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ATP INHIBITION OF *PHYCOMYCES* PYRUVATE KINASE: A KINETIC STUDY OF THE INHIBITORY EFFECTS ON THE ALLOSTERIC KINETICS SHOWN BY THE ENZYME

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Studies on ATP effects on the allosteric kinetics shown by pyruvate kinase from *Phycomyces blakesleeanus* NRRL 1555 (−) are reported. Phosphoenolpyruvate showed an allosteric ATP-dependent substrate inhibition. The results supported the existence of spatially distinct catalytic binding sites and the inhibitory binding sites for phosphoenolpyruvate, and ATP showed opposite heterotropic effects with respect to these two types of binding site. With respect to Mg^{2+} ions, ATP caused a negative heterotropic effect. The global inhibitory effect of ATP was in agreement with the predictions postulated by the two-state concerted-symmetry model of Monod, Wyman and Changeux.

KEY WORDS: ATP, allosteric inhibition, *Phycomyces*, pyruvate kinase.

INTRODUCTION

Pyruvate kinase (EC 2.7.1.40) is a key regulatory enzyme and the regulation of its activity is very important in the control of glycolysis and gluconeogenesis. From mammals four types of pyruvate kinase isoenzymes have been isolated: L-, M_1-, M_2- and R-type[1]. *Phycomyces blakesleeanus* pyruvate kinase exhibits L-type kinetic properties: it shows sigmoidal saturation kinetics with respect to substrates, phosphoenolpyruvate and Mg^{2+} ions, whereas with ADP, normal hyperbolic saturation kinetics are observed[2,3] in the absence of effectors at pH 7.5. Fructose 1,6-bisphosphate, L-alanine and H^+ ions are also allosteric effectors of the *Phycomyces* enzyme, and we have reported that most of the effects observed can be explained[4,5] by the concerted-symmetry model of allosteric control of Monod *et al*[6]. All pyruvate kinases of the "allosteric" class are allosterically inhibited by ATP[1,7,8]. For liver pyruvate kinase, a decrease in pH value causes a reduction in the inhibitory action of ATP[9]. However, low concentrations of ATP may activate pyruvate kinase, as has been described for the enzyme from yeast[9], *Carcinus maenas* hepatopancreas[10], and *E. coli* (type I enzyme). In addition, for yeast pyruvate kinase, the activation by low ATP concentrations was more pronounced at pH 6.0[9].

In earlier papers[2,3] we described studies of the allosteric inhibition by ATP of *Phycomyces* pyruvate kinase. We were interested by the study of the interactions between ATP and each of the reaction substrates, phosphoenolpyruvate, ADP and

† Correspondence

Mg^{2+} ions, and between ATP and fructose 1,6-bisphosphate. The elucidation of these interactions may be of interest for a more complete understanding of pyruvate kinase regulation. The existence of two types of binding sites for phosphoenolpyruvate, which we propose, may be of importance in the ATP inhibition, mainly at acidic pH values because *Phycomyces* grows under acidic pH conditions.

This paper reports the results obtained from an exhaustive study of the ATP effect on the allosteric interactions of *Phycomyces* pyruvate kinase.

MATERIALS AND METHODS

Biochemical reagents

Fructose 1,6-bisphosphate (tetracyclohexylammonium salt), NADH (disodium salt), ADP (disodium salt), phosphoenolpyruvate (monocyclohexylammonium salt), rabbit muscle lactic dehydrogenase type II (EC 1.1.1.27) and ATP (disodium salt) were purchased from the Sigma Chemical Co., St. Louis, Missouri, USA. Mes buffer (4-morpholineethanesulfonic acid) was provided by Boehringer Mannheim, Mannheim, F.R.G. All other chemicals used were standard analytical grade and provided by Merck, Darmstadt, F.R.G.

Phycomyces blakesleeanus wild-type strain NRRL 1555(−) was used. See De Arriaga *et al.*[12] for liquid minimal medium and general method of cultivation. The mycelia were obtained by filtration at 48 h of growth. Pyruvate kinase (EC 2.7.1.40) from the mycelium of *P. blakesleeanus* was purified according to Del Valle *et al.*[4].

Enzyme assay

Standard pyruvate kinase activity was determined according to the continuous lactate dehydrogenase-coupled assay method[13] for measurement of NADH oxidation. In a final volume of 1.0 ml the assay mixture contained 0.250 mM NADH, 0.5 mM ADP, 2.5 mM phosphoenolpyruvate, 7.5 mM of Mg^{2+} ions added as $MgCl_2$, 30 units of lactate dehydrogenase and 50 μl of the purified pyruvate kinase preparation suitably diluted in 50 mM sodium phosphate buffer, pH 7.5. Activity was measured at 30°C by the decrease in A_{340} associated with NADH oxidation in a Beckman model 35 spectrophotometer equipped with a recorder and temperature control unit. Under these conditions the molar absorption coefficient for NADH was $6.05 \times 10^3\,M^{-1}\,cm^{-1}$. One unit of enzyme activity is defined as the amount of enzyme which produced 1 μMol of pyruvate per minute under standard conditions. Protein was determined by the method of Lowry *et al.*[14] with bovine serum albumin as standard.

For kinetic analysis, the assay conditions were similar to those stated above, except that the concentrations of substrates or of effectors were varied as indicated in the description of individual experiments. The Mg^{2+}_{free} concentration was estimated in all cases by calculation, allowing for its chelation with ADP, ATP, phosphoenolpyruvate, and fructose 1,6-bisphosphate by using the dissociation constants given by Martell and Schwarzenbach[15], Wold and Ballou[16] and McGilvery[17], respectively. Other references to the concentration of substrates and effectors are in terms of their total chemical concentrations.

Analysis of kinetic data

When hyperbolic kinetics were obtained, the initial rate data were fitted to the Michaelis-Menten equation using Wilkinson's unweighted hyperbolic least-square method[18] after a graphic check to ascertain that the double-reciprocal plots of the reaction rate against substrate concentration were linear. When sigmoidal kinetics were obtained, the kinetic parameters, the hill coefficient (h) and the concentration of substrate (or effector) giving half-maximal velocity ($[S]_{0.5}$) were obtained by checking the Hill equation by means of the method of Silanova *et al.* as described by Kurganov[19] and/or by a Hill plot of the kinetic data using the Enzfitter computer program[20]. Allosteric substrate inhibition kinetics were analyzed according to Kurganov[21] from $\ln (V_{max} - v)/v$ vs ln [substrate inhibitor] plot, V_{max} being the generally accepted value of V_{max} for the ascending branch of the velocity curve, i.e. the maximum value of the velocity for the hyperbolic branch, and v the velocity at any specified inhibitory substrate concentration. The maximal slopes (h) of the above Hill plots were estimated by fitting the experimental inhibition data to straight lines by the least-squares method; in addition, the substrate inhibition constant values $[I]_{0.5}$, were determined as the abscissa intercept when the ordinate was zero.

In order to calculate the degree of sigmoidicity of plots of velocity against [ATP], the curves were analyzed from $\ln v_o - v_i/v_i$ vs ln [ATP] according to Kurganov[22], where v_o and v_i are the rates of the enzymatic reaction in the absence and presence of the inhibitor, respectively, and assuming that the limiting value of the reaction rate as $[ATP] \rightarrow \infty$ was zero. $[ATP]_{0.5}$ is the concentration of inhibitor at which $(v_o - v_i) = v_o/2$, i.e. the ATP concentration that gave half-maximal inhibition.

RESULTS AND DISCUSSION

The effect of ATP on the saturation curves with respect to each one of the substrates, ADP, phosphoenolpyruvate and Mg^{2+} ions was studied at pH 7.5. At 2.5 mM phosphoenolpyruvate and 7.5 mM Mg^{2+}_{free} ions, the presence of ATP does not alter the hyperbolic kinetics with respect to ADP as the variable substrate. A Dixon plot (Figure 1) illustrates the ATP inhibition pattern with respect to ADP. The secondary replot of slopes against 1/[ADP] gives a straight line through the origin, and a Cornish-Bowden plot[23] of [ADP]/v against [ATP] (plot not shown) gave parallel straight lines which are those expected for a simple linear competitive inhibition[24]. From these data we have calculated an apparent K_I value of 6.67 ± 0.65 mM for ATP.

Figure 2 shows the influence of ATP on positive homotropic Mg^{2+} ion interactions with *Phycomyces* pyruvate kinase. An increase in the ATP concentration produces a shift of the $[S]_{0.5}$ value for Mg^{2+}_{free} ions to higher values and a decrease in the positive cooperativity as indicated by the decrease in the Hill coefficient value (h), which tends to unity. The Hill coefficients run frum $h = 3.0$ in the absence of ATP to $h = 1.8$ at 8.mM ATP, whereas the $[S]_{0.5}$ value increases from 1.90 mM to 6.20 mM under the same conditions (Table I).

When phosphoenolpyruvate was the variable substrate the presence of ATP caused a great change in the positive homotropic interactions shown by phosphoenolpyruvate (Figure 3) as indicated by the increased Hill coefficient values in the presence of effectors. The Hill coefficient value approached 4 ($h = 3.6$) when ATP was 12.5 mM whereas the $[S]_{0.5}$ value also shifted to higher phosphoenolpyruvate

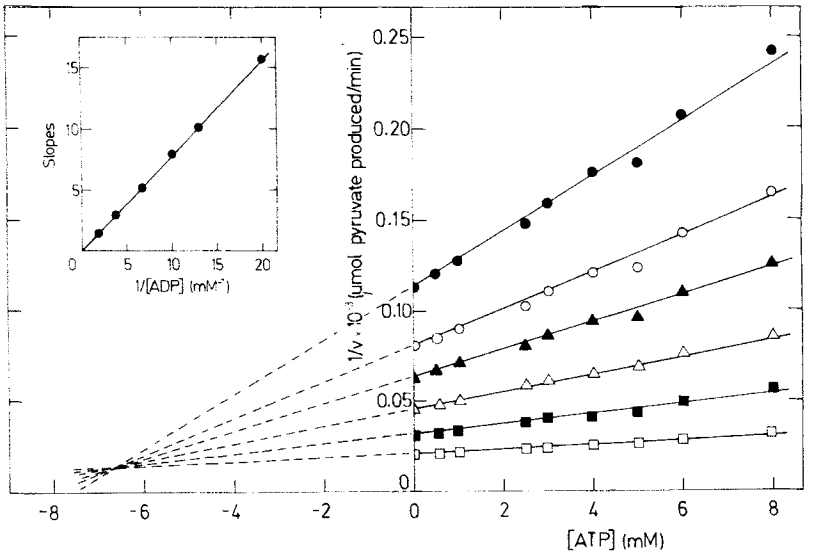

FIGURE 1 Inhibition by ATP of the forward reaction catalyzed by *P. blakesleeanus* pyruvate kinase with ADP as the variable substrate. The assays were performed at 30°C in 50 mM sodium phosphate buffer, pH 7.5, with constant concentrations of phosphoenolpyruvate and Mg^{2+}_{free} ions of 3.5 mM and 7.5 mM, respectively. The concentrations of ADP used were: ●, 0.05 mM; ○, 0.075 mM; ▲, 0.1 mM; △, 0.15 mM; ■, 0.25 mM and □, 0.5 mM. Inset: Secondary replot of the data.

concentrations. Table I summarizes the allosteric parameters with respect to phosphoenolpyruvate and Mg^{2+}_{free} ions at pH 7.5. In all cases V_{max} was not changed by this allosteric effector. These heterotropic interactions brought about by ATP on the homotropic interactions shown by phosphoenolpyruvate and Mg^{2+}_{free} ions are compatible with the effect produced by a negative heterotropic effector in an allosteric K-system according to the concerted-symmetry model proposed by Monod *et al.*[6]. The effects produced by ATP are in agreement with an exclusive binding of phosphoenolpyruvate to the high-affinity conformational state (R) of the *Phycomyces* pyruvate kinase and a non-exclusive binding of Mg^{2+}_{free} ions, as we have previously reported as judged by the effect of L-alanine, fructose 1,6-bisphosphate and H^+ ions on *Phycomyces* enzyme[4,5]. The two-state concerted-symmetry model of Monod *et al.*[6] appears to be in agreement with the allosteric behaviour followed by pyruvate kinase from rabbit muscle[25,26], type M enzyme from leucocytes[27] and yeast[28,29], whereas an extended model of Monod *et al.*[6], which permits the occurrence of one symmetrical hybrid state R_2T_2, explains the kinetic data obtained from *Saccharomyces carlsbergensis*[8].

On the other hand, from Figure 3, substrate inhibition by phosphoenolpyruvate appears to be dependent on ATP concentration. The phosphoenolpyruvate concentration at which substrate inhibition is detectable decreases as the ATP concentration increases. In addition, according to Kurganov[21], substrate inhibition by phosphoenolpyruvate is allosteric in nature. In the region of substrate inhibition we calculated Hill coefficient values higher than unity ($h = 3.15$ in the absence of ATP) indicating the degree of kinetic cooperativity with respect to phosphoenolpyruvate. As the ATP concentration is increased, the Hill coefficient for phosphoenolpyruvate

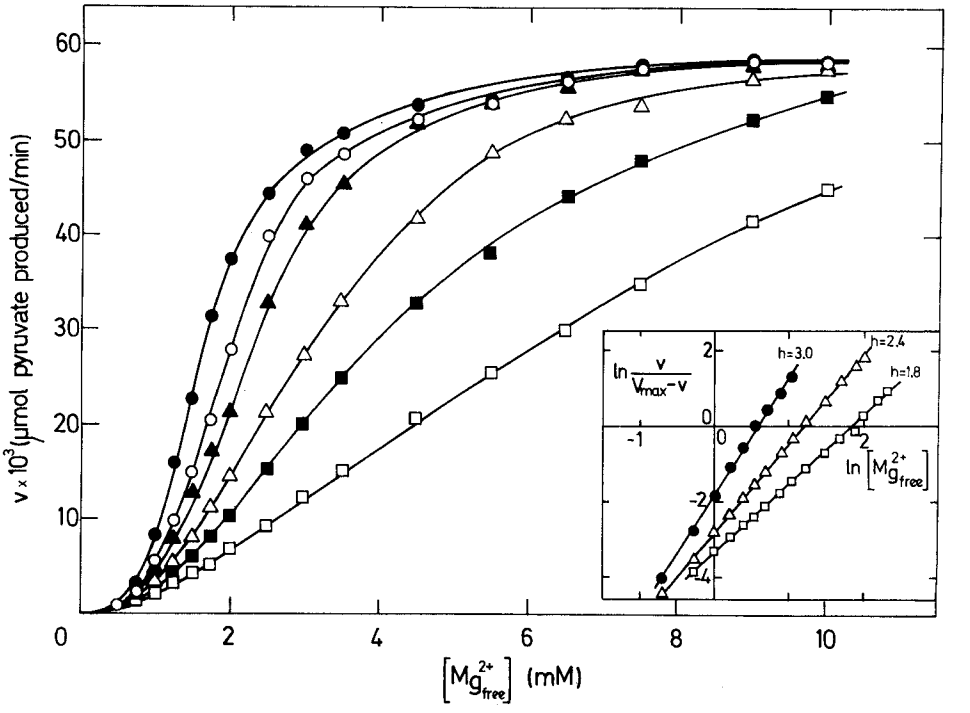

FIGURE 2 Effect of ATP on the positive homotropic interactions shown by *Phycomyces* pyruvate kinase with respect to Mg^{2+}_{free} ion concentration. The experiments were carried out at 30°C in 50 mM sodium phosphate buffer, pH 7.5, with constant concentrations of ADP and phosphoenolpyruvate of 0.5 mM and 2.5 mM, respectively. The ATP concentrations used were: ●, control; ○, 0.5 mM; ▲, 1 mM; △, 2.5 mM; ■, 5 mM; and □, 8 mM. Inset: Hill plots of the data corresponding to ATP concentrations of 0; 2.5 and 8 mM.

TABLE I

Effect of ATP on the allosteric parameters calculated from the phosphoenolpyruvate and Mg^{2+} ion saturation curves obtained with *Phycomyces* pyruvate kinase at pH 7.5. The $[S]_{0.5}$ and h values were calculated from the data shown in Figures 2 and 3 as described in Materials and Methods.

	$[S]_{0.5}$ (mM)	h
Phosphoenolpyruvate as substrate (0.1 mM – 3 mM concentration range)		
Control	1.20	2.60
+ 5 mM ATP	1.30	2.70
+ 8 mM ATP	1.50	2.80
+ 10 mM ATP	1.60	3.10
+ 12.5 mM ATP	2.00	3.60
Mg^{2+} ions as substrate (0.25 mM – 10 mM concentration range)		
Control	1.90	3.00
+ 0.5 mM ATP	2.20	2.95
+ 1 mM ATP	2.50	2.80
+ 2.5 mM ATP	3.30	2.40
+ 5 mM ATP	4.20	2.15
+ 8 mM ATP	6.20	1.80

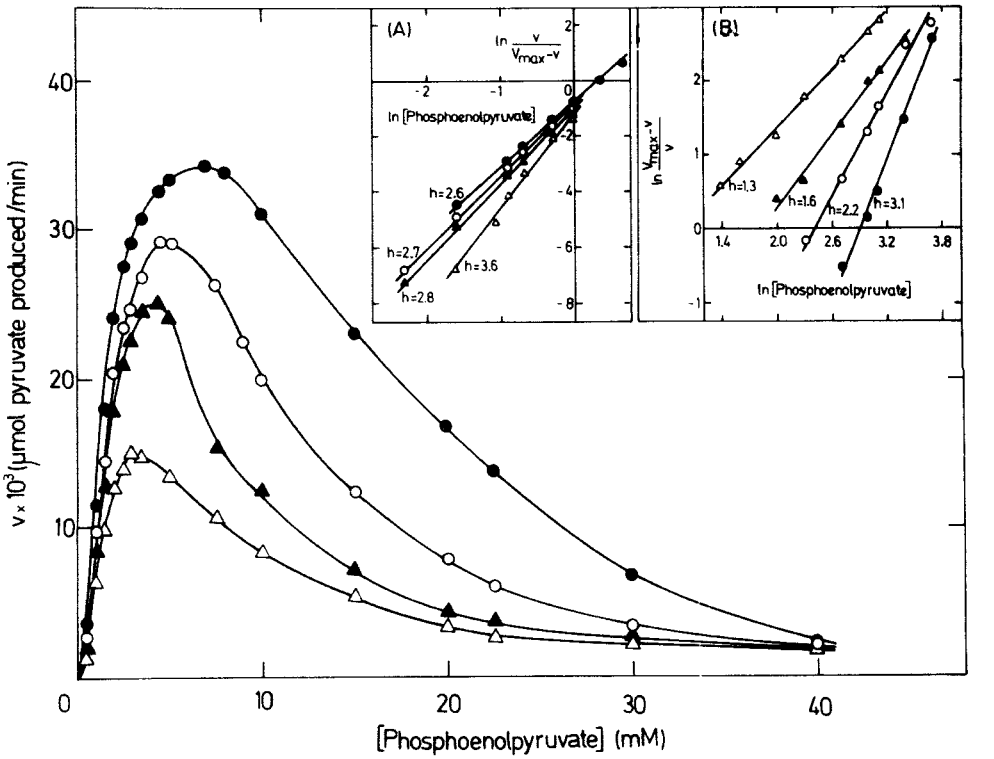

FIGURE 3 Effect of ATP on the positive homotropic interactions shown by *Phycomyces* pyruvate kinase with respect to phosphoenolpyruvate concentration. The experiments were performed at 30°C in 50 mM sodium phosphate buffer, pH 7.5, with ADP and Mg^{2+}_{free} ions concentrations held constant at 0.5 mM and 7.5 mM, respectively. The ATP concentrations used were: ●, control; ○, 5 mM; ▲, 8 mM and △, 12.5 mM. The lines at 6 mM ATP and 10 mM ATP have been omitted for clarity, but were included in the analysis. Inset: Hill plots of the data corresponding to: (A), the phosphoenolpyruvate concentration range at which substrate inhibition was not detectable and (B), the substrate inhibition range. The kinetic data were analyzed as described in Materials and Methods.

as an inhibitor decreased (Figure 3 inset (B)), while simultaneously substrate inhibition increased (Figure 3). The Hill coefficients fun from $h = 3.15$ in the absence of ATP to $h = 1.3$ at 12.5 mM ATP, whereas the $[I]_{0.5}$ value decreases from 18.45 mM to 2.60 mM under the same conditions (Table II), indicating a possible heterotropic

TABLE II

Effect of ATP on the interactions shown by phosphoenolpyruvate as an inhibitor. The h, and $[I]_{0.5}$ values were calculated from the data shown in Figure 3 as described in Materials and Methods.

	$[I]_{0.5}$(mM)	h
Phosphoenolpyruvate as substrate inhibitor		
Control	18.45	3.15
+5 mM ATP	11.4	2.25
+6 mM ATP	8.25	1.80
+8 mM ATP	6.05	1.60
+12 mM ATP	2.60	1.30

TABLE III

Effect of the pH and ATP on the kinetic cooperativity shown by phosphoenolpyruvate as a substrate inhibitor. The experiments were carried out at 30°C at fixed concentrations of ADP (0.5 mM) and Mg^{2+}_{free} ions (7.5 mM) in MES and phosphate buffers at the pH values shown above. The data were analyzed as described in Materials and Methods.

Buffer	h	$[I]_{0.5}$(mM)	h	$[I]_{0.5}$(mM)
	[ATP] : 0 mM		[ATP] : 5 mM	
MES pH 6.0	3.10	8.75	2.10	3.20
MES pH 7.5	3.10	19.80	2.25	14.15
phosphate pH 6.0	2.80	1.90	–	–
phosphate pH 7.5	3.15	18.50	2.25	11.40

effect of ATP on the kinetic cooperativity with respect to phosphoenolpyruvate as an inhibitor. According to Kurganov[21] we considered the "allosteric substrate inhibition" as the result of the occurrence of spatially distinct effector sites, at which binding of the phosphoenolpyruvate results in a reduction in the catalytic efficiency of the active sites. The heterotropic effect shown by ATP was distinct with respect to phosphoenolpyruvate as substrate (negative heterotropic effector) and to phosphoenolpyruvate as inhibitor (apparent positive heterotropic effector), and it thus appears reasonable to suppose the existence of two types of binding sites for phosphoenolpyruvate. On the other hand, hydrogen ions increase the affinity of the phosphoenolpyruvate with respect to inhibitory binding sites, indicating a positive heterotropic effect of protons on the kinetic cooperativity with respect to substrate inhibition. The results are summarized in Table III. The set of experiments at two pH values, pH 6.0 and 7.5, were carried out in a 50 mM MES buffer, because phosphate buffer favours phosphoenolpyruvate substrate inhibition at acidic pH values. The kinetic behaviour of *Phycomyces* pyruvate kinase was the same in the non-inhibitory phosphoenolpyruvate concentration range in both buffers. As can be seen in Table III, in the presence of ATP, the kinetic cooperativity with respect to phosphoenolpyruvate inhibitory binding sites was weakened (the Hill coefficient value and the $[I]_{0.5}$ value decreased). It was apparent that the positive heterotropic effect shown by ATP is higher at acidic pH values. The $[I]_{0.5}$ value at pH 6.0 is 4-fold lower than at pH 7.5.

We have previously postulated two types of binding sites for phosphoenolpyruvate according to the results obtained from the study of the pH effect on *Phycomyces* pyruvate kinase[5], namely inhibitory which are spatially different and catalytic binding sites, and the results obtained here with ATP appear to support this hypothesis. The existence of two types of binding sites for phosphoenolpyruvate has been also suggested for rabbit muscle enzyme[30,31] *C. maenas* muscle enzyme[32] and *C. maenas* hepatopancrease enzyme[33].

The antagonism between phosphoenolpyruvate and ATP was also evident. We have tested the shapes of the inhibitory plots of the enzymatic reaction rate against ATP concentrations in the presence of different fixed phosphoenolpyruvate concentrations. Phosphoenolpyruvate, in the concentration range at which substrate inhibition was not appreciable, increases the ATP concentration that gives half-maximal inhibition ($[ATP]_{0.5}$) and the positive cooperativity towards ATP (Table IV). However, when phosphoenolpyruvate concentration rises within the substrate inhibition concentration range, there is a decrease in the positive cooperativity towards ATP (the Hill coefficient value shifts to lower values) with a simultaneous decrease in

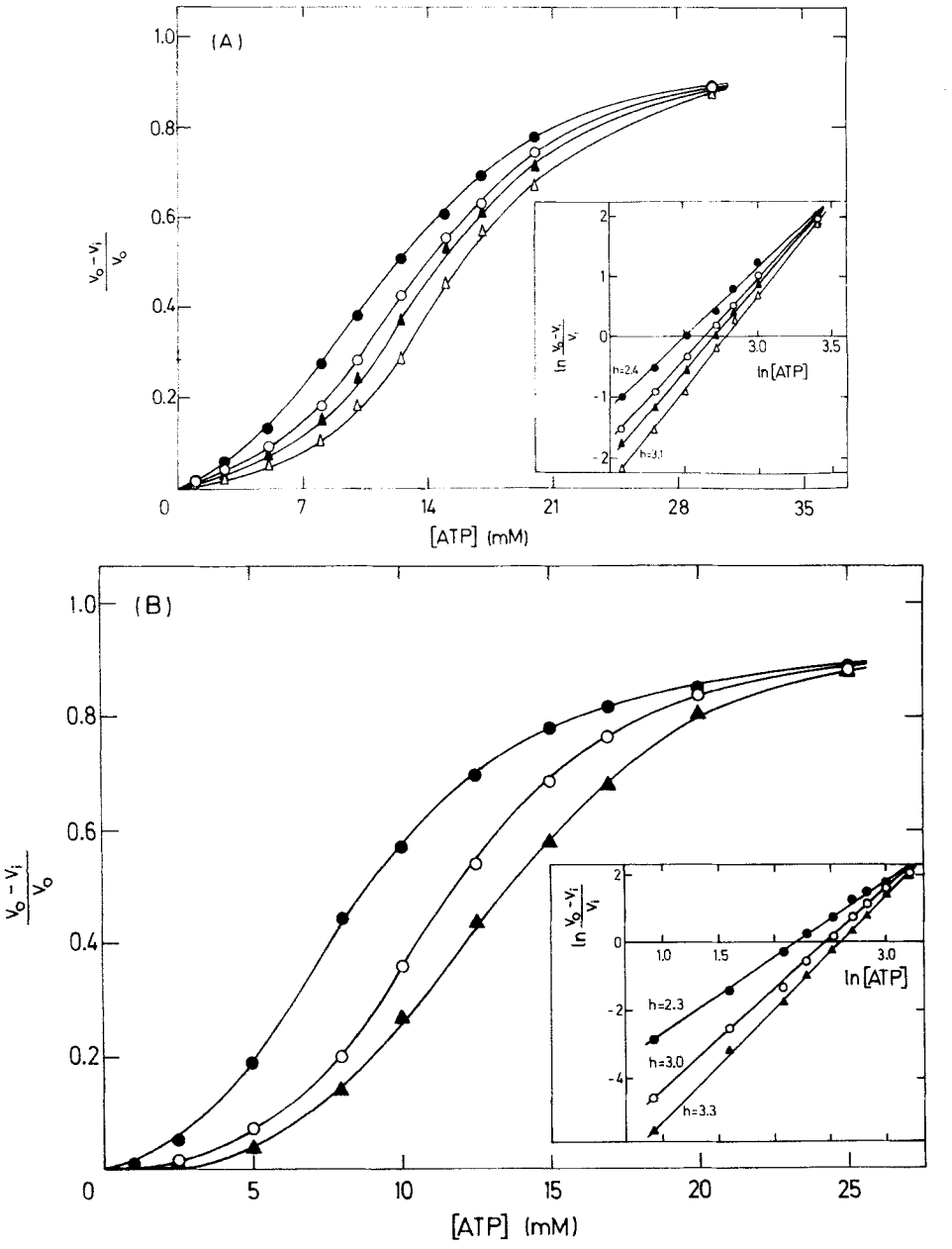

FIGURE 4 Effect of fructose 1,6-bisphosphate on the inhibition of pyruvate kinase from *P. blakesleeanus* by ATP. The experiments were performed in a 50 mM sodium phosphate buffer, pH 7.5, at 30°C, fixed concentrations of ADP (0.5 mM) and Mg^{2+}_{free} ions (7.5 mM). A) At fixed 2.5 mM phosphoenolpyruvate the fructose 1,6-bisphosphate concentrations used were: ●, 0; ○, 10 μM; ▲, 100 μM and △, 500 μM. B) At fixed 8 mM phosphoenolpyruvate, the fructose 1,6-bisphosphate concentrations used were: ●, 0; ○, 1 mM and ▲, 5 mM. v_i is the rate of the enzymatic reaction in the presence of ATP, and v_o is the rate in the absence of ATP but in the presence of fructose 1,6-bisphosphate at the corresponding concentrations. Inset: Hill plots of the data analysed as described in Materials and Methods.

TABLE IV

Effect of phosphoenolpyruvate on the dependence of the reaction rate catalysed by *Phycomyces* pyruvate kinase with respect to ATP concentrations. The ATP saturation curves were obtained in a 50 mM sodium phosphate buffer 7.5 at 30°C at fixed concentrations of ADP (0.5 mM) and Mg^{2+}_{free} ions (7.5 mM) in the presence of the different fixed phosphoenolpyruvate concentrations indicated below. The data were analyzed as described in Materials and Methods.

Variable [ATP] (0–30 mM)

[Phosphoenolpyruvate]	*h*	$[ATP_{0.5}$(mM)
0.5	2.0	9.2
1	3.05	18.5
2	2.65	15.0
2.5	2.40	13.0
8	2.30	11.0
10	2.15	10.0
15	1.93	7.5

the $[ATP]_{0.5}$ value (Table IV) as expected. On the other hand, we have not detected that low concentrations of ATP activate *Phycomyces* pyruvate kinase which is not the case for yeast enzyme[28] or *C. Maenas* hepatopancrease enzyme[10].

Like phosphoenolpyruvate, fructrose 1,6-bisphosphate also weakened the inhibitory action of ATP (Figure 4). At 2.5 mM phosphoenolpyruvate, the positive cooperativity of ATP was increased as indicated by the shift in the Hill coefficient from 2.4 (in the absence of the effector) to 3.1 (in the presence of 0.5 M effector), while the $[ATP]_{0.5}$ value also shifted towards higher values (Table V). However, at a high phosphoenolpyruvate concentration (8 mM), the activator was less effective in counteracting the inhibition by ATP, high concentrations of this being necessary for an appreciable effect (Table V). The results show that as the concentration of ATP is increased, the inhibitory effect on the reaction rate tends to the same value, irrespective of the presence of phosphoenolpyruvate or fructose 1,6-bisphosphate.

The results reported in this paper appear to indicate that the negative heterotropic effects shown by ATP on *Phycomyces* pyruvate kinase can be interpreted on the basis of the two-state concerted-symmetry model of Monod *et al*[6]. In addition, the existence of the inhibitory binding sites and catalytic binding sites for phosphoenolpyruvate

TABLE V

Effect of fructose 1,6-bisphosphate on the dependence of the reaction rate catalyzed by *Phycomyces* pyruvate kinase with respect to ATP concentrations. The ATP saturation curves were obtained in a 50 mM sodium phosphate buffer, pH 7.5, at 30°C at fixed concentrations of ADP (0.5 mM) and Mg^{2+}_{free} ions (7.5 mM) in the presence of the different fixed phosphoenolpyruvate and fructose 1,6-bisphosphate concentrations indicated below. The data were analyzed as described in Materials and Methods.

Variable [ATP] (0–30 mM)

[Phosphoenolpyruvate] : 2.5 mM			[Phosphoenolpyruvate] : 8 mM		
[Fructose 1,6-bisphosphate] (mM)	*h*	$[ATP]_{0.5}$(mM)	[Fructose 1,6-bisphosphate] (mM)	*h*	$[ATP]_{0.5}$(mM)
0	2.40	13.0	0	2.3	11.0
0.01	2.60	14.0	0.5	2.5	11.8
0.10	2.80	15.0	1	3.0	12.0
0.50	3.10	16.3	5	3.3	13.5

which we propose strengthened the global negative heterotropic effect of ATP with respect to this substrate, since the affinity of phosphoenolpyruvate by catalytic binding sites decreases, while its affinity by inhibitory binding sites increases.

References

1. Hall, E.R. and Cottam, G.L. (1978) *Int. J. Biochem.*, **9,** 785.
2. Busto, F., Del Valle, P., De Arriaga, D. and Soler, J.(1985) *Int. J. Biochem.*, **17,** 253
3. Busto, F., Del Valle, P. and Soler, (1988) *J. Biochem. Cell. Biol.*, **66,** 148
4. Del Valle, P., De Arriaga, D., Busto, F. and Soler, J. (1986) *Biochim. Biophys. Acta,* **874,** 193
5. De Arriaga, D., Busto, F., Del Valle, P. and Soler, J. (1989) *Biochim. Biophys. Acta,* (in press)
6. Monod, J., Wyman, J. and Changeux, J.P. (1965) *J. Mol. Biol.*, **12,** 88
7. Wieker, H.-J. and Hess, B. (1971)*Biochemistry,* **10,** 1243
8. Johannes, K.-J. and Hess, B. (1973) *J. Mol. Biol.* **76,** 181
9. Seubert, W. and Schoner, W. (1971) *Curr. Top. Cell. Regul.*, **3,** 237
10. Giles, I.G., Poat, P.C. and Munday, K.A. (1977) *Biochem. J.*, **165,** 97
11. Markus, M., Plesser, T., Boiteux, A., Hess, B. and Malcovati, M. (1980) *Biochem. J.*, **189,** 421
12. De Arriaga, D., Teixidó, F., Busto, F. and Soler, J. (1984) *Biochim. Biophys. Acta,* **784,** 158
13. Bücher, T. and Pleiderer, G. (1955) *Meth. Enzymol.*, **1,** 435
14. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) *J. Biol. Chem.*, **193,** 265
15. Martell, A.E. and Schwarzenbach, S. (1965) *Helv. Chim. Acta,* **39,** 653
16. Wold, F. and Ballou, C.E. (1957) *J. Biol. Chem.*, **227,** 301
17. McGilvery, R.W. (1965) *Biochemistry,* **4,** 1924
18. Wilkinson, G.N. (1961) *Biochem. J.*, **80,** 324
19. Kurganov, B.I. (1982) *Allosteric Enzymes. Kinetic Behaviour* (Yakovlev, V.A., ed.), pp. 32–37, New York, Wiley-Interscience.
20. Leatherbarrow, R.J. (1987) *Enzfitter Manual*, Amsterdam, Elsevier Science Publishers.
21. Kurganov, B.I. (1982) in *Allosteric Enzymes. Kinetic Behaviour* (Yakovlev, V.A., ed.), pp. 49–54, New York, Wiley-Interscience.
22. Kurganov, B.I. (1982) in *Allosteric Enzymes. Kinetic Behaviour* (Yakovlev, V.A., ed.), pp. 56–60, New York, Wiley-Interscience.
23. Cornish-Bowden, A. (1974) *Biochem. J.*, **137,** 143
24. Segel, I.H. (1975) in *Enzyme Kinetics* (Segel, I.H., ed.), Chap. 3, pp. 100–125, New York, Wiley-Interscience Publication.
25. Oberfelder, R.W., Lee, L.L.-Y. and Lee, J.C. (1984) *Biochemistry,* **23,** 3813
26. Oberfelder, R.W., George Barisas, B. and Lee, J.C. (1984) *Biochemistry,* **23,** 3822
27. Van Berkel, Th. J.C. and Koster, J.F. (1973) *Biochim. Biophys. Acta,* **293,** 134
28. Haeckel, R., Hess, B., Lauterborn, W. and Wüster, K.H. (1968) *Hoppe-Seyler's Z. Physiol. Chem.*, **349,** 699
29. Hunsley, J. and Suelter, C.H. (1969) *J. Biol. Chem.*, **244,** 4819
30. Reynard, A.M., Hass, L.F., Jacobsen, D.D. and Boyer, P.D. (1961) *J. Biol. Chem.*, **236,** 2277
31. Mildvan, A.S. and Cohn, M. (1966) *J. Biol. Chem.*, **241,** 1178
32. Newton, C.J., Poat, P.C. and Munday, K.A. (1976) *Biochem. Soc. Trans.*, **4,** 1158
33. Giles, I.G. and Poat, P.C. (1980) *Biochem. J.*, **185,** 289