The Phosphofructokinase of *Dictyostelium discoideum**

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ABSTRACT: Phosphofructokinase from the slime mold *Dictyostelium discoideum* was purified 50-fold by a combination of $(NH_4)_2SO_4$ fractionation and by selective adsorption and elution using Cγ gel. The enzyme had a pH optimum of about 7.5. Unlike the analogous enzyme from other organisms, the slime mold phosphofructokinase had simple kinetic properties. The reaction rates for fructose 6-phosphate and adenosine triphosphate followed Michaelis–Menten kinetics and there was no evidence for sigmoidicity. Neither adenosine triphosphate nor citrate inhibited the reaction rate, nor was there activation or change in kinetics on addition of adenosine monophosphate, adenosine 3′,5′-cyclic monophosphate, inorganic phosphate, or fructose 6-phosphate. Adenosine diphosphate, pyrophosphate, and fructose 1,6-diphosphate inhibited the reaction rate. Inhibition by the first two compounds was competitively overcome by adenosine triphosphate; inhibition by the latter compound was competitively overcome by fructose 6-phosphate. Inhibition by adenosine diphosphate and inorganic pyrophosphate was not modified by adenosine monophosphate, 3′,5′-adenosine monophosphate, inorganic phosphate, or citrate. The phosphofructokinase reaction was completely dependent upon the presence of Mg^{2+} and NH_4^+, but a higher concentration of the former ion was required for an equivalent increase in the reaction rate.

The regulatory pattern of the slime mold phosphofructokinase suggests an unusual physiological function. Evidence is discussed suggesting that this enzyme does not play a central role in energy-yielding metabolism of the slime mold.

Phosphofructokinase (ATP[1]–D-fructose 6-phosphate 1-phosphotransferase, EC 2.7.1.11) is an enzyme which has been extensively studied by many workers (see reviews by Atkinson, 1966; Stadtman, 1966; Wood, 1966). Its kinetics are very complex and the reaction rate can be modified by many compounds. In general ATP and citrate act as inhibitors while F-6-P, FDP, AMP, 3′,5′-AMP, ADP, P_i, and NH_4^+ act as activators. Depending on the source of the enzyme, the reaction rate may be altered by all or only some of these compounds. The regulatory properties of this enzyme are consistent with its important role in energy-yielding metabolism. Evidence obtained from *in vivo* and *in vitro* studies indicates that PFKase is a key enzyme in regulating the balance between glycolysis and gluconeogenesis and is also responsible for the Pasteur effect.

The slime mold *Dictyostelium discoideum* multiplies as a free living amoeba. Upon exhaustion of the food supply the amoebae aggregate and differentiate to form a multicellular fruiting body containing spore cells. During differentiation there is a decrease in soluble glycogen, protein, and RNA, and a rapid synthesis of cell wall material (a cellulose–glycogen complex), mucopolysaccharide, and trehalose (for reviews, see Wright, 1963, 1968). In view of these changes it appeared desirable to study specific reactions known to be involved in the control of glycolysis and gluconeogenesis. PFKase was selected as the initial enzyme for investigation; its presence in *D. discoideum* has been reported by Cleland and Coe (1968). These authors have shown that there is only a slight change in activity in extracts obtained at the amoeba and the preculmination stages.

Materials and Methods

Materials. Glucose, F-6-P (barium salt), FDP, nucleotides (acid form), dithiothreitol, PEP, citric acid, isocitrate, α-KG (acid form), and the enzymes used in the assays were obtained from Calbiochem. Streptomycin sulfate was obtained from Parke Davis and Co. All other chemicals were obtained from Fisher Scientific Co. The barium salt of F-6-P was converted into the sodium salt by addition of Na_2CO_3 and removal of $BaCO_3$ by centrifugation. Unless otherwise stated sodium salts of acids were used.

Assays. All assays were performed at 25° and completed within 7 min, using a Beckman DU spectrophotometer with a Gilford recorder. The reaction mixture consisted of 5 mM $MgCl_2$, 2 mM NH_4Cl, 1 mM dithiothreitol, 0.2 mM NADH, 0.5 mM F-6-P, 0.2 mM ATP, and either 50 mM imidazole-HCl buffer (pH 7.0) or 50 mM Tris-HCl buffer (pH 8.0). PFKase was

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[1] Abbreviations used that are not listed in *Biochemistry 5*, 1445 (1966), are: PFKase, phosphofructokinase; PEP, phosphoenolpyruvate; α-KG, α-ketoglutarate.

TABLE I: Enzyme Purification.[a]

Fraction	Vol (ml)	Protein (mg/ml)	Sp Act.	Recovy (%)	Purifcn Factor
A	50	41	0.085	100	1
B	48.5	37	0.093	96	1.1
$(NH_4)_2SO_4$	63	5	0.44	80	5.2
Cγ	1.5	5.4	4.4	20	51.7

[a] All assays performed at pH 8.0 as described in Methods.

measured by two methods, both dependent upon observation of the rate of NADH oxidation followed at 340 nm. In the first FDP formation was measured by coupling with excess adlolase, triose phosphate isomerase, and α-glycerophosphate dehydrogenase (Racker, 1947). In the second method ADP formation was measured in the presence of 0.2 mM PEP by coupling with excess pyruvate kinase and lactic dehydrogenase (Kornberg and Pricer, 1951). $(NH_4)_2SO_4$ was removed from the assay enzymes by dialysis against 0.05 M Tris-HCl buffer (pH 7.5). When pyruvate kinase was dialyzed the buffer also contained 10 mM KCl. Unless otherwise stated the assay measuring FDP formation was used throughout these experiments. A unit of enzyme was defined as the amount catalyzing the formation of 1 μmole of FDP/min, at pH 8.0 and 25°. In experiments designed to test the stoichiometry of the PFKase reaction, F-6-P was measured by coupling with phosphohexoisomerase and glucose 6-phosphate dehydrogenase. ATP was determined in the presence of glucose with hexokinase and glucose 6-phosphate dehydrogenase. In both cases NADP reduction was measured at 340 nm. Protein was assayed by the method of Lowry *et al.* (1951).

Enzyme Preparation. Cells of *D. discoideum* were grown as previously described (Ward and Wright, 1965). With a few noted exceptions, the cells were harvested at the late stage of aggregation in 0.05 M Tris-HCl buffer (pH 8.0), washed twice in the same buffer, collected by centrifugation, and stored at −15°. All operations involved in enzyme purification were performed at 4°. Within 1-week harvesting the frozen cells were thawed, suspended in the same buffer, and centrifuged at 30,000*g* for 30 min. Table I summarizes the enzyme purification. The supernatant solution was diluted with buffer until the protein concentration was about 40 mg/ml (fraction A). Nucleic acids were removed by addition of 0.4 ml of Streptomycin sulfate (0.4 g/ml) per 10 ml of crude extract, over a period of 5 min, with stirring. After agitating for 30 min the precipitate was collected by centrifugation at 20,000*g* for 20 min (fraction B). All subsequent centrifugations were performed at 20,000*g* for 15 min. To the supernatant, an equal amount of saturated $(NH_4)_2SO_4$ was added over a period of 5 min (FDP phosphatase is

TABLE II: Compounds Which Do Not Modify the Activity of PFKase in Fresh Extracts.[a]

Concentration (mM) of Fixed Substrate(s)	Concentration Range (mM) of Tested Compounds
F-6-P (0.1)	ATP (0.1–10)[b]
	GTP (0.1–4)
	ITP (0.1–4)
F-6-P (4)	UTP (0.1–4)
	CTP (0.1–4)
F-6-P (0.1)	
ATP (0.1)	F-6-P (0.1–4)[c]
	AMP (0.1–4)
F-6-P (0.1)	3′,5′-AMP (0.1–4)
ATP (10)	P_i (0.1–4)
	Citrate (0.1–4)
F-6-P (4)	Isocitrate (0.1–4)
ATP (0.1)	

[a] Fresh extracts prepared and assayed at pH 7.0 as described in Methods. [b] All nucleoside triphosphates were tested at saturating levels. [c] Lower concentrations were nonsaturating; increase in activity at higher levels is as expected from Michaelis–Menten kinetics.

TABLE III: Stoichiometry of the PFKase Reaction.[a]

Reaction Component	Initial Concn (μmoles/ml)	Final Concn (μmoles/ml)	Net Changes (μmoles)
F-6-P	1.13[b]	0.26	−0.87
	(1.21)[c]	(0.48)	(−0.73)
ATP	1.07	0.27	−0.80
	(1.05)	(0.34)	(−0.71)
FDP	0.00	0.82	+0.82
	(0.00)	(0.75)	(+0.75)
ADP	0.00	0.72	+0.72
	(0.00)	(0.68)	(+0.68)

[a] The reaction was carried out in a 0.05 M imidazole buffer (pH 7.0) with 5 mM $MgCl_2$, terminated after 10 min, and the reaction components were assayed in the same buffer as described in Methods. [b] $(NH_4)_2SO_4$ fraction. [c] Cγ fraction (in parentheses).

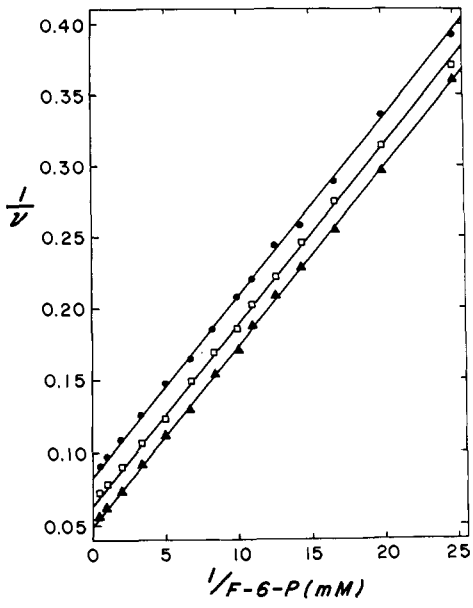

FIGURE 1: Effect of ATP on kinetics of F-6-P utilization. (●) 0.025 mM ATP, (○) 0.05 mM ATP, (▲) 0.2 mM ATP, v = mμmoles/min. Assay performed using the C_γ fraction at pH 7.0, as described in Methods, in the presence of 3 mM Na_2HPO_4–KH_2PO_4.

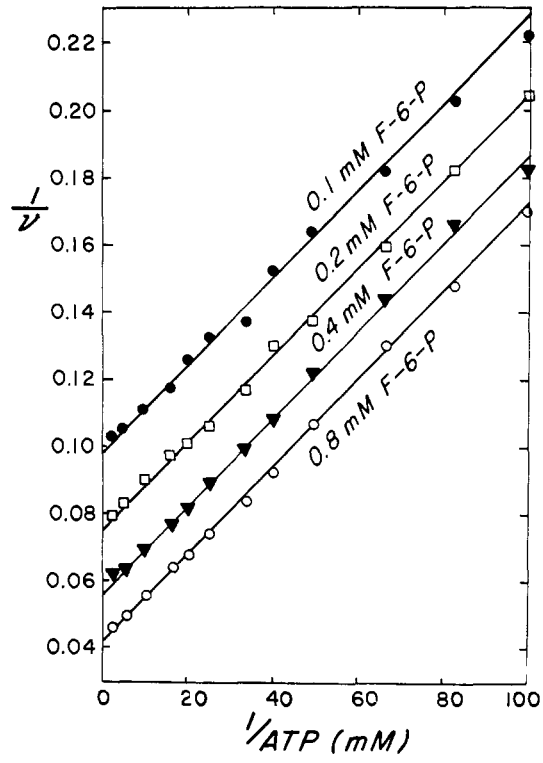

FIGURE 2: Effect of F-6-P on kinetics of ATP utilization. v = mμmoles/min. Assay performed with the C_γ fraction at pH 7.0, as described in Methods, in the presence of 4 mM Na_2HPO_4–KH_2PO_4.

precipitated above 50% saturation). Agitation was continued for an additional 15 min and the suspension was centrifuged. The pellet was suspended in 0.05 M Na_2HPO_4–KH_2PO_4 buffer (pH 7.0) to a concentration of 5 mg of protein/ml [$(NH_4)_2SO_4$ fraction]. Alumina C_γ gel (Colowick, 1955) was slowly added to give a final gel/protein ratio of 0.1 (w/w). After agitating for 15 min the mixture was centrifuged and the pellet was discarded. Additional C_γ gel was slowly added until a cumulative gel/protein ratio of 1.0 was obtained. The mixture was stirred for 15 min, centrifuged, washed once in distilled water, and suspended in 0.1 M Na_2HPO_4–KH_2PO_4 (pH 7.0). After agitating for 20 min the suspension was centrifuged and the supernatant fraction was used in experiments (C_γ fraction). Routinely an over-all purification of 45–55-fold was readily obtained. At 4° the C_γ fraction lost 55% of its activity at pH 8.0 and 85% of its activity at pH 7.0, after 72 hr, in 0.05 M Na_2HPO_4–KH_2PO_4 buffer. When the $(NH_4)_2SO_4$-precipitated fraction was directly used in experiments, the pellet was resuspended in 0.05 M Tris-HCl buffer (pH 8.0). This enzyme preparation lost only about 10% of its activity after 72 hr at 4°.

Results

Experiments with Freshly Prepared Extracts. In order to minimize any possible changes of the enzyme due to aging or freezing and thawing, the cells were also harvested in buffer containing 5 mM $MgCl_2$, 1 mM F-6-P, 1 mM ATP, and 1 mM dithiothreitol. After passage through a French pressure cell (30,000 psi) the suspension was centrifuged at 30,000g for 30 min and the supernatant fraction was used immediately. Repeated attempts were made to demonstrate activation of the enzyme (Mansour, 1963; Stone and Mansour, 1967) by preincubation of the freshly prepared extract with an equal volume of a mixture consisting of 50 mM imidazole buffer (pH 7.0), 20 mM $MgCl_2$, 4 mM ATP, 4 mM ADP, 4 mM AMP, and 10 mM K_2HPO_4. At 5-min intervals the activity was assayed at pH 7.0 with the following substrate concentrations: 0.1 mM F-6-P, 0.1 mM ATP; 0.1 mM F-6-P, 10 mM ATP; and 5 mM F-6-P, 0.1 mM ATP. Similar preincubation experiments were performed with freshly prepared crude extracts containing 5 mM $MgCl_2$ and 1 mM dithiothreitol as well as either 1 mM ATP, 1 mM ADP, or 1 mM F-6-P. The final dilution of the preincubation mixture in the assays was greater than 1:100. All attempts to increase enzyme

TABLE IV: Kinetic Constants for F-6-P and ATP at Different Concentrations of the Corresponding Substrate.[a]

K_m for F-6-P (M)	Concn of ATP (M)	K_m for ATP (M)	Concn of F-6-P (M)
1.5×10^{-4}	0.25×10^{-4}	1.3×10^{-5}	1.0×10^{-4}
1.9×10^{-4}	0.5×10^{-4}	1.7×10^{-5}	2.0×10^{-4}
2.3×10^{-4}	1.0×10^{-4}	2.3×10^{-5}	4.0×10^{-4}
2.5×10^{-4}	2.0×10^{-4}	3.1×10^{-5}	8.0×10^{-4}

[a] Data obtained from Figures 1 and 2.

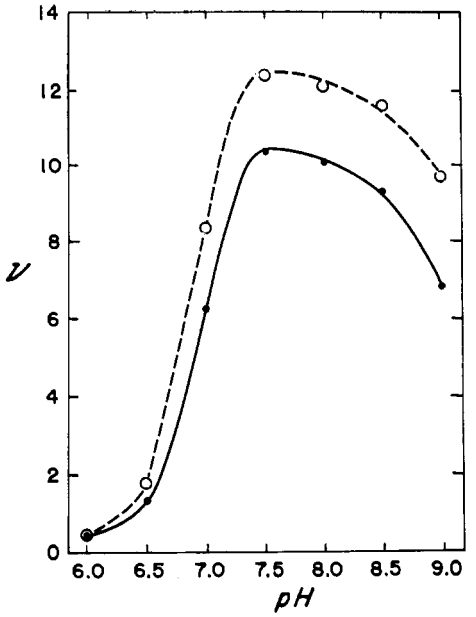

FIGURE 3: Effect of pH on the reaction rate. Solid line: C_γ fraction, broken line: $(NH_4)_2SO_4$ fraction, v = mμmoles/min. Assay mixture consisted of 50 mM NaH_2PO_4, and 50 mM Tris adjusted to the appropriate pH with HCl, 20 mM KCl, 5 mM $MgCl_2$, 1 mM dithiothreitol, 0.4 mM F-6-P, 0.2 mM ATP, 0.2 mM NADH, and the assay enzymes.

activity and/or modify its response to F-6-P and ATP were unsuccessful. During the incubation period there was identical loss of enzyme activity when assayed with 0.1 or 10 mM ATP. The same fraction of activity was also lost when the assay was performed with 0.1 or 5 mM F-6-P. In these experiments saturating levels of ATP were used. This was not the case for F-6-P. The

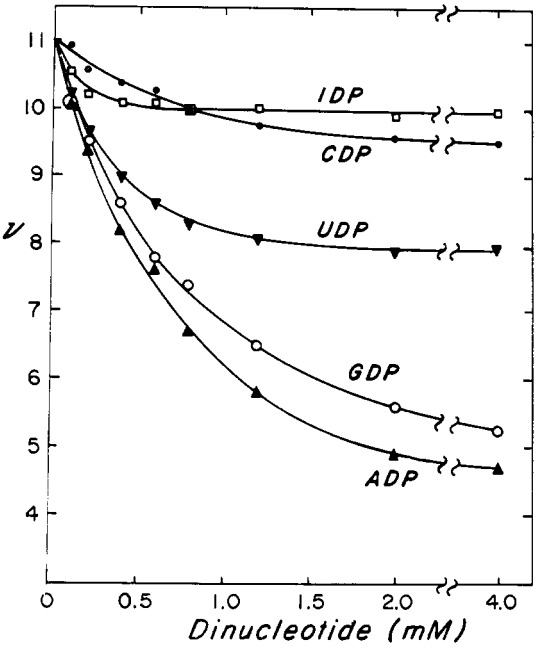

FIGURE 4: Inhibition of the reaction rate by various dinucleotides. v = mμmoles/min. Assay performed at pH 7.0, using the $(NH_4)_2SO_4$ fraction with 0.1 mM F-6-P and 0.1 mM ATP, as described in Methods.

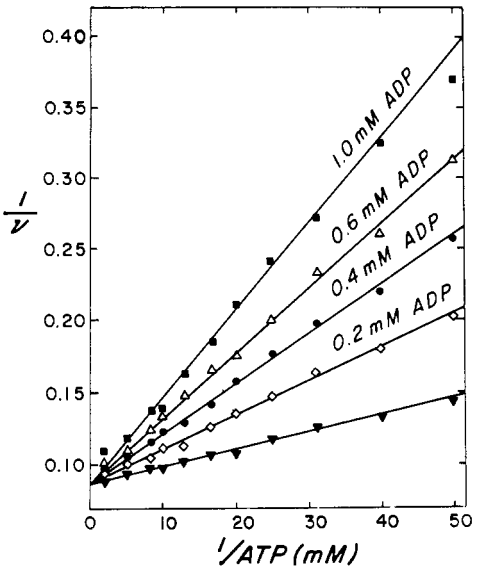

FIGURE 5: Effect of ADP on kinetics of ATP utilization. (▼) No added ADP, v = mμmoles/min. Assayed using the $(NH_4)_2SO_4$ fraction, with 0.1 mM F-6-P, as described in Methods.

higher activity with 5 mM F-6-P as compared with 0.1 mM F-6-P is the same as expected from Michaelis–Menten kinetics (see below).

Various compounds, some known to modify the activity of PFKase, were tested (Table II). With the exception of F-6-P, none of these compounds affected the rate of the reaction at the concentrations tested. The standard deviation between determinations was 5%. The results were the same with extracts prepared from late aggregation or culmination stages and harvested with or without 5 mM $MgCl_2$, 1 mM F-6-P, 1 mM ATP, and 1 mM dithiothreitol.

Stoichiometry of the PFKase-catalyzed reaction was determined using the $(NH_4)_2SO_4$ and the C_γ fractions (Table III). The reaction mixture contained 50 mM imidazole-HCl buffer (pH 7.0), 5 mM $MgCl_2$, 2 mM NH_4Cl, and 1 mM dithiothreitol. After a 10-min incubation an equal amount of cold 0.7 M $HClO_4$ was added. The mixture was brought to pH 7.0 with 5 N KOH and the

TABLE V: K_m and V_{max} of Nucleoside Triphosphates Used as Substrates by the PFKase.[a]

Trinucleotides	K_m (M × 10^{-5})	% of V_{max} Obtained with ATP
ITP	3.4	94
ATP	3.0	100
GTP	2.5	80
UTP	2.2	72
CTP	1.6	90

[a] $(NH_4)_2SO_4$ fraction assayed at pH 7.0, with 0.8 mM F-6-P as described in Methods.

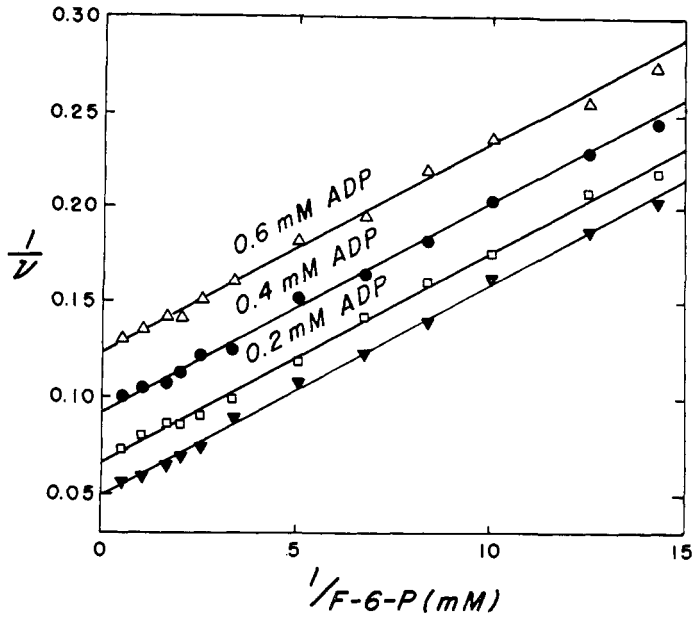

FIGURE 6: Effect of ADP on kinetics of F-6-P utilization. (▼) No added ADP, v = mμmoles/min. Assayed using the $(NH_4)_2SO_4$ fraction with 0.1 mM ATP, as described in Methods.

$KClO_4$ was removed by centrifugation. $MgCl_2$, NH_4Cl, and dithiothreitol were added to a final concentration of 5, 2, and 1 mM, respectively. To different aliquots of the supernatant solution appropriate purine nucleotides and assay enzymes were added in order to measure the four compounds involved in this reaction. A control mixture without enzyme was incubated and assayed as described.

Kinetics with Respect to F-6-P and ATP. Plots of $1/v$ *vs.* 1/F-6-P gave parallel lines, with different intercepts, for each concentration of ATP (Figure 1). Similar kinetics were observed for ATP (Figure 2). As the concentration of ATP was raised the V_{max} and K_m for F-6-P increased; an increase in F-6-P resulted in a higher V_{max} and K_m for ATP (Table IV). Experiments performed with the Cγ and the $(NH_4)_2SO_4$ fractions at pH 7.0 gave comparable K_m values. Similar results were obtained with either of these fractions at pH 8.0. GTP, ITP, UTP, and CTP were all used as substrates by the PFKase. Table V gives the K_m and V_{max} of these substrates. The pH optimum of the enzyme was found to be about 7.5 (Figure 3).

Inhibition by ADP and PP_i. Various dinucleotides were tested for their ability to modify the rate of the PFKase reaction (Figure 4). ADP and GDP were the best inhibitors, CDP and IDP had little effect, and UDP was intermediate. AMP, 3′,5′-AMP, GMP,

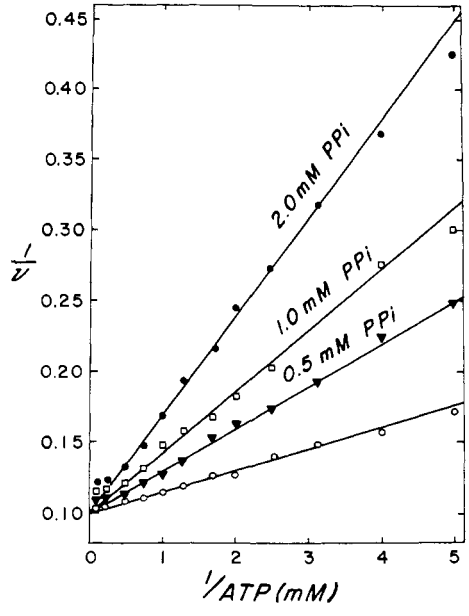

FIGURE 7: Effect of PP_i on kinetics of ATP utilization. (○) No PP_i added, v = mμmoles/min. Assayed with the $(NH_4)_2SO_4$ fraction at pH 7.0, with 0.1 mM F-6-P, as described in Methods.

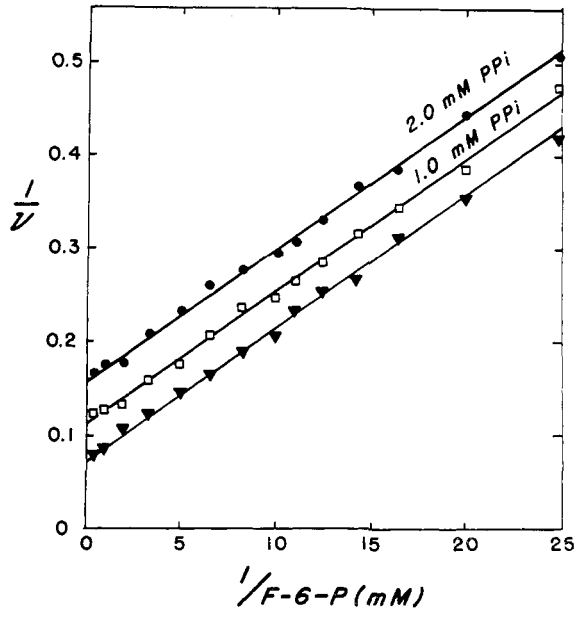

FIGURE 8: Effect of PP_i on kinetics of F-6-P utilization. (▼) No added PP_i, v = mμmoles/min. Assayed with the $(NH_4)_2SO_4$ fraction, at pH 7.0, with 0.05 mM ATP, as described in Methods.

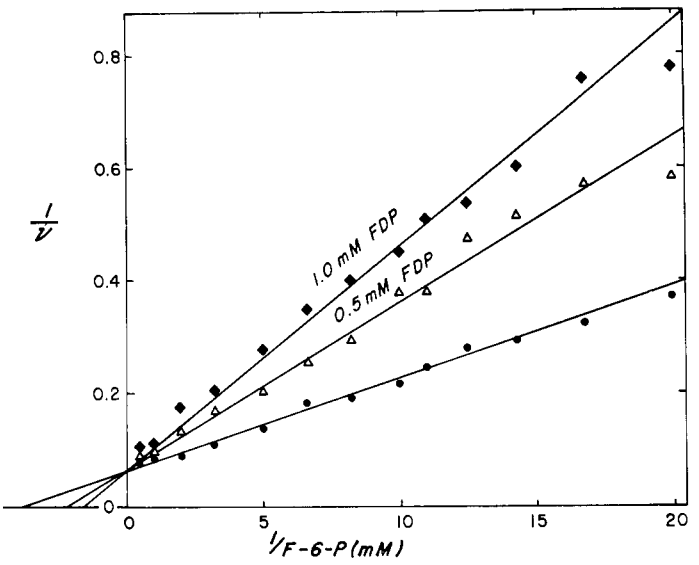

FIGURE 9: Effect of FDP on kinetics of F-6-P utilization. (●) No FDP added, v = mμmoles/min. Assayed with the $(NH_4)_2SO_4$ fraction, at pH 7.0 with 0.1 mM ATP, as described in Methods using the ADP assay system.

TABLE VI: Effect of Trinucleotides, Mononucleotides, and Other Compounds on Inhibition by ADP, GDP, and PP_i.[a]

	Per cent Activity after Addition of Inhibitor			
Concn (mM)	No Addn	And 2 mM ATP, GTP, ITP, CTP, or UTP	And 2 mM AMP, 3′,5′-AMP, GMP, IMP, CMP, or UMP	And 4 mM Citrate, Isocitrate, α-KG, PEP, or P_i
ADP (1)	52	82–92	46–51	50–53
GDP (1)	58	81–100	53–58	55–58
PP_i (2)	46	76–90	42–47	45–48

[a] $(NH_4)_2SO_4$ fraction assayed at pH 7.0 as described in Methods. Substrate concentrations were 0.4 mM F-6-P and 0.1 mM ATP.

IMP, CMP, and UMP were not inhibitory, the curves obtained being similar to IDP. In the presence of 1 mM AMP or 1 mM 3′,5′-AMP, the curve for ADP inhibition was identical with that for ADP alone. Citrate, isocitrate, α-KG, PEP, and P_i had no effect on the reaction rate when tested in the same range as ADP. The pattern of dinucleotide inhibition was similar when GTP, ITP, CTP, or UTP was used as the substrate.

Inhibition by ADP was competitively overcome by ATP (Figure 5). Addition of ADP increased the K_m for ATP without affecting the V_{max}. The K_i with 0.2, 0.4, 0.6, and 1.0 mM ADP was 0.20, 0.22, 0.22, and 0.25 mM, respectively. ADP was an uncompetitive inhibitor (Cleland, 1963b) with respect to F-6-P (Figure 6). Such inhibition gave parallel lines with higher intercepts (indicative of a lower K_m and V_{max} for F-6-P) as the inhibitor concentration was increased. PP_i, like ADP, was a competitive inhibitor with respect to ATP (Figure 7) and uncompetitive with respect to F-6-P (Figure 8). The K_i with 0.5, 1.0, and 2.0 mM PP_i was 0.50, 0.53, and 0.55 mM, respectively.

The effect of various trinucleotides, mononucleotides, and other compounds on inhibition by ADP, GDP, and PP_i was determined. Table VI gives the results. It is clear that the inhibition by ADP, GDP, and PP_i is overcome by all the trinucleotides tested and is unaffected by the mononucleotides as well as by citrate, isocitrate, α-KG, PEP, and P_i.

Inhibition by FDP. Using the assay for ADP it was found that FDP inhibits the PFKase reaction and the inhibition is overcome competitively by F-6-P (Figure 9). The K_i with 0.5 and 1.0 mM FDP was calculated to be 0.62 and 0.69 mM, respectively. FDP acts as an uncompetitive inhibitor with respect to ATP (Figure 10).

Stimulation of the Reaction Rate by NH_4^+. The activity of PFKase was dependent on the presence of NH_4^+ and Mg^{2+} (Table VII). K^+ could replace NH_4^+ but a much higher concentration was required for comparable activity. SO_4^{2-} was inhibitory at 5 mM. There was no stimulation or inhibition by NaCl. The K_m for NH_4Cl was 0.28 mM (Figure 11) and remains the same upon variation of F-6-P and ATP. Inhibition of enzyme activity by ADP and PP_i did not affect the

TABLE VII: Relative Rates of the PFKase Reaction in the Presence of Some Anions and Cations.[a]

Variable Compd (mM)	NH_4Cl	$(NH_4)_2SO_4$	KCl	NaCl	2 mM NH_4Cl Na_2SO_4	2 mM NH_4Cl NaCl	2 mM NH_4Cl $MgCl_2$
0	0	0	0	0	104	100	0[b]
2	100[a]	102	22	0	100	102	N.T.[c]
5	98	90	40	0	83	98	100
10	100	81	55	0	70	104	N.T.[c]

[a] Assayed at pH 7.0 with 0.1 mM F-6-P and 0.1 mM ATP as described in Methods. All values are expressed as per cent of reaction rate with 2 mM NH_4Cl as the only addition. The $(NH_4)_2SO_4$ fraction was used after dialysis against 50 mM Tris-HCl (pH 7.5). [b] $MgCl_2$ omitted from assay mixture. [c] Not tested.

K_m for NH_4Cl (Figure 11). Within the tested range of NH_4Cl, the per cent inhibition of enzyme activity by ADP and PP_i remained constant. Variation of NH_4Cl concentration did not change the K_m for F-6-P (Figure 12) or ATP (Figure 13).

The Cγ fraction is relatively unstable and was obtained in poor yields. Due to this fact the $(NH_4)_2SO_4$ fraction was used in many of the experiments. The main features of this enzyme, such as its inhibition by ADP, PP_i, and FDP, as well as activation by NH_4^+, were all observed with the Cγ fraction at pH 7.0.

Discussion

The PFKase of *D. discoideum* had simpler kinetic properties than any of the PFKases so far studied. There was no evidence for sigmoidicity; all the reactions followed Michaelis–Menten kinetics. The enzyme was not inhibited by ATP or activated by F-6-P. Unlike the PFKase from some organisms (Mansour, 1963; Stone and Mansour, 1967) the slime mold enzyme was fully active upon isolation.

The effect of the concentration of one substrate on the rate of the reaction varied with the concentration of the other substrate in such a manner as to give rise to parallel lines in a double-reciprocal plot. Similar kinetics have been observed with yeast (Viñuela *et al.*, 1963), muscle (Sols and Salas, 1966), and calf lens (Lou and Kinoshita, 1967) PFKase. This type of kinetics is characteristic of the so-called Ping-Pong mechanism (Cleland, 1963a). Both ADP and PP_i were competitive inhibitors with respect to ATP; this inhibition was overcome by all the trinucleotides tested (Table VI). The activity present after the addition of both ADP and PP_i was the same as the product of the remaining activities after each compound was added singly (tested at 0.1, 0.2, 0.6, and 1.0 mM). These two facts were consistent with ADP and PP_i acting on the same site. The kinetic data suggest that the slime mold PFKase has three sites: a site for NH_4^+, a substrate site for F-6-P, and a substrate site for ATP.

The slime mold PFKase is not inhibited by citrate, nor is it activated by F-6-P, AMP, or P_i. Similar results have been obtained with the PFKases from other organisms. For example, the calf lens enzyme is not activated by F-6-P (Lou and Kinoshita, 1967); citrate has no effect on the *Escherichia coli* enzyme (Blangy *et al.*, 1968); P_i has no effect on the avocado (Lowry and Passonneau, 1964) and yeast (Ramaiah *et al.*, 1964) enzymes; and AMP has no effect on the PFKases from avocado (Lowry and Passonneau, 1964) and *E. coli* (Atkinson, 1966). Like the slime mold enzyme those of parsley (Lowry and Passonneau, 1964), carrot (Dennis and Coultate, 1966), and Brussels sprout (Dennis and Coultate, 1967) are inhibited by ADP. Under their conditions of assay, Blangy *et al.* (1968) did not find ATP inhibition of the *E. coli* PFKase. They were, however, able to demonstrate ADP inhibition which was competitively overcome by ATP (as in the slime mold enzyme). NH_4^+ stimulation of the PFKase reaction has been observed in the enzymes from mammals (Uyeda and Racher, 1965), yeast (Sols and Salas, 1965), and the slime mold. It is evident from the above discussion that the various individual properties of the slime mold enzyme are found in the PFKases from other organisms. Only in the slime mold, however, are all of these properties combined in one enzyme.

In comparison with the PFKases from other orga-

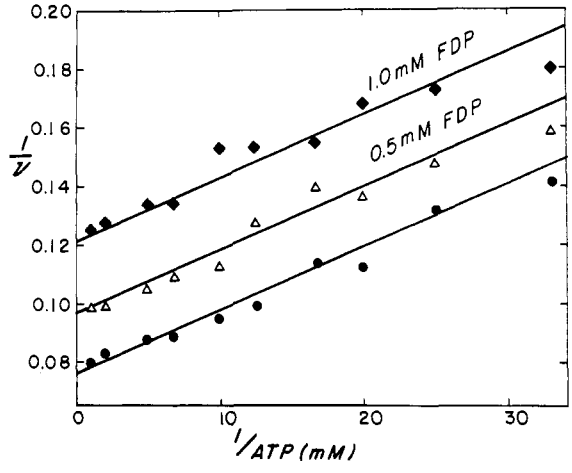

FIGURE 10: Effect of FDP on kinetics of ATP utilization. (●) No FDP added, v = mμmoles/min. Assayed with the $(NH_4)_2SO_4$ fraction, at pH 7.0, as described in Methods using the ADP assay system.

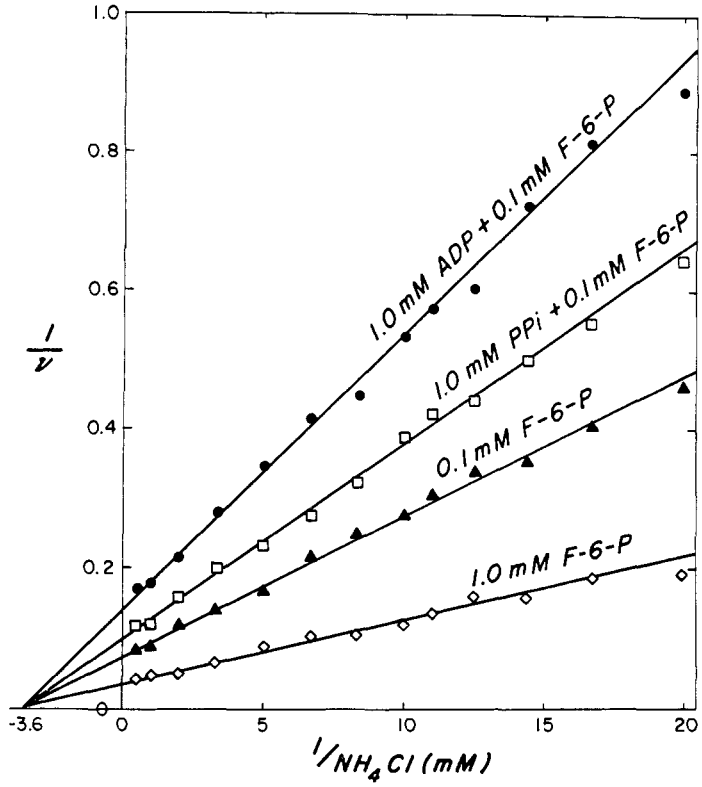

FIGURE 11: Effect of F-6-P, ATP, ADP, and PP_i on the K_m for NH_4Cl, v = mμmoles/min. Assayed at pH 7.0, with 0.1 mM ATP, as described in Methods. The $(NH_4)_2SO_4$ fraction was used after dialysis against 50 mM Tris-HCl (pH 7.5). A plot for 0.1 mM F-6-P and 1.0 mM ATP is not shown due to its close proximity to the 0.1 mM F-6-P and 0.1 mM ATP plot.

nisms, the slime mold PFKase is weakly regulated. ADP, PP_i, and FDP inhibition may be of significance in understanding the role of this enzyme in metabolism. If so, the inhibition pattern is inconsistent with that expected from an enzyme which serves primarily as a controlling point in energy-yielding metabolism and, in fact, resembles the type of control expected in a biosynthetic enzyme. The unusual regulatory pattern of the PFKase implies an altered physiological function.

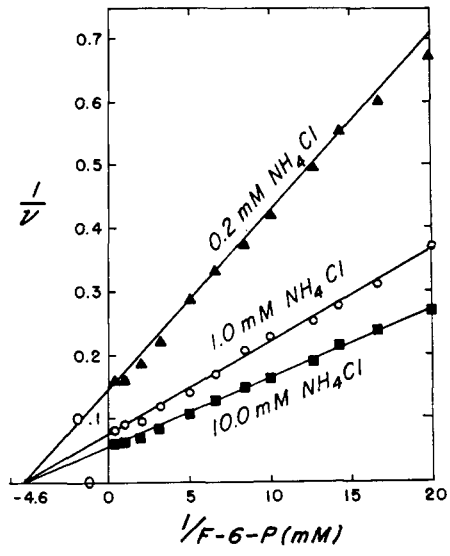

FIGURE 12: Effect of NH_4Cl on kinetics of F-6-P utilization. v = mμmoles/min. Assayed at pH 7.0 with 0.1 mM ATP, as described in Methods. The $(NH_4)_2SO_4$ fraction was used after dialysis against 50 mM Tris-HCl (pH 7.5).

Considerable evidence suggests that this enzyme does not play a central role in the energy-yielding metabolism of the slime mold. A variety of observations, some direct and some circumstantial, suggests that in the amoeboid form the slime mold grows mainly at the expense of proteins and amino acids and that glucose neither decreases the doubling time nor increases the total cell yield (Sussman and Bradley, 1954; Gezelius, 1962). Following the growth phase of the life cycle, the formation of fruiting bodies is greatly delayed in cells which have grown in the absence of glucose (Sussman and Bradley, 1954). These observations may be interpreted to mean that during growth glucose is converted to a storage product, such as glycogen, which is later used as a source of precursors for the synthesis of polysaccharides, which accumulate during fruiting body formation. In fact, the extent of glycogen depletion during differentiation can roughly account for the new polysaccharide formed (Wright, 1968). Thus gluconeogenesis may not be of primary importance in this system, as is strongly indicated by the extremely low level of FDP phosphatase (Cleland and Coe, 1968). Protein may serve as the main energy source as is suggested by its rapid drop and the increase of CO_2 evolution from [^{14}C]-glutamate as differentiation progresses (Wright, 1963). In comparison with [^{14}C]glutamate, [^{14}C]glucose is much more readily incorporated into cell wall material (Pannbacker and Wright, 1967). All these observations are consistent with a low level of gluconeogenesis from amino acids, with direct utilization of hexose units for cell wall synthesis, and with amino acids and proteins serving as the main energy sources for both growth and differentiation. The PFKase reaction may serve as a source of triose and acetate units for biosynthetic

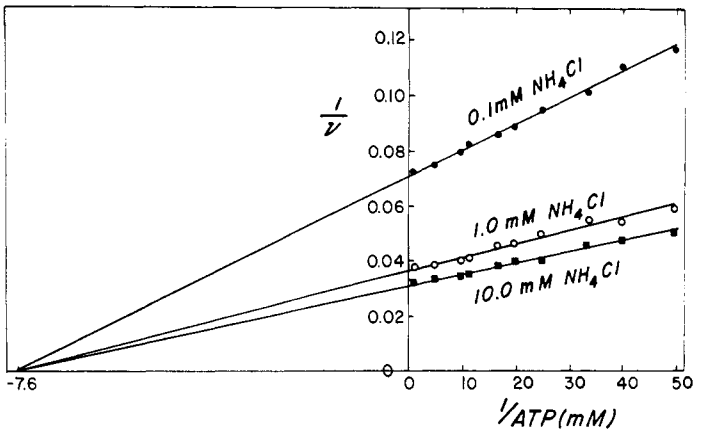

FIGURE 13: Effect of NH_4Cl on kinetics of ATP utilization, v = mμmoles/min. Assayed at pH 7.0, with 0.1 mM F-6-P, as described in Methods. The $(NH_4)_2SO_4$ fraction was used after dialysis against 50 mM Tris-HCl (pH 7.5).

reactions or even for supplementary energy-yielding metabolism under conditions in which there is excess glucose. The latter may take place in amoebae which are replete with glycogen, but still have glucose in the environment, or in differentiating cells having an amount of glycogen in excess of that required for the synthesis of various hexose-containing end products of differentiation. Such an enzyme would be expected to have the type of weak regulation exhibited by the slime mold PFKase.

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