of *H. sapiens* ADPGK were purified and kinetic parameters determined. A single crystal was obtained from a truncated variant of *M. musculus* ADPGK, which diffracted to 2.1 Å. The structure of *M. musculus* ADPGK could be solved by molecular replacement using the known crystal structures of the archaeal ADPGKs for initial phasing. It proved to be quite similar to the archaeal, ADPGKs, despite the low sequence identity. The combined data in this work improves our understanding of the conservation of the structure-function relationship of eukaryotic ADPGKs.

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Abbreviations and symbols

˚C	Temperature in centigrade
Å	Ångström (10^{-10} m)
aa	Amino acid
ADP	Adenosine diphosphate
ADPGK	ADP-dependent glucokinase
ADPPFK	ADP-dependent phosphofructokinase
Amp	Ampicillin
AMP	Adenosine monophosphate
AMPCP	Adenosine 5′-(α,β-methylene) diphosphate
ATP	Adenosine triphosphate
BLAST	Basic Local Alignment Search Tool
bp	Base pair (of DNA)
BSA	Bovine serum albumin
BTP	Bis-tris propane buffer
Cam	Chloramphenicol
CD	Circular dichroism
cDNA	Complementary DNA, experimentally derived from messenger RNA
cv	Column volume (geometric column volume of a chromatography column)
DNA	Deoxyribonucleic acid
DTT	Dithiothreitol
EDTA	Ethylenediaminetetraacetate
g	Gram, unit of weight
g	Gravitational force
GK	Glucokinase

h	Hour
HK	Hexokinase
HRP	Peroxidase from horseradish
IMAC	Immobilised metal ion affinity purification
IPTG	Isopropyl β-D-1-thiogalactopyranoside
K	Temperature in Kelvin
Kan	Kanamycin
kDa	Kilodalton
K_i	Dissociation constant for an enzyme inhibitor
K_m	Michaelis-Menten constant
LB broth	Luria-Bertani broth
LB/Amp	Luria-Bertani broth with ampicillin selection marker
m	Metre
M	Molar
min	Minute
MTT	Thiazolyl blue tetrazolium bromide
NAD^+ or NADH	Nicotinamide adenine dinucleotide (oxidised or reduced)
$NADP^+$ or NADPH	Nicotinamide adenine dinucleotide phosphate (oxidised or reduced)
NMR	Nuclear magnetic resonance
OD_{600}	Optical density at wavelength of 600 nm
PAGE	Polyacrylamide gel electrophoresis
PCR	Polymerase chain reaction
pdb	Protein data bank file format (*.pdb)
PDB	Protein Data Bank for protein structures
pH	Measurement of acidity or basicity of an aqueous solution
P_i	Inorganic phosphate

pI	Isoelectric point
PMS	Phenazine methosulfate
PP_i	Pyrophosphate
psi	Pounds per square inch
RMSD	Root-mean-square deviation
RNAi	RNA interference
rpm	Revolutions per minute
SAXS	Small angle X-ray scattering
SDS	Sodium dodecyl sulphate
sec	Second
Str	Streptomycin
TAE	Tris-acetate-EDTA buffer
TBS	Tris-Buffered Saline solution
TBST	Tris-Buffered Saline solution with additional Tween 20 detergent
TCEP	Tris(2-carboxyethyl)phosphine
Tet	Tetracycline
U	Enzyme unit, 1 U = 1 μmol min^{-1}
U/mg	Specific enzyme activity, 1 U/mg = 1 μmol min^{-1} mg^{-1}
V	Enzyme velocity
(v/v)	Volume per volume
V_{max}	Maximum enzyme velocity
(w/v)	Weight per volume