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THE ROLE OF PYRUVATE KINASE IN THE REGULATION
OF GLYCOLYSIS AND GLUCONEOGENESIS
IN PROPIONIBACTERIUM SHERMANII

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John Beresford SMART

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ABSTRACT

Pyruvate kinase catalyses the main irreversible reaction of glycolysis in Propionibacterium shermanii since the ATP-dependent phosphofructokinase is largely replaced by a pyrophosphate-dependent phosphofructokinase catalysing a reversible reaction. Measurement of activity of several glycolytic enzymes in glucose-, glycerol- and lactate-grown cells showed that pyruvate kinase activity is much higher than that of pyruvate, orthophosphate dikinase which catalyses the reversible interconversion of PEP and pyruvate.

This poses problems in cells grown on lactate where gluconeogenesis must operate to supply hexoses and pentoses for biosynthesis. The regulatory properties of this enzyme were accordingly studied.

The partially purified (140-fold) pyruvate kinase displayed sigmoidal kinetics for both of its substrates, phosphoenolpyruvate (PEP) and ADP. At pH 7.5 the interaction coefficient (n_H) for PEP saturation in the absence of effectors was in the range 1.9-2.5 while for ADP saturation it was 1.7-2.1. The pyruvate kinase was shown to be activated by glucose 6-phosphate (G6P) at non-saturating (0.5 mM) PEP concentrations but other glycolytic and hexose monophosphate pathway intermediates and AMP were without effect. Half-maximal activation was obtained at 1 mM G6P. The presence of G6P decreased both the $PEP_{0.5V}$ and $ADP_{0.5V}$ values and the Hill interaction coefficient for each substrate. The enzyme was strongly inhibited by ATP and inorganic phosphate (P_i) at all PEP concentrations. At non-saturating (0.5 mM) PEP half-maximal inhibition was obtained at 1.8 mM ATP and 1.4 mM P_i . The inhibition of both P_i and ATP could be largely overcome by G6P. The G6P activation and other regulatory properties of the enzyme were pH dependent.

On the basis of this in vitro study it was suggested that the activity of pyruvate kinase in vivo is determined by the balance between activators and inhibitors such that it is inhibited by ATP and P_i during gluconeogenesis while, during glycolysis, the inhibition is relieved by G6P. Such a mechanism requires that the G6P concentration should be sufficiently high when cultures are grown on glucose or glycerol but not on lactate.

To verify this proposed mechanism the in vivo concentrations of a number of selected glycolytic intermediates were measured in P.shermanii growing under a range of nutritional conditions using batch and continuous cultures. The pyruvate kinase activator, G6P, was maintained at levels of 1-2 mM in lactate-grown cells but, when growing in the presence of high levels of glucose or glycerol, G6P was present at levels between 5 and 16 mM in the cell.

F6P and FBP were always present at levels below 1.0 mM and 0.2 mM respectively in lactate- and glucose-grown cells but attained significantly higher levels on glycerol-grown cells. At high concentrations of glucose where high G6P levels were found, F6P was present at concentrations much lower than would be expected from the equilibrium constant of the phosphoglucoisomerase; this was not the case when high G6P levels were attained by growth on glycerol. The reason for this is not known.

Levels of the inhibitor of pyruvate kinase activity, ATP, were maintained in the range 1-2 mM under all the conditions studied. At the in vivo levels of the substrates PEP and ADP this level of ATP would not by itself maintain pyruvate kinase in an inactive state in the presence of the 1-2 mM G6P shown to be present in lactate-grown cells. The other inhibitor, P_i , must also be important but the in vivo levels of this effector were not determined.

A reinvestigation of the pyruvate kinase at the in vivo levels of substrates (PEP and ADP) and effectors (G6P and ATP) found in the cell indicated that, at levels above 10 mM P_i , concentrations of 5-15 mM G6P (as found in cells growing on glucose and glycerol) were required to activate the enzyme while concentrations of 1-2 mM G6P (as found in lactate-grown cells) were not able to overcome the inhibition by ATP and P_i . A concentration of 10 mM P_i in the cell does not seem unreasonable and at this concentration the proposed mechanism for control of the pyruvate kinase in vivo would be able to operate.

During this investigation data were also collected on growth yields and carbon balances in both batch and continuous cultures during growth on the three substrates lactate, glucose and glycerol.

Succinate was found to account for up to 26% of the products in glucose cultures but was only present at negligible levels in lactate cultures. Accumulation of succinate in the glucose cultures was highest in the later stages of growth of batch cultures and in carbon-limited continuous cultures when the G6P level was low. This suggests that regulation of pyruvate kinase by G6P may also serve to determine the relative flux via the PEP:carboxytransphosphorylase and pyruvate kinase enzymes - the carboxytransphosphorylase functioning to provide PP_i for the PP_i -dependent phosphofructokinase in glycolysis.

It is also proposed that G6P may be involved in partitioning glucose metabolism via glycolysis and the hexose monophosphate pathway - high internal G6P concentrations favouring flow through the hexose monophosphate pathway and thus bypassing the PP_i -dependent phosphofructokinase - however, this is highly speculative.

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LIST OF ABBREVIATIONS

ADP	adenosine 5'-diphosphate
AMP	adenosine 5'-monophosphate
ATP	adenosine 5'-triphosphate
Bis-trispropane	2 bis[tris (hydroxymethyl) methylamino]-propane
CDP	cytidine 5'-diphosphate
CM-	carboxymethyl-
CoA	coenzyme A
DE-, DEAE-	diethylaminoethyl-
DHAP	dihydroxyacetone phosphate
DNase	deoxyribonuclease
ED	Entner-Doudoroff
EDTA	ethylenediamine tetra-acetic acid
EMP	Embden -Meyerhof-Parnas
FBP	fructose 1,6-bisphosphate
F6P	fructose 6-phosphate
Ga 3-P, Glyceraldehyde 3-P	} glyceraldehyde 3-phosphate }
GDP	guanosine 5'-diphosphate
glycerol 3-P	glycerol 3-phosphate, α -glycerophosphate
G6P	glucose 6-phosphate
GTP	guanosine 5'-triphosphate
HEPES	N-2-hydroxyethyl piperazine-N'-2-ethanesulphonic acid
HMP	hexose monophosphate
IDP	inosine 5'-diphosphate
MES	2[N-morpholino]ethane sulphonic acid
NAD	nicotinamide adenine dinucleotide
NADH	nicotinamide adenine dinucleotide, reduced

NADP	nicotinamide adenine dinucleotide phosphate
NADPH	nicotinamide adenine dinucleotide phosphate, reduced
n_H	Hill interaction coefficient
OD ₅₄₀	optical density at 540 nm
OAA	oxaloacetic acid
PEP	phosphoenolpyruvate
6-PG	6-phosphogluconate
3-PGA	3-phosphoglyceric acid
P_i	inorganic phosphate
PP_i	inorganic pyrophosphate
ribose 5-P	ribose 5-phosphate
ribulose 5-P	ribulose 5-phosphate
Tricine	N-tris (hydroxymethyl) methylglycine
Tris	tris (hydroxymethyl) aminomethane
UDP	uridine 5'-diphosphate
V_{max}	maximum velocity
<u>E.coli</u>	<u>Escherichia coli</u>
<u>N.crassa</u>	<u>Neurospora crassa</u>
<u>S.lactis</u>	<u>Streptococcus lactis</u>