Enzymes Associated with the Complications of Diabetes Mellitus

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Abstract

Diabetes mellitus (DM) is a metabolic disease resulting from failures in the production or response to the hormone insulin. Much of the pathogenesis and mortality attributed to DM are due to the long-term complications of hyperglycaemia, which is characteristic of the disease. This thesis presents structural and functional studies of two previously uncharacterised human enzymes, dihydrodipicolinate synthase-like protein (DHDPSL) and D-xylulokinase (XK). Both enzymes were revealed to have unexplored associations with DM.

DHDPSL is distantly related (≈25% sequence identity) to a family of Schiff base-dependent aldolases that include dihydrodipicolinate synthase and *N*-acetylneuraminate lyase. Despite these distant homologies the biological function of DHDPSL is unknown. It also does not map to any known metabolic pathway in humans, but is targeted to the mitochondrial compartment consistent with the presence of a mitochondrial targeting sequence. There are also strong associations between mutations in the *Dhdpsl* gene and primary hyperoxaluria type III a rare disorder of endogenous oxalate production.

The DHDPSL crystal structure was determined by X-ray crystallography utilising *in situ* proteolysis of a fusion of DHDPSL with maltose-binding protein for crystallisation. Two apo-forms and six Schiff base complexes with potential ligands were analysed at best to 2.0 Å resolution and with an R_{free} of 18.3%. DHDPSL is folded as $(\alpha/\beta)_8$-barrel with a C-terminal subdomain and forms a tetramer in the crystal. The structural consequences of the disease-relevant DHDPSL mutations were analysed and were found to largely affect the C-terminal subdomain. Findings also showed that DHDPSL acts as an oxaloacetate decarboxylase and is therefore likely to be a bifunctional oxaloacetate decarboxylase/4-hydroxy-2-ketoglutarate aldolase present in the liver and kidney mitochondria. Overall, these results revealed the presence of a potentially significant metabolic pathway in mitochondria whereby oxaloacetate can be converted to pyruvate.

XK has a potential role in the regulation of *de novo* lipogenesis in the liver that has gained little previous attention. Excessive hepatic lipid accumulation is linked to impaired insulin response and the development of DM. XK was identified and produced recombinantly in *Eschericia coli* aided by molecular chaperones. Crystals suitable for structural analysis were obtained after five generations of repeated seeding. Five crystal structures were used to analyse substrate binding, the best of which was determined at 2.0 Å resolution with an

R_{free} of 17.8%. XK assumes an actin-like two-domain FGGY sugar kinase fold. The most striking feature revealed by the XK molecular structure was a dramatic domain movement that must accompany catalysis. A competitive inhibitor of XK, 5-deoxy-5-fluoro-D-xylulose (K_i of 25.4 μM) was also functionally validated and structurally analysed.

A supplemental structural study of a major hypoxic response protein of unknown function, Rv1738, from the causative agent of tuberculosis, *Mycobacterium tuberculosis* is also described. This study presents the first novel protein structure to be determined by the racemic protein crystallography method. The Rv1738 structure exposes a relationship with a family of ribosome-inhibiting stress-response proteins that may be indicative of its function.

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Contents

List of Figures

List of Tables

List of Abbreviations

2OG	α-ketoglutarate
3BP	3-bromopyruvate
3C	human rhinovirus-14 3C protease
3PY	3-hydroxypyruvate
5FX	5-deoxy-5-fluoro-D-xylulose
A	absorbance
ACF	auto-correlation function
ADP	adenosine diphosphate
AMP-PCP	5′-adenylyl (β,γ-methylene)diphosphate
AMP-PNP	5′-adenylyl imidodiphosphate
ATP	adenosine triphosphate
atXK	*Arabadoposis thaliana* D-xylulokinase
AU	asymmetric unit
BLAST	basic local alignment search tool
btXK	*Bos taurus* D-xylulokinase
C	Coulomb
CHAPS	3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate
ChREBP	carbohydrate response element binding protein
CoA	coenzyme A
CV	column volume
DHDPS	dihydrodipicolinate synthase
DHDPSL	dihydrodipicolinate synthase-like protein
DM	diabetes mellitus
DMSO	dimethylsulfoxide
EC	Enzyme Commission
ecXK	*Escherichia coli* D-xylulokinase
EDTA	ethylenediaminetetraacetic acid
GX	glucuronate-xylulose pathway
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
hisMBP-3C-DHDPSL	3C protease-cleavable polyhistidine-tagged *E. coli* maltose binding protein fusion of human dihydrodipicolinate synthase-like protein
hisMBP-DHDPSL	polyhistidine-tagged *E. coli* maltose binding protein fusion of human dihydrodipicolinate synthase-like protein
HPF	ribosome hibernation promoting factor
hsp70	heat-shock protein 70
IPTG	isopropyl-β-D-thiogalactopyranoside

J	Joule
K	Kelvin
KD(P)GA	bifunctional 2-keto-3-deoxy-(6-phospho)-gluconate aldolase
KDGA	2-keto-3-deoxygluconate specific aldolase
KDGD	5-dehydro-4-deoxyglucarate dehydratase
KHG	4-hydroxy-2-ketoglutarate
LB	lysogeny broth
LSSR	local structure similarity restraints
LXRα	liver X receptor α
MES	2-(*N*-morpholino)ethanesulfonic acid
MWCO	molecule-weight cutoff
NADH	reduced nicotinic acid adenine dinucleotide
NAL	*N*-acetylneuraminate lyase
NCBI	National Center for Biotechnology Information
NCS	non-crystallographic symmetry
NYSGXRC	New York Structural GenomiX Research Center
OAA	oxaloacetate
OD	optical density
ORF	open reading frame
PEG	polyethylene glycol
PF2K-Pase	bifunctional 2-keto-3-deoxy-(6-phospho)-gluconate aldolase
PFK	phosphofructokinase
PP2A	protein phosphatase 2A
ppm	parts per million
PSI-BLAST	position-specific iterative BLAST
PYR	pyruvate
ROS	reactive oxygen species
RXR	retinoid X receptor
S	Svedberg
SDS-PAGE	sodium docecyl sulfate polyacrylamide gel electrophoresis
SEC	size exclusion chromatography
SeMet	selenomethionine
TAE	Tris/acetate EDTA
TAG	triacylglycerol
TB	tuberculosis
TCEP	*tris*(2-carboxyethyl)phosphine
TEV	tobacco etch virus NIa protease
TLS	translation, libration, screw
Tris	*tris*(hydroxymethyl)aminomethane
TSP	3-trimethylsilylpropanoate
UDP	uridine diphosphate
XK	human D-xylulokinase
XK-5FX	human D-xylulokinase 5-deoxy-5-fluoro-D-xylulose-bound crystal structure
XK-ADP-XUL	human D-xylulokinase–ADP and D-xylulose complex crystal structure
XK-Apo	human D-xylulokinase apoenzyme crystal structure
XK-XUL	human D-xylulokinase–D-xylulose complex crystal structure

Xu1P	D-xylulose 1-phosphate
Xu5P	D-xylulose 5-phosphate
XUL	D-xylulose
xyl	D-xylulose