T. Solomos*

Botany School

University of Cambridge, England

Observations on yeast pyruvate kinase activity in vivo

Received July 21, 1970

Abstract

The results indicate that the activity of pyruvate kinase in intact yeast cells is greatly influenced by fructose 1,6 diphosphate. Furthermore, it is suggested that the permeability properties of the cellular membranes may be an important factor in regulating the activity of PK.

Pyruvate kinase (PK) has often been considered to be a controlling enzymatic step in glycolysis (1 - 3). Kinetic studies with purified enzyme from various sources have demonstrated that PK shows properties of a regulatory enzyme being modified by various effectors (4 - 7). Thus, the yeast enzyme shows co-operative kinetics towards the activating kations K^+, NH_4^+, Mg^{2+} and PEP (4, 5). FDP changes the sigmoid kinetics of the enzyme, with respect to the above activating ions and PEP, to hyperbolic. In the case of ions, the sigmoid to hyperbola transition occurs by altering either K_m and V_{max} (4) or K_m only (5). The evidence regarding the effect of FDP on the activity of PK has not been conclusively demonstrated in vivo. However, PK has been shown to

Abbreviations: Phosphoenolpyruvate PEP, 3-phosphoglyceric acid 3-PGA, Pyruvate kinase PK, fructose 1,6 diphosphate FDP

*Present address: Plant Biochemistry,
South Laboratory,
University of Alberta,
Edmonton. Alberta. Canada.

be a cross-over point in the transient phase of changes in the rate of glycolytic flux (1, 2). The above evidence, although indicative of an activation of PK under conditions of increased glycolytic flux, does not identify with certainty the factors which cause this activation. In the present work we offer data which indicate, firstly, that in intact baker yeast cells PK is activated by FDP, and, secondly, that the state of cytoplasmic membranes may affect in vivo the activity of the enzyme. Although the effect of cytoplasmic membranes on PK activity is not clear, we postulate that it is manifested by the segregation of the activating ions.

Methods and Material

D.C.L. yeast was bought from a local supplier. The cells were suspended in 0.1 M phosphate buffer pH 5.4 and aerated for 18 - 20 hours at room temperature. The cells were then washed twice with cold distilled water and suspended in 0.02 M citrate buffer pH 5.4. The cell concentration was 10% wet weight/volume. All the experiments were carried out at 8°C. Phosphate esters were estimated by pipetting samples into TCA solution of 6% final concentration The cells were centrifuged, washed once more with 5% TCA and the subsequent determination of the phosphate esters was carried out by using specific enzymes (8, 9).

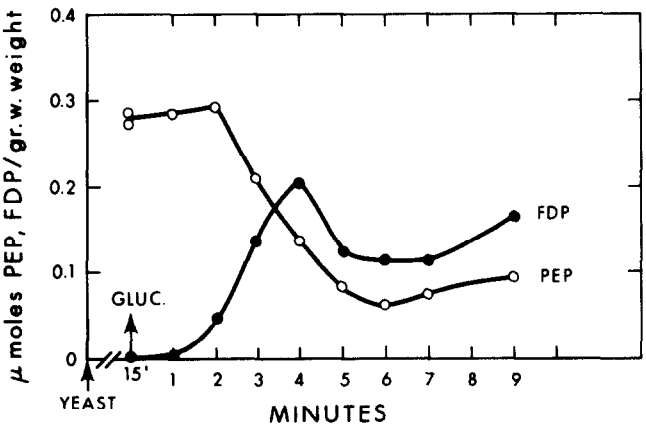

Fig. 1. Baker's yeast was aerated for 20 hours in 0.1 M. phosphate buffer pH 5.4 and 0.01 mM glucose. The cells were then washed and suspended in 0.02 M. citrate buffer pH 5.4 at 8°C. After 15 minutes of constant aeration, 0.1 M. glucose final concentration was added.

Results and Discussion

It is known that starved baker yeast cells do not show endogenous fermentation and their rate of respiration is low (10, 11). The estimation of phosphate esters shows that the hexose phosphate pool is also very low, whereas the levels of both phosphoenol pyruvate and 3-phosphoglyceric acid are several times higher than in either yeast respiring or fermenting glucose (Table 1).

Table 1

μ Moles/gr. wet weight*

Metabolite	Starved cells	Glucose oxidising cells	Glucose Fermenting cells
FDP	-	0.520	0.610
G6P	0.008	1.40	1.30
PEP	0.230	0.035	0.040
3-PGA	0.560	0.150	0.145
ATP	1.50	1.85	1.600
ADP	0.90	0.70	0.70

*The values are the average steady state values from six experiments.

It seems, therefore, that in intact starved baker yeast cells PK is relatively inactive. Taking into consideration the facts that, firstly, the preceding glycolytic enzymes up to phosphoglycerate kinase are easily reversible, and, secondly, that in all our experiments PEP and 3-PGA show parallel changes, it can be argued that the high level of 3-PGA reflects an inactivation of PK as well. Similar observations have also been made previously (12).

With the addition of glucose under aerobic conditions PEP initially either does not change at all, or else it even increases, and then, after about two minutes, starts falling rapidly (Figs. 1, 2). This decrease in PEP is always associated with an increase in fructose 1,6 diphosphate. The pattern of changes in fructose 1,6 diphosphate is different when glucose is added under anoxic conditions. Thus, instead of the slow initial rise, it rises rapidly within two minutes after the addition of glucose. This increase in fructose 1,6 diphosphate is associated with a steep decrease in PEP levels (Fig. 3). Adenosine diphosphate is not likely to activate PK in intact yeast cells as, although it rises appreciably at the initial stages of glucose addition, PEP does not decrease (10). Neither can the inactivity of PK be explained in terms of lack of ADP

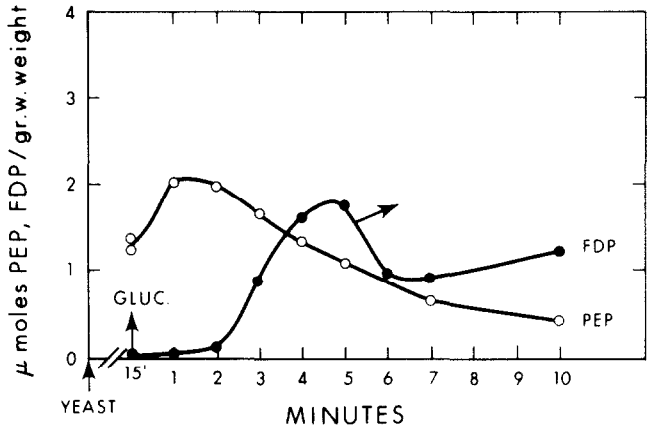

Fig 2. As Fig. 1.

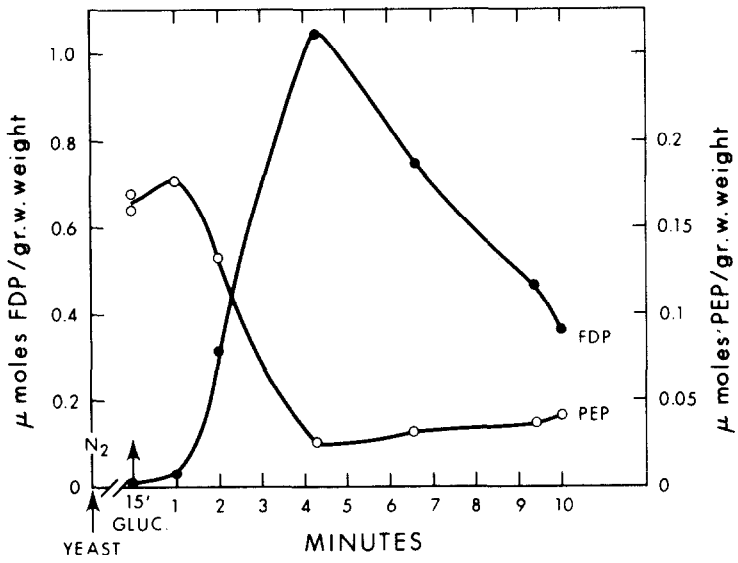

Fig. 3. Starved baker yeast as Fig. 1 was suspended in 0.02 M. citrate buffer pH 5.4 at 8°C. Nitrogen was bubbled through for 15 minutes, then 0.1 M. glucose final concentration was added.

---

as it is present at a higher concentration in starved yeast than in glucose utilizing cells (Table 1). We have also found that the air to nitrogen transition of glucose respiring yeast cells is followed by cyclic changes in fructose 1,6 phosphate, other glycolytic intermediates and adenosine nucleotides (10). We always found that in the first cycle fructose 1,6 phosphate and phosphoenol

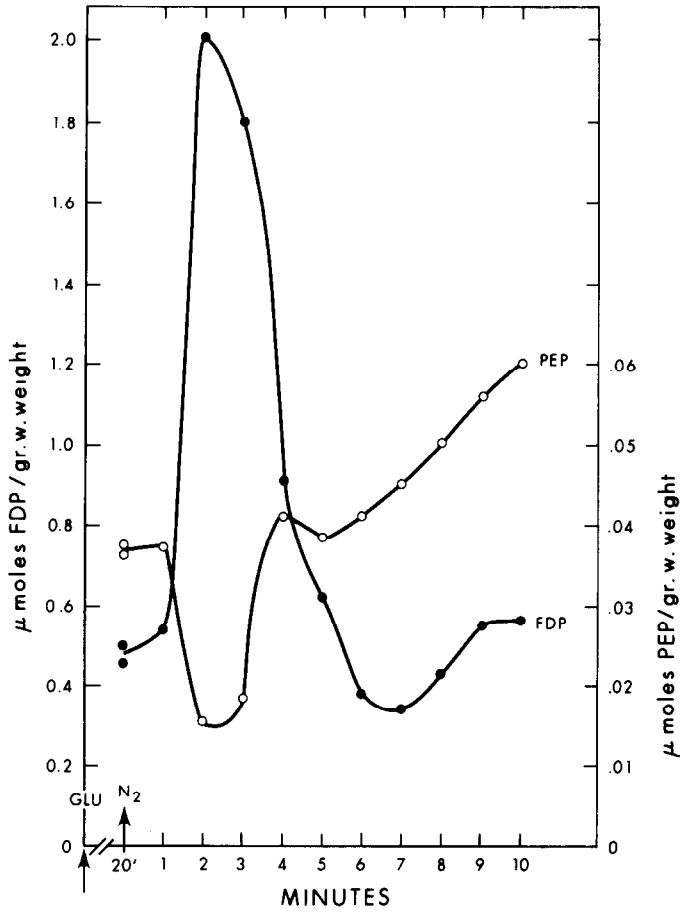

Fig. 4. Starved yeast cells, as in Fig. 1, were suspended in 0.02 M. citrate buffer pH 5.4 at 8°C. After 15 minutes of constant aeration, 0.1 M. glucose was added. The yeast suspension was made anearobic after 20 minutes by bubbling nitrogen through.

---

pyruvate were out of phase. Thus the peak in FDP coincides with the trough in PEP (Fig. 4). This inverse pattern of changes in FDP and PEP, at the time when the rate of alcohol production increases (10), and moreover despite the fact that from one mole of FDP, two moles of PEP are formed, indicates that PK is greatly activated.

The above results suggest that the activity of PK is strongly influenced by FDP in intact baker yeast cells. It should also be pointed out that the steady state level of FDP in both glucose respiring and fermenting yeast is always

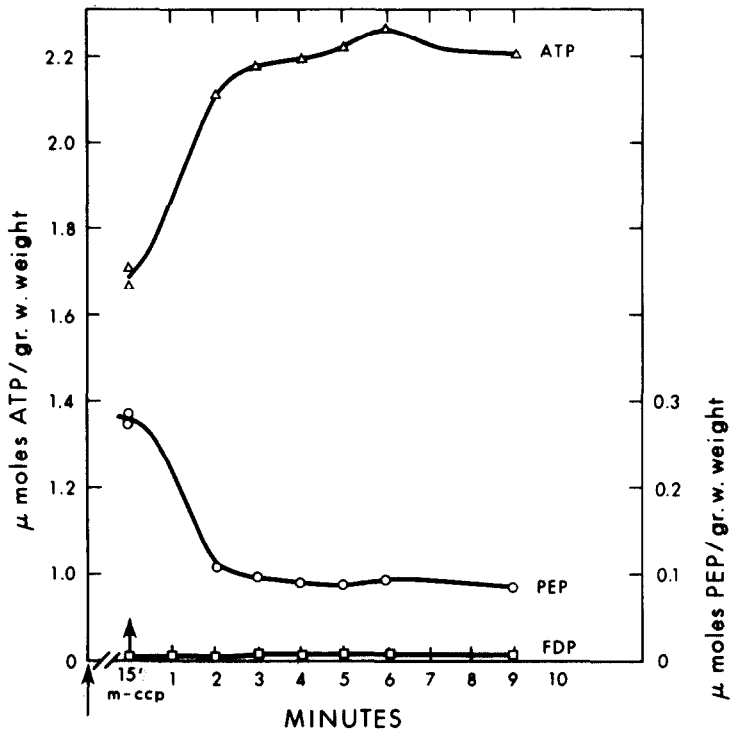

Fig. 5. Starved yeast cells, as in Fig. 1, were suspended in 0.02 M. citrate buffer pH 5.4. After 15 minutes of aeration, 10^{-5} M. carbonylcyanide m-chlorophenylhydrazone final concentration was added.

much higher than the threshold value which appears to be required for PK's activation. A similar inverse pattern of changes in FDP and PEP under conditions of an increase in the rate of glycolysis has been observed with plant tissues (2, 13).

We also found that if starved yeast was treated with either 2,4 dinitrophenol or carbonylcyanide m- chlorophenylhydrazone, PK was activated, as both PEP and 3-PGA fell rapidly. From the phosphate esters known to influence the activity of PK, only ATP, which is a negative effector of the enzyme, increased (Figs. 5, 6).

Unless the uncouplers themselves activate the enzyme, the most likely explanation would be that the cellular distribution of the essential activating ions is altered by the addition of uncouplers. It is known that with artificial biological membranes 2,4 dinitrophenol alters their permeability characteristics (14). Also, an efflux of K^+, although not pronounced, has been observed after

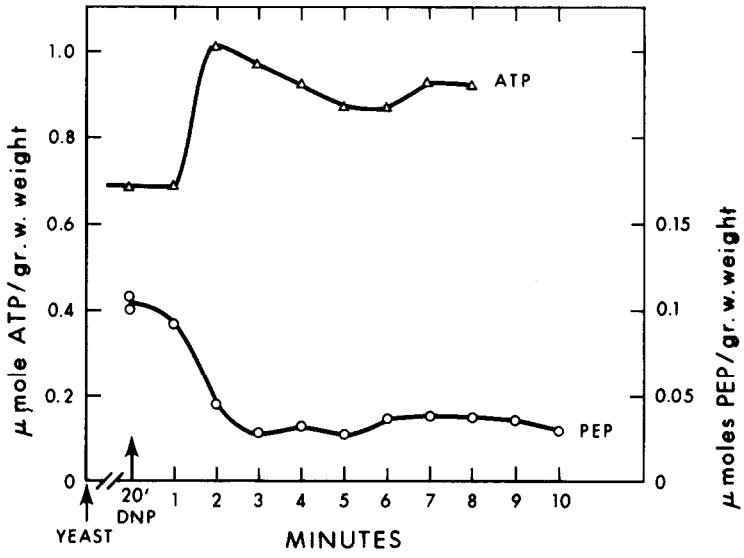

Fig. 6. To a suspension of starved baker yeast cells as in Fig. 5, 10^{-4} M. 2,4 dinitrophenol final concentration was added.

---

the addition of DNP to isolated mitochondria (15). The alternative explanation of the enzyme being converted to an FDP insensitive, as has been found with purified enzyme from rat epididymal adipose tissue (16), does not seem likely as the activation of PK is very rapid (Figs. 5, 6).

Acknowledgements

I wish to thank Dr. J. Barker for advice, Mr. R. Jakes and Mr. M. Askam for technical assistance, and the Broodbank Managers for a Fellowship.

1. Homes, F.A., Arch. Bioch. Biophys., 108, 36 (1964).
2. Barker, J., Khan, M.A.A. and Solomos, T., New Phytol., 66, 577 (1967).
3. Hess, B., In M.D. Mesarovic: Systems Theory and Biology, New York, pp. 88 (1968).
4. Hess, B., and Haenckel, R., Nature, 214, 848 (1967).
5. Hinsley, R.J. and Suelter, H.C., J. Biol. Chem., 244, 4819 (1970).
6. Maeba, P. and Sanwal, D.B., J. Biol. Chem., 243, 488 (1969).
7. Tanaka, T., Harano, Y., Sue, F., Morimura, H., J. Biochemistry (Tokyo), 62, 71 (1967).

8. Barker, J., Jakes, R., Solomos, T., Younis, E.M. and Sherwood, A.F., J. Exp. Bot., 15, 284 (1964).

9. Bergmeyer, H., Methods of Enzymatic Analysis, Academic Press, London (1963)

10. Solomos, T. and Barker, J., in preparation.

11. Berke, L.H. and Rothstein, A., Arch. Bioch. Bioph., 72, 380 (1957).

12. Pye, E.K., "Studies on the Mechanism of the Pasteur effect in yeast", Ph.D. thesis, University of Manchester (1964).

13. Solomos, T., J. Exp. Botany, in press.

14. Hopfer, V., Lehninger, L.A. and Thomson, E.T., Proc. Nat. Acad. Sci., 59, 484 (1968).

15. Chappell, B.J. and Haarhoff, N.K. (1967), In Biochemistry of Mitochondria, ed. E.C. Slater, Z. Kaniuga and L. Wojtczak, Academic Press, London and New York.

16. Pogson, I.C., Bioch. Bioph. Res. Com., 30, 297 (1969).

17. Preis, J., Ghosh, H.P. and Wittkop, S., In Biochemistry of Chloroplasts, ed. Goodwin, T., Academic Press, London and New York, 1967.