A COMPARATIVE STUDY OF PHOSPHOFRUCTOKINASE AND TAGATOSE 6-PHOSPHATE KINASE FROM *STREPTOCOCCUS LACTIS*

A THESIS PRESENTED IN PARTIAL
FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE
OF DOCTOR OF PHILOSOPHY IN BIOCHEMISTRY AT
MASSEY UNIVERSITY

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1982

ABSTRACT

In the lactic streptococci glucose is metabolised to lactic acid via the Embden-Meyerhof-Parnas (EMP) pathway. Metabolism of lactose and galactose in these organisms involves participation of the D-tagatose 6-phosphate pathway in which galactose 6-phosphate is metabolised to triose phosphates via tagatose derivatives.

Phosphofructokinase (ATP : D-fructose 6-phosphate 1-phosphotransferase, E.C. 2.7.1.11) catalyses the ATP-dependent phosphorylation of fructose 6-phosphate in the EMP pathway. The analogous reaction in the tagatose 6-phosphate pathway, phosphorylation of tagatose 6-phosphate with ATP, is catalysed by a specific enzyme, tagatose 6-phosphate kinase. While phosphofructokinase (PFK) is known to be a major regulatory enzyme in carbohydrate metabolism in most organisms, little is known of the regulatory properties of tagatose 6-phosphate kinase (T6PK).

PFK and T6PK were purified from *Streptococcus lactis* C_{10} PFK was purified to homogeneity (364-fold purification) by affinity chromatography on Blue-dextran-Sepharose. Unlike PFK, T6PK did not bind to Blue-dextran-Sepharose : a 136-fold purification was achieved using ammonium sulphate fractionation, gel filtration, and ion exchange chromatography.

A study of some of the properties of PFK and T6PK from *S. lactis* C_{10} showed that these two enzymes are distinct proteins with different physical and kinetic characteristics.

S. lactis PFK is a tetramer (MW 145,000 daltons) of identical subunits of molecular weight 33,500 daltons. It therefore appears structurally similar to other bacterial PFKs.

T6PK from *S. lactis* has a molecular weight of approximately 114,000 daltons, a value similar to that of *Staphylococcus aureus* T6PK which is a dimer.

S. lactis PFK exhibited the co-operative binding of F6P and inhibition by high concentrations of ATP relative to F6P which is typical of most bacterial and mammalian PFKs. $F6P_{0.5}$ and K_m (MgATP) values were 0.28 mM and 0.18 mM respectively. ADP stimulated PFK activity, shifting the sigmoidal saturation curve to a more hyperbolic form, with a corresponding decrease in n_H. Ammonium and

potassium ions also activated PFK, while activity was inhibited by AMP, PEP, FBP, T6P and inorganic phosphate. In contrast to PFK, T6PK showed no co-operative binding of sugar phosphate substrate and was less sensitive than PFK to ATP inhibition. K_m values for T6P and MgATP were 0.16 mM and 0.4 mM respectively. Apart from ammonium and potassium ions, no activators of T6PK were found. Activity was inhibited by ADP, PEP, and FBP. PFK and T6PK could catalyse phosphorylation of both F6P and T6P although the enzymes showed a much greater affinity for their natural substrate. Maximum velocities attained were higher with the natural substrate than when the other sugar phosphate was used as substrate.

Both enzymes showed similar pH optima and divalent cation requirement.

Levels of PFK, T6PK, and Galactokinase (Gal K), enzymes of the Embden-Meyerhof-Parnas, Tagatose 6-phosphate, and Leloir pathways respectively, were measured in strains of *S. lactis*, *S. cremoris*, *S. diacetylactis* and *S. faecalis* grown on different sugars. Growth on lactose and galactose induced increased levels of T6PK and Gal K activity, galactose generally inducing higher levels of T6PK than lactose.

In most strains, addition of glucose to media containing lactose or galactose resulted in lowered activities of Gal K, comparable to those in glucose-grown cells. In contrast, T6PK activity was generally not suppressed by growth on glucose plus lactose, while in growth on glucose plus galactose, T6PK activity was approximately 50% of the activity in cells grown on glucose alone.

PFK activity was generally unaffected by the sugar in the growth medium.

In spite of changes in specific activities of PFK and T6PK throughout the growth period of *S. lactis*, the ratio of PFK : T6PK remained fairly constant.

The properties of *S. lactis* PFK and T6PK are compared to those of these enzymes in other bacteria, and the possible role of T6PK in regulation of carbohydrate metabolism in *S. lactis* is discussed.

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ACKNOWLEDGEMENTS

I would like to thank my supervisors, Dr C.H. Moore and Dr G.G. Pritchard, for their support and advice throughout the preparation of this thesis. Their continual interest and encouragement was much appreciated.

I am also grateful to:

Dr T.D. Thomas and Dr V.L. Crow (N.Z. Dairy Research Institute) for gifts of purified tagatose 6-phosphate and tagatose 1,6-bisphosphate aldolase.

Dr C.H. Moore for synthesising tagatose 6-phosphate.

Dr J.G. Robertson (Applied Biochemistry Division, D.S.I.R.) for performing the stain for phosphatase activity.

Other members of the Department of Chemistry, Biochemistry and Biophysics at Massey University with whom I have held many helpful discussions, and thus received valuable comments.

Also, particular thanks are extended to Mrs Valerie Oram for typing this manuscript.

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ABBREVIATIONS

A^{280}, A^{540}	absorbance at 280 nm and 540 nm
ADP	adenosine 5'-diphosphate
AMP	adenosine 5'-monophosphate
ATP	adenosine 5'-triphosphate
bisacrylamide	N, N'-methylene bisacrylamide
β-gal	β-D-galactosidase
β-P-gal	phospho-β-D-galactosidase
BPB	bromophenol blue
BSA	bovine serum albumin
cAMP	3', 5' cyclic AMP
CM	carboxymethyl
CTP, GTP, TTP, ITP, UTP	5'-triphosphates of cytosine, guanine, thymine, inosine, and uracil
DEAE	diethyl aminoethyl
DHAP	dihydroxy acetone phosphate
E. coli	*Escherichia coli*
ED	Entner-Doudoroff
EDTA	ethylene diamine-tetra acetic acid
EMP	Embden-Meyerhof-Parnas
F6P	fructose 6-phosphate
$F6P_{0.5}$	concentration of F6P giving half maximal velocity
FBP	fructose 1,6-bisphosphate
$\Delta G°$	change in free energy
G3P	glyceraldehyde 3-phosphate
G6P	glucose 6-phosphate
G6PDH	glucose 6-phosphate dehydrogenase
GBP	glucose 1,6-bisphosphate
Gal	galactose
Gal 6P	galactose 6-phosphate
Gal K	galactokinase

Glu	glucose
∝GPDH	∝-glycerophosphate dehydrogenase
HEPES	N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid
HMP	hexose monophosphate
K_i	inhibitor constant
K_m	Michaelis-Menten constant
Lac	lactose
LDH	lactate dehydrogenase
MES	2-(N-morpholino)ethanesulfonic acid
MW	molecular weight
NAD^+	nicotinamide adenine dinucleotide
NADH	reduced nicotinamide adenine dinucleotide
$NADP^+$	nicotinamide adenine dinucleotide phosphate
NADPH	reduced nicotinamide adenine dinucleotide phosphate
NBT	nitroblue tetrazolium
ND	not determined
n_H	Hill coefficient
ox	oxidised
PEG	polyethylene glycol
PEP	phospho-enol pyruvate
PFK	phosphofructokinase
PGA	phosphoglyceric acid
PGI	phospho glucose isomerase
P_i	inorganic phosphate
PK	pyruvate kinase
PMS	phenazine methosulphate
PP_i	inorganic pyrophosphate
PS	protamine sulphate
red	reduced
R5P	ribose 5-phosphate
R_F	relative mobility

$S_{0.5}$	concentration of substrate required to give half maximal velocity
SDS	sodium dodecyl sulphate
Sn	supernatant
T6P	tagatose 6-phosphate
T6PK	tagatose 6-phosphate kinase
TEMED	N, N, N', N' - tetramethylene diamine
TPI	triose phosphate isomerase
Tris	tris (hydroxymethyl) amino methane
Tris-glycerol buffer	50 mM Tris HCl containing 20% (v/v) glycerol, 5 mM EDTA, 5 mM $MgCl_2$, and 10 mM 2-mercapto ethanol
u	In all figures, u represents the Greek letter μ, the symbol for 'micro'
V_{max}, V_m	maximum velocity

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