CONTROL OF PHOSPHOFRUCTOKINASE [PFK] ACTIVITY IN CONDITIONS SIMULATING THOSE OF GLYCOLYSING YEAST EXTRACT

A. BETZ and E. SEL'KOV

Institut für Molekulare Biologie, Biochemie und Biophysik, 3301 Stöckheim, Germany and Institute of Biophysics, USSR Academy of Science, Puschino na Oke, USSR

Received 1 March 1969

1. Introduction

Kinetic studies on very dilute purified enzymes, in conditions of optimal pH and substrate concentrations, are very useful for the characterisation of these isolated biocatalysts. But they give only very limited information about their action *in situ*. This is especially true regarding metabolic control phenomena.

In this dilemma we are very fortunate that in carefully prepared cell-free extracts of yeast cells, control of glycolysis persists with characteristics comparable to those of the intact living cell [1]. This extract is substantially free of mitochondria and other particles, but it contains all the cytoplasmic enzymes and cofactors. In this homogeneous system we can be certain that substrates and cofactors are present at the enzyme site in the concentrations analysed. Conditions in this extract are not identical with those of living cells, but insofar as control characteristics are similar, we may compare the two systems.

2. Material and methods

The preparation of cell-free extracts from *Saccharomyces carlsbergensis* has been described [1–3]. Metabolites were assayed enzymatically [4] using improved methods [5,6]. The enzymes used were purchased from Boehringer and Comp., Tutzing.

In experiments on the kinetics of yeast PFK sampling methods were used throughout, since the reaction had to be followed in the presence of both reaction products. Yeast PFK was incubated with ATP and F-6-P, in the presence of other substances as indicated, in 40 mM TRA buffer pH 6.5. Samples of the reaction mixture were taken at 0.3 and 6 min after the addition of the enzyme and deproteinized by injection of 2 ml into 1 ml of 15% $HClO_4$. After centrifugation, the clear supernatant was neutralized with KOH and TRA buffer to pH 7.0–7.2.

After freezing and thawing, the potassium perchlorate was removed by centrifugation and the supernatant was assayed for F-6-P. The velocity of the enzyme reaction was constant for more than 10 min, provided that no one of the substrates was limiting.

3. Results and discussion

3.1. *Concentrations of metabolic intermediates in yeast cells and cell-free extract*

Table 1 illustrates the maximal and mininal concentrations of intermediates during oscillatory glycolysis. Since there is only a small dilution by buffer in the preparation of the extract (up to a maximal 13%), it is not surprising that many substances are found in the same range of concentration in both the living cell and the cell-free system. Adenosine nucleotides are somewhat less concentrated in the extract, reasonably since mitochondria are absent. Hexose monophosphates are about 25% more concentrated in the extract. Since glycolysis persists, their concentration depends on the control actually occurring in this system. FDP and both triose phosphates are 4 times more concentrated in the cell-free system. This difference is large enough to suggest some difference in the con-

Table 1
Maximal and minimal concentrations of intermediates in oscillatory controlled glycolysis (from ref. [7]).

Substance	Yeast cells (μmoles/g)		Yeast extract (μmoles/ml)	
	max.	min.	max.	min.
ATP	1.90	1.40	1.60	0.70
ADP	0.52	0.18	0.45	0.11
AMP	0.16	0.02	0.16	0.06
G-6-P	1.25	0.80	1.95	0.51
F-6-P	0.33	0.22	0.55	0.15
FDP	1.60	0.65	6.60	5.10
DAP	0.43	0.27	1.67	1.15
GAP	0.10	0.05	0.44	0.24
Pyruvate	0.75	0.45	0.58	0.32

trol of glycolysis in the two systems.

Indications of such a difference also come from the phase relations in the two systems. In fig. 1, our earlier data on phase relations in yeast cells [8] are compared with Cassuto's [9] data on yeast extract. In both systems NADH is out of phase with ATP, and F-6-P is out of phase with FDP. That means, that in both cells and extract, control at the PFK level is effective. But pyruvate, which in living cells is clearly in phase with NADH, is retarded in the cell-free system, which indicates some limiting step in the extract between 1,3-diphosphoglycerate and pyruvate. This step need not necessarily be actively controlled, it might only be a "bottle neck". A limiting step could explain why pyruvate and NADH are not exactly synchronised in the extract. It would also explain the slight differences between cells and extract in the phases of ATP and FDP, and the accumulation of FDP, DAP and GAP in the cell-free system. But besides these minor differences, control at the PFK step is common to both systems.

Fig. 2 summarizes some common control features. During the accumulation of NADH, glucose incorporation into yeast cells is maximal [10]. A high [FDP] / [DAP] · [GAP] quotient indicates that the influx into FDP is higher than the efflux from GAP [10], and in this phase pyruvate or acetaldehyde induce maximal phase shifting in cell suspensions [11]. Simultaneously, FDP synthesis is maximal in the extract [3]. During the oxidative half cycle all these reactions are minimal, only ATP synthesis [3] is maximal in the extract. These observations demonstrate that control of glycolysis, as far as it depends on PFK, is essentially identical in both systems.

3.2. *Kinetics of yeast phosphofructokinase*

Yeast PFK is inhibited by rather low ATP concentrations, as in the enzyme from other organisms. Its

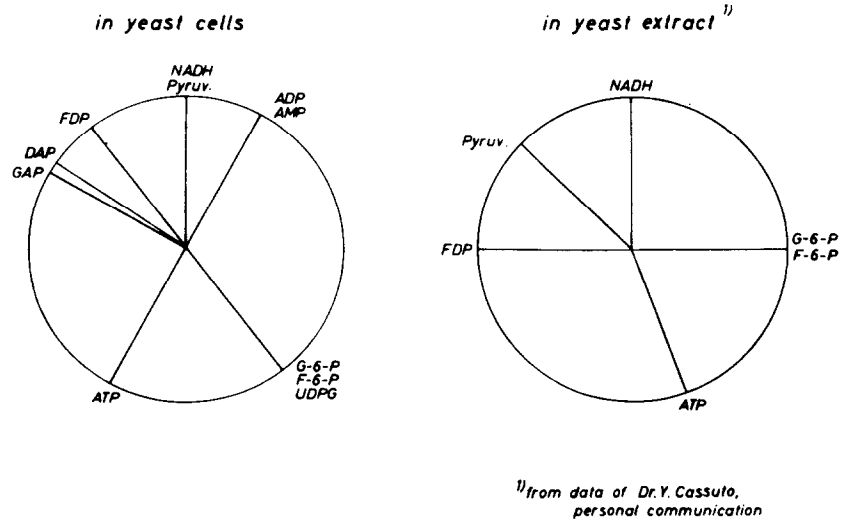

Fig. 1. Schema of phase relations of glycolytic intermediates in yeast cells [8] and yeast extract [9].

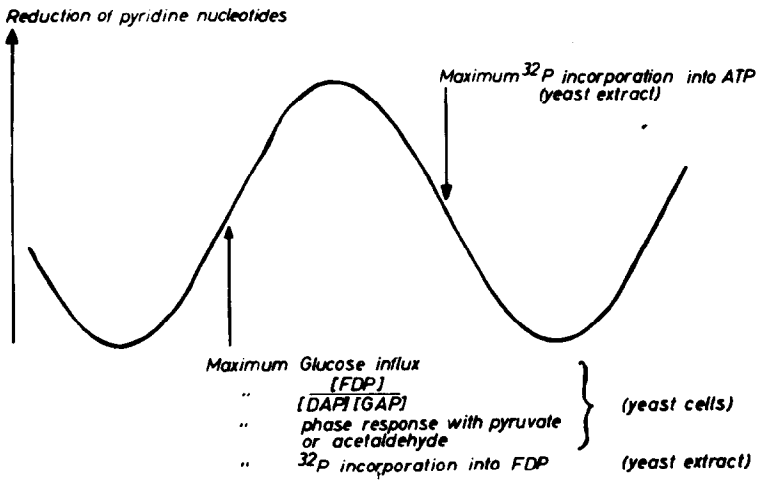

Fig. 2. Schema of pulsatory flux in glycolysis.

activity is restored by higher concentrations of the second substrate, F-6-P and to some extent it is activated by the reaction product FDP and to a much larger extent by AMP [12,7].

In contrast to PFK from other sources, yeast PFK is not stimulated by ADP. In the Hill plot coordination numbers of 2 can be demonstrated for ATP, AMP and FDP; $n = 4$ was found for F-6-P [13]. All substrates and effectors become inhibitory at slightly elevated concentrations.

The effect of these chemicals becomes different, if kinetics are studied in the presence of all the other effectors in concentrations similar to those found in the extract. As most of the earlier work on yeast PFK has been done at pH 7.5, we, too, first used this pH in the multifactor system. In this work pH = 7.5 was used, because freshly prepared extracts are of this pH, and also it is nearer the pH optimum of yeast PFK (6.7). There is no substantial difference in kinetics between pH 6.5 and 7.5.

We used a reaction medium with similar concentrations as the extract, containing ATP 1 mM, ADP 0.3 mM, AMP 0.1 mM, F-6-P 0.3 mM, FDP 8 mM, Mg^{2+} 10 mM and PO_4^{2-} 10 mM. The last two ions were at smaller concentrations than in the extract because of their limited solubility in the absence of complexing agents.

Fig. 3a represents the dependence of v on ATP concentration. There is evidently some inhibition by an overshoot of this substrate, but under our conditions almost maximal activity persists with concentrations of ATP from 0.5 mM to 2.5 mM, whereas in the absence of FDP, ADP and AMP the inhibitory effect of ATP starts at 0.1 mM [12]. Under "extract conditions" PFK activity is almost maximal over the range of physiological fluctuations in ATP concentrations. Thus in oscillatory control of glycolysis the ATP concentration is not a decisive factor, FDP activitates PFK, as seen from fig. 3b, but only to a very limited extent. With concentrations of 2 mM and higher, PFK is maximally activated. In yeast extracts, where FDP is fluctuating within the concentration range 5 to 7 mM, this chemical has no control at the PFK step. In this series of experiments ADP was as inefficient as described earlier [12]. Fig. 3c shows the activating effect of AMP in the concentration range which really coincides with the physiological one. The enzyme is activated by a factor of 1.5 if the AMP concentration rises from 0.05 to 0.1 mM.

In fig. 3d the dependence of PFK activity on the concentration of F-6-P is shown. There is a considerable enhancement, by a factor of 2.5, if F-6-P is raised from 0.25 to 0.5 mM.

At substrate and effector concentrations similar to those of the cell free extract, only AMP and F-6-P are effective activators of yeast PFK. The efficient concentration range of both chemicals coincides with the fluctuations actually occurring during oscillatory control in yeast extract. Both substances controlling the activity of PFK are dependent on other reactions.

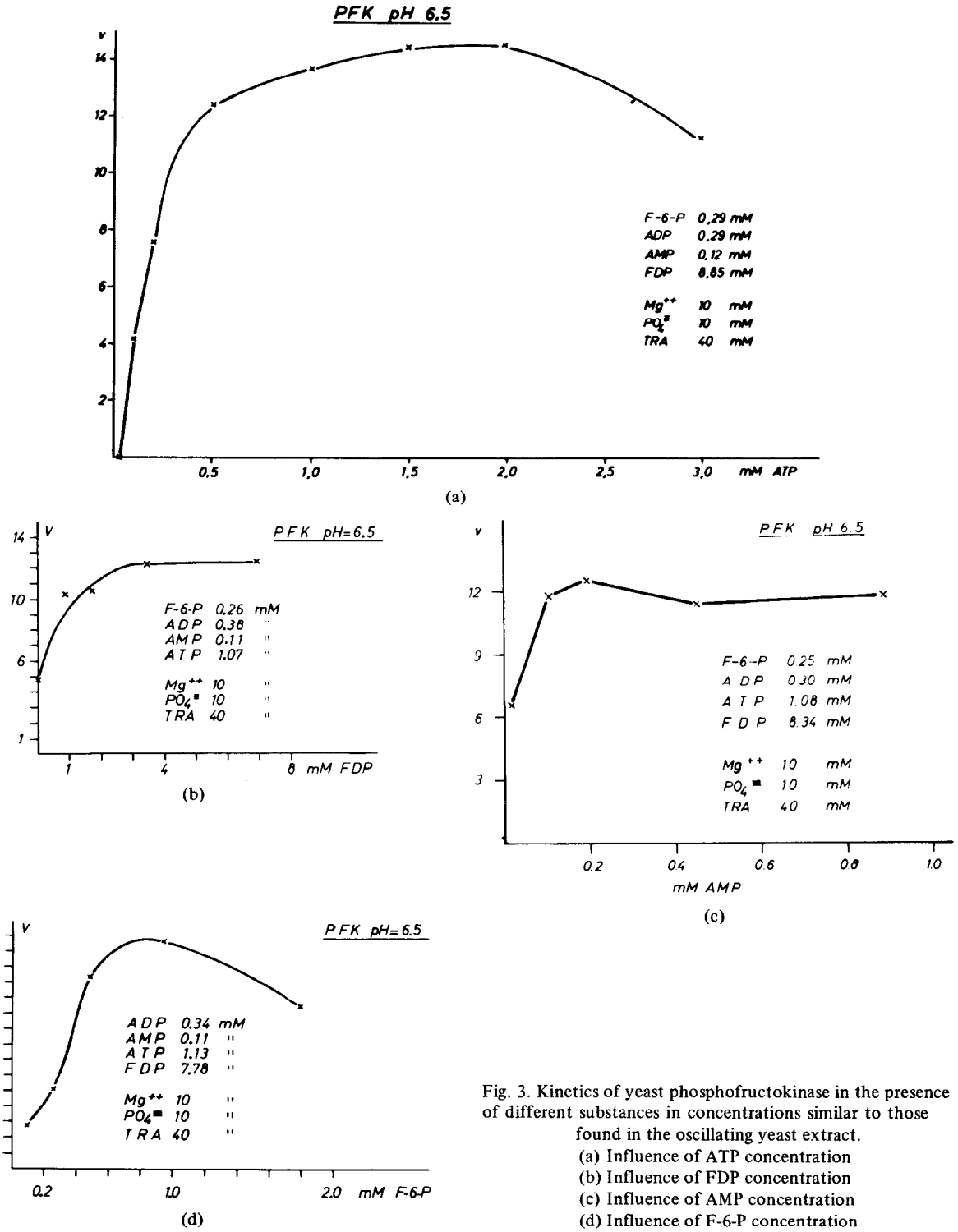

Fig. 3. Kinetics of yeast phosphofructokinase in the presence of different substances in concentrations similar to those found in the oscillating yeast extract.
(a) Influence of ATP concentration
(b) Influence of FDP concentration
(c) Influence of AMP concentration
(d) Influence of F-6-P concentration

AMP is always in phase with ADP and adenylate kinase is active enough in yeast cells to keep the concentrations not far from equilibrium. F-6-P is always in phase with G-6-P. Its concentration depends on the influx from hexokinase or phosphorylase reactions and the efflux through the PFK reaction. As control is rather drastic at the PFK step and the activation factors observed for AMP and F-6-P are rather low, we should look for the possibility that activation of different reactions could be sequentially additive.

References

[1] B.Chance, B.Hess and A.Betz, Biochem. Biophys. Res. Commun. 16 (1964) 182.
[2] K.Pye and B.Chance, Proc. Natl. Acad. Sci. 55 (1966) 888.
[3] A.Betz, European J. Biochem. 4 (1968) 354.
[4] H.U.Bergmeyer, Methoden der enzymatischen Analyse (Verlag Chemie, 1962).
[5] R.W.Estabrook and P.K.Maitra, Analyt. Biochem. 3 (1962) 369.
[6] P.K.Maitra and R.W.Estabrook, Analyt. Biochem. 7 (1964) 472.
[7] A.Betz and C.Moore, Arch. Biochem. Biophys. 120 (1967) 268.
[8] A.Betz, in: Quantitative Biology of Metabolism, ed. A.Locker (Springer-Verlag, Berlin, Heidelberg, New York, 1968) p. 205.
[9] Y.Cassuto, personal communication.
[10] A.Betz, Physiol. Plant. 19 (1966) 1049.
[11] A.Betz and J.-U.Becker, in: Symposium on Short Term Oscillations, eds. B.Chance and B.Hess (Academic Press, 1969) in press.
[12] C.L.Moore, A.Betz and B.Chance, in: Control of Energy Metabolism, eds. B.Chance, R.W.Estabrook and J.R. Williamson (Academic Press, 1965) p. 97.
[13] A.Betz, in: Symposium on Short Term Oscillations, eds. B.Chance and B.Hess (Academic Press, 1969) in press.