REGULATION OF PYRUVATE KINASE DURING GLYCONEOGENESIS IN *SACCHAROMYCES CEREVISIAE*

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1. Introduction

Due to the equilibrium of the reaction catalysed by pyruvate kinase and the enzyme's low affinity for pyruvate [1] it is unlikely to participate in glyconeogenesis. However, with substrates entering the pathway prior to PEP, the rate of conversion of this intermediate to pyruvate will influence the rate of glyconeogenesis. Therefore, it appears important that the rate of the pyruvate kinase reaction should be low under such conditions.

The investigation reported here was carried out in order to assess the state of the pyruvate kinase reaction during glyconeogenesis from ethanol by non-growing cells of *Saccharomyces cerevisiae*. The results indicate that conversion of PEP to pyruvate was controlled at a low level, due to a low concentration of the allosteric activator FDP and substrate PEP [2, 3].

2. Materials and methods

Chemicals of analytical grade were purchased from E. Merck AG, Darmstadt and enzymes and substrates from C.F. Boehringer and Söhne GmbH, Mannheim. The yeast, a laboratory strain of *Saccharomyces cerevisiae*, was grown in a mineral salts medium [4] containing 0.25% Difco yeast extract and 1% ethanol and harvested in the logarithmic phase of growth. The cells were washed twice with distilled water and once with buffer, then suspended in 50 mM imidazol-HCl buffer (pH 6.4), 50 mM KCl, to a concentration of 5 g wet wt/100 ml. This cell suspension was stirred rapidly, under an atmosphere of oxygen, in a reaction vessel thermostatically controlled at 25°. Following 30 min equilibration a sample was withdrawn for determination of the endogenous level of metabolites after which ethanol was added at an initial concentration of 100 mM and samples withdrawn after 10, 11 and 12 min. Glucose was then added at an initial concentration of 50 mM and samples withdrawn after 10, 11 and 12 min. Glyconeogenesis from ethanol was measured as the rate of total polysaccharide synthesis. The polysaccharide content of the cells was determined as described previously [5] by the phenolsulphuric acid method of Dubois et al. [6].

Metabolites were assayed in neutralized perchloric acid extracts of the cell suspensions according to procedures contained in [7]. Cell extracts used to assay pyruvate kinase activity were prepared in a Braun disintegrator [8]. 5 g wet wt of cells, made up to 30 ml with 50 ml imidazol-HCl buffer (pH 6.4), 50 mM KCl, 30 mM $MgSO_4$ were shaken for 1 min at a frequency of 4000/min whilst cooling with liquid CO_2. After centrifuging for 10 min at 50,000 *g* the supernatant was used to assay enzyme activity.

The metabolite quotient Γ = [F6P/G6P] was used as an indicator of the direction of flux in the glycolytic enzyme system according to the principle described

Abbreviations:

G6P	: glucose-6-phosphate
F6P	: fructose-6-phosphate
FDP	: fructose-1,6-diphosphate
PEP	: phosphoenol pyruvate
ATP	: adenosine triphosphate
ADP	: adenosine diphosphate
AMP	: adenosine monophosphate
NADH	: reduced nicotinamide adenine dinucleotide
$K_{0,5}$(PEP)	: concentration of phosphoenol pyruvate giving half the maximal velocity.

earlier [9]. A high concentration of ethanol was already present in the cell suspension at the time of glucose addition. Due to this it was not practical to measure ethanol production as an indication of whether glucose reversed the direction of flux in the glycolytic enzyme system from glyconeogenesis to glycolysis. Therefore, under steady state conditions, the metabolite quotient Γ = [F6P/G6P] of the phosphoglucose isomerase catalyzed reaction was used as an indicator of the direction of flux in the glycolytic enzyme system. At 25° $K_{eq} = [\overline{F6P}/\overline{G6P}] = 0.28$ [10] and is independent of the H^+ ion and metal ion concentration [11] so that knowledge of these parameters *in vivo* is not necessary. Thus when Γ = [F6P/G6P] is greater than the equilibrium value of 0.28, flux is in the direction of glyconeogenesis and in the direction of glycolysis when it is less than 0.28.

3. Results

3.1. *Metabolite pattern*

Table 1 summarizes metabolite levels during endogenous metabolism, glyconeogenesis, and glycolysis. The level of G6P and F6P are given together with the calculated values of Γ = [F6P/G6P] during the steady states attained after addition of ethanol and glucose. During metabolism of ethanol alone Γ = [F6P/G6P] was higher than the equilibrium value of 0.28, indicating flux in the direction of glyconeogenesis. This was confirmed by the measured rate of polysaccharide synthesis which was equivalent to 2.5 μmoles glucose/min/g wet wt. Following addition of glucose Γ = [F6P/G6P] was less than 0.28, indicating a switch from glyconeogenesis to glycolysis. Table 1 also contains the levels of FDP, PEP, citrate and adenine nucleotides. ATP, ADP and AMP were essentially the same in all three conditions whilst citrate was highest during glycolysis. The level of FDP was very low in the endogenous condition and was only two times higher in the glyconeogenic condition. This low level of FDP during glyconeogenesis is similar to that reported in *S. cerevisiae* growing on ethanol [12]. Following the switch to glycolysis FDP increased to approximately one hundred times the glyconeogenic value. In contrast to FDP the level of PEP was high in the endogenous condition, decreased markedly after addition of ethanol and was even lower during glycolysis.

3.2. *Properties of pyruvate kinase*

Table 2 shows the apparent affinity for PEP in the absence and presence of FDP, together with the maximum catalytic activity, of the pyruvate kinase in

Table 1
Metabolic levels (in μmoles per gram wet weight) during endogenous metabolism, glyconeogenesis and glycolysis.

	Endogenous		Glyconeogenesis (min after ethanol add.)			Glycolysis (min after glucose add.)		
			10	11	12	10	11	12
G6P	> 0		0.60	0.59	0.54	1.84	1.87	1.86
F6P	> 0		0.18	0.18	0.16	0.46	0.44	0.45
$\Gamma = \frac{F6P}{G6P}$	–		0.30	0.31	0.30	0.25	0.24	0.24
FDP		0.01	0.03	0.02	0.02	2.45	2.20	2.40
PEP		1.80	0.21	0.23	0.23	0.12	0.13	0.13
Citrate		1.50	1.30	1.20	1.30	2.20	2.10	2.30
ATP		1.60	1.80	1.70	1.60	1.60	1.60	1.70
ADP		1.30	1.00	1.05	1.08	1.00	1.00	0.96
AMP		0.30	0.16	0.18	0.20	0.16	0.18	0.18

Table 2
Some kinetic properties of the pyruvate kinase in cell free extracts of yeast grown on ethanol and glucose.

		$K_{0.5}$ (PEP) (mM)		IU/g wet wt
		without FDP	with 2 mM FDP	
ethanol	A	5.5	0.19	165
	B	6.2	0.22	150
glucose	A	6.0	0.19	480
	B	6.0	0.20	440

The yeast was harvested during logarithmic growth upon either 1% ethanol or 1% glucose. Cell free extracts were prepared as described under Material and methods. Assay conditions: 50 mM imidazol-HCl buffer (pH 6.4), 50 mM KCl, 30 mM $MgSO_4$, 0.25 mM NADH, lactate dehydrogenase (freed from NH_4) 10 IU/ml, 25° $K_{0.5}$ (PEP) was measured in the presence of 5 mM ADP. IU/g wet wt was calculated from plots of the reciprocal of the initial velocity, in the presence of 2 mM FDP, against the reciprocal of the concentration of PEP.

cells grown on ethanol and glucose. As might be expected, activation of the enzyme by FDP and its affinity for PEP was independent of the growth conditions. In contrast the maximum catalytic activity was three times lower in cells grown upon ethanol. The activity of pyruvate kinase in these cells was much higher than that measured in similar cells by other workers who in addition found little difference between the activity in ethanol and glucose grown *S. cerevisiae* [12]. The differences are probably due to extraction and assay conditions.

4. Discussion

Interpretation of the results presented here depends upon the assumption that pyruvate kinase has similar properties in the intact cell to those observed *in vitro*. In a previous investigation it was demonstrated that in *S. cerevisiae* the affinity of the enzyme for PEP is dependent upon the intracellular concentration of FDP [13]. Therefore assumptions necessary for a qualitative assessment seem to be valid.

The fact that the activity of pyruvate kinase was lower in ethanol grown cells should facilitate glyconeogenesis. However, the high potential activity in the cells points to the requirement for an additional means of controlling the rate of the reaction. In agreement with previous findings [13, 14] PEP was high during endogeneous metabolism which, since flux is low under such conditions, indicates that pyruvate kinase was in its low affinity state. This must be due to the low level of FDP would also permit ATP and citrate to excert some inhibitory effect [2]. FDP did increase following addition of ethanol but such an increase would not be expected to increase the affinity of pyruvate kinase relative to the endogenous condition [15, 16]. In contrast, during glycolysis FDP was high enough to overcome the inhibitory effect of both ATP and citrate and to convert the enzyme to its high affinity state [2]. This is indicated by the fact that glycolysis occurred with a lower level of PEP than that present during glyconeogenesis.

The results support the view that during glyconeogenesis pyruvate kinase occurs in the state which has a low affinity for PEP. In addition the level of this substrate was low relative to its half-saturating value in the absence of FDP. The combination of these factors must result in a low rate of conversion of PEP to pyruvate. It should be pointed out, however, that the measured rate of polysaccharide synthesis, which was equivalent to the conversion of 5 μmoles PEP/min/g wet wt, was low in relation to the potential activitity of pyruvate kinase (150–165 μmoles/min/g wet wt). Thus, although the enzyme must be controlled so as to allow glyconeogenesis to proceed, its rate under such conditions may exert a fine control upon the rate of glyconeogenesis.

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