Vol. 111, No. 1, 1983 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
February 28, 1983 Pages 294-300

SIMILARITY OF ACTIVATION OF YEAST PHOSPHOFRUCTOKINASE BY AMP AND FRUCTOSE-2,6-BISPHOSPHATE

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Received January 14, 1983

SUMMARY: Phosphofructokinase from yeast is effectively activated by AMP and fructose-2,6-bisphosphate by increasing the affinity of the enzyme to fructose-6-phosphate and the maximum activity toward this substrate. The enzyme is activated by AMP and fructose-2,6-bisphosphate both at high and at low concentrations of ATP. The half maximum stimulation concentrations of AMP and fructose-2,6-bisphosphate are about 200 µM and 2 µM, respectively. At saturating concentrations of AMP and fructose-2,6-bisphosphate similar maximum activities were observed in the dependence of enzyme activity on the concentrations of fructose-6-phosphate. The fructose-6-phosphate affinity is more enhanced by fructose-2,6-bisphosphate than by AMP.

The activity of phosphofructokinase (EC 2.7.1.11) is influenced by a variety of ligands. For the regulation of cellular metabolism the activation of the enzyme by AMP and Fru-2,6-P_2 is considered most important (1,2). The effect of AMP contributes to the homeostasis of ATP (3), while Fru-2,6-P_2 is involved in the coordination of glycolysis and gluconeogenesis (2). Recently, Fru-2,6-P_2 has been detected in yeast cells under glycolytic conditions (2,4). Yeast phosphofructokinase was found to be very sensitive to Fru-2,6-P_2 (5,6). According to (5) Fru-2,6-P_2 does not affect the maximum activity with respect to Fru-6-P, whereas Bartrons et al. (6) described an enhancement of the maximum activity by this effector. Fru-2,6-P_2 was found to increase the affinity of the enzyme to AMP (7). AMP was reported to increase the affinity of yeast phosphofructokinase to Fru-6-P and to relieve the ATP inhibition without exerting an effect on the maximum activity (8-11). The activation of phosphofructokinase by AMP prevailed at high concentrations of ATP and non-saturating Fru-6-P. In this work the activation pattern of AMP and Fru-2,6-P_2 was

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investigated under conditions at which phosphofructokinase is most sensitive against AMP (8). The conditions are compatible to those used for the investigation of the cooperation of enzymes in reconstituted systems (3). An attempt for a quantitative interpretation of the effects of the two activators was made by using phenomenological equations.

MATERIALS AND METHODS: Phosphofructokinase from Saccharomyces cerevisiae was prepared according to (12) and Fru-2,6-P_2 according to (13). Biochemicals and auxiliary enzymes were purchased from Boehringer. The kinetic experiments were carried out with a coupled enzyme assay using pyruvate kinase and lactate dehydrogenase. The reaction mixture (1.0 ml, 25°C) contained 100 mM imidazol/HCl, pH 6.6, 25 mM KH_2PO_4/K_2HPO_4, 10 mM $MgCl_2$, 100 mM KCl, 0.25 mM NADH, 0.5 mM phosphoenolpyruvate, Fru-6-P and ATP as indicated, lactate dehydrogenase (2 units), pyruvate kinase (2 units) and phosphofructokinase (0.5 µg). Fructose-1,6-bisphosphatase (0.2 units) were added for continuous regeneration of Fru-6-P. Phosphofructokinase and the auxiliary enzymes were dialysed and diluted with imidazol buffer before use. The reaction was started by addition of ATP. Enzyme activity is expressed as units per ml enzyme solution (25 µg protein/ml). The kinetic data were compared with the following functions:

$$v = V \frac{([\text{Fru-6-P}]/K)^{n_H}}{1 + ([\text{Fru-6-P}]/K)^{n_H}} \qquad (1)$$

Equ. 1 is the Hill equation. V denotes the maximum activity of the enzyme with respect to Fru-6-P, K is the half maximum rate constant, n_H is the Hill number.

$$v = V \frac{[\text{ATP}]}{(K_S + [\text{ATP}])} \frac{(1 + [\text{ATP}]/_R)^n}{(1 + [\text{ATP}]/K_R)^n + L_o(1 + [\text{ATP}]/K_T)^n} \qquad (2)$$

Equ. 2 used for the phenomenological description of the ATP velocity curves was derived from the allosteric model of Monod et al. (14). V is the maximum activity of the enzyme at the applied concentration of Fru-6-P, K_R and K_T are the affinity constants for ATP in the active R and the inactive T state, respectively, L_o is the allosteric constant. For treatment, an octameric structure of the enzyme (n = 8), and for substrate affinity a numerical value of K_S = 30 µM (15) were taken into account.

$$v = V_o + (V - V_o) \frac{([X]/K)^{n_H}}{1 + ([X]/K)^{n_H}} \qquad (3)$$

Equ. 3 is a generalization of the Hill equation. X denotes AMP or Fru-2,6-P_2, V_o is the activity of the enzyme in absence of the respective effector, V gives the activity at infinite concentrations of X, K is the effector concentration causing half maximum activation, n_H is the Hill coefficient of the activation process.

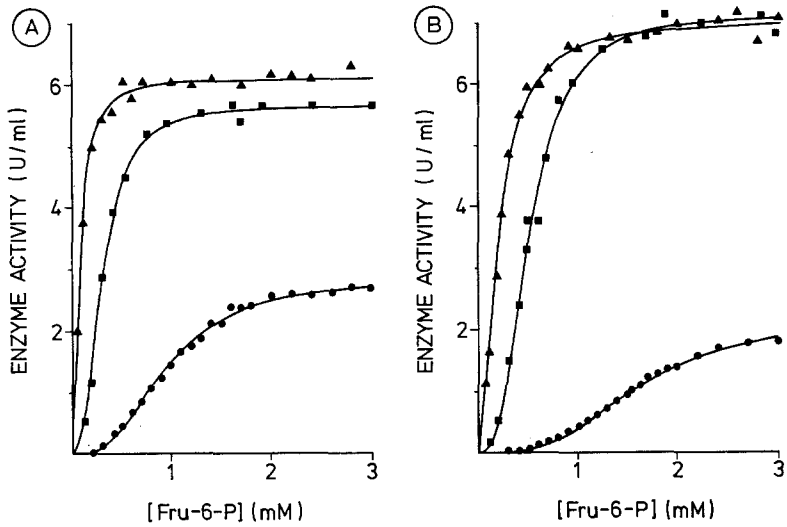

Fig. 1: Dependence of enzyme activity on the concentration of fructose-6-phosphate in presence of AMP and fructose-2,6-bisphosphate

A :[ATP]= 0.3 mM; B :[ATP]= 3.0 mM

● no additions; ■ [AMP]= 1 mM; ▲[Fru-2,6-P_2]= 20 µM

Kinetic parameters according to Equ. 1:

	A			B		
	V (U/ml)	K (mM)	n_H	V (U/ml)	K (mM)	n_H
●	2.91	0.96	2.6	2.26	1.65	2.8
■	5.74	0.30	2.5	7.23	0.51	2.7
▲	6.21	0.08	1.7	7.13	0.11	1.7

The constants have been estimated by a least square procedure using equally weighted data (16).

RESULTS AND DISCUSSION: Fig. 1 shows the dependence of phosphofructokinase activity on the concentration of Fru-6-P in the presence of AMP and Fru-2,6-P_2. The Fru-6-P velocity curves determined in the absence of the two activators exhibit nearly identical sigmoidality at low and at high concentrations of ATP. Both AMP and Fru-2,6-P_2 decrease the Michaelis constant (K) for Fru-6-P and increase the maximum velocity with respect to this substrate. Fru-2,6-P_2 decreases the Michaelis constant much stronger than AMP. In the presence of either AMP or Fru-2,6-P_2 similar maximum activities were obtained significantly higher than those measured in the absence of the activators. The attained maximum activity is not further increased when the two activators are added together (not shown). In contrast to earlier findings (9,11) under the conditions used in this study

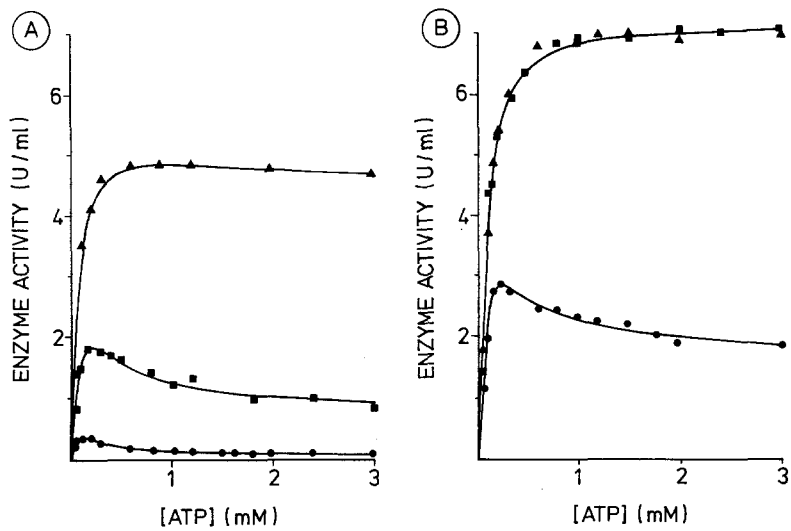

Fig. 2: Dependence of enzyme activity on the concentration of ATP in presence of AMP and fructose-2,6-bisphosphate
A : [Fru-6-P] = 0.3 mM; B : [Fru-6-P] = 3.0 mM
● no additions; ■ [AMP] = 1 mM; ▲ [Fru-2,6-P_2] = 20 µM
Kinetic parameters according to Equ. 2:

	A				B			
	V (U/ml)	K_R (mM)	K_T (mM)	L_o	V (U/ml)	K_R (mM)	K_T (mM)	L_o
●	6.79	0.26	0.54	4.76	7.94	0.24	0.43	0.39
■	6.20	0.27	0.51	0.52	8.17	0.30	0.48	0.02
▲	6.20	0.27	0.57	0.02	8.10	0.31	0.48	0.02

the enzyme is also strongly activated by AMP at non-inhibiting ATP concentrations.

The sigmoidality of the curves is not significantly changed by AMP and ATP, whereas Fru-2,6-P_2 causes a diminution of the Hill coefficient. However, also Fru-2,6-P_2 does not convert completely the sigmoidal Fru-6-P velocity curves into hyperbolic ones even not at high concentrations.

Fig. 2 represents the dependence of the enzyme activity on the concentration of ATP. At low concentrations of Fru-6-P the activation by Fru-2,6-P_2 exceeds the effect of AMP (Fig. 2 A). In the presence of AMP an inhibition of the enzyme by ATP remains detectable, but by Fru-2,6-P_2 the ATP inhibition is abolished. At saturating concentrations of Fru-6-P the ATP inhibition is fully relieved by the two effectors (Fig. 2 B). Remarkably, at high Fru-6-P no difference between the activities in the presence of either AMP or Fru-2,6-P_2 is observed. The

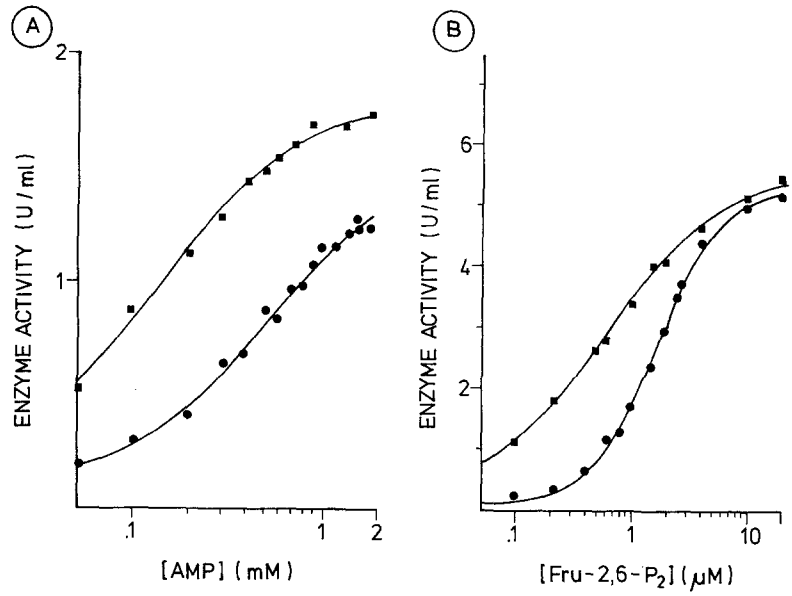

Fig. 3: Dependence of enzyme activity on the concentration of AMP (A) and fructose-2,6-bisphosphate (B)
[Fru-6-P]= 0.3 mM; ■ [ATP]= 0.3 mM; ● [ATP]= 3.0 mM
Kinetic parameters according to Equ. 3:

	A				B			
	V_o (U/ml)	V (U/ml)	K (mM)	n_H	V_o (U/ml)	V (U/ml)	K (μM)	n_H
■	0.18	1.81	0.14	1.1	0.18	5.62	0.60	1.0
●	0.11	1.55	0.52	1.2	0.11	2.37	1.65	1.5

data shown in Fig. 2 were described by Equ. 2 which is derived from the model of Monod et al. (14) by assuming that ATP is substrate and inhibitor. According to Equ. 2, AMP and Fru-2,6-P_2 influence the allosteric equilibrium (L_o) without changing the maximum activity (V). Yeast phosphofructokinase is two orders of magnitude more sensitive to Fru-2,6-P_2 than AMP (Fig. 3). The activation constants (K) for Fru-2,6-P_2 and AMP are in the ranges of 0.6 - 1.7 μM and 0.1 - 0.5 mM, respectively. By lowering ATP the enzyme becomes more sensitive toward AMP and Fru-2,6-P_2.
In previous investigations (8-10) no evidence for an AMP induced enhancement of the maximum activity with respect to Fru-6-P was obtained. Accordingly, the actions of AMP were interpreted in terms of a K-system and related to a relief of the ATP inhibition. The increase of the maximum activity and the strong activation at non-inhibiting ATP concentrations point to a more complex action of AMP on yeast phosphofructokinase (Figures 1 and 3).

The results of this contribution concerning the effects of Fru-2,6-P_2 differ from the data published in (5). These differences are apparently due to proteolytic modification of the commercial enzyme (12) used in (5). The results of Bartrons et al. (6) are qualitatively in accordance with our findings. A quantitative comparison, however, is not feasible because in (6) a non-cooperative response of the enzyme activity toward Fru-6-P was obtained even in the absence of Fru-2,6-P_2.

As shown in this study a similarity exists between the activating effects of AMP and Fru-2,6-P_2: both affect the Michaelis constant for Fru-6-P and the maximum activity toward this substrate. There is no simple way to find out whether the activating effects of AMP and Fru-2,6-P_2 involve an increase of the catalytic constant (V-system). A mechanistic interpretation of the data shown in Fig. 2 might suggest that AMP and Fru-2,6-P_2 influence mainly the allosteric equilibrium without increasing the catalytic constant; however, the model of Monod et al. (14) is not in accord with the observed invariance of the Hill coefficient with respect to AMP and ATP (Fig. 1)(9). For a deeper insight into the activation mechanism of AMP and Fru-2,6-P_2 the data have to be analyzed by a rate equation integrating the actions of the two substrates and the activators. This requires a thorough analysis of the kinetic cooperation of the two effectors on the enzyme.

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