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## Regulation of glycolysis in *Trypanosoma brucei*: hexokinase and phosphofructokinase activity

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### Summary

The kinetic properties of the glycosomal hexokinase (HK)<sup>2</sup> and phosphofructokinase (PFK) from *Trypanosoma brucei* bloodstream forms were investigated. Hexokinase has a very high affinity for glucose ( $K_m = 17 \mu\text{M}$ ) and exhibits a broad pH optimum with a maximum at pH 7.8. No indications have been found for regulation of HK activity. Phosphofructokinase behaves as an allosteric protein with respect to its substrate, fructose-6-phosphate. 5'-AMP acts as a positive allosteric effector. The apparent  $K_m$  for 5'-AMP is extremely low ( $7 \mu\text{M}$ ). The other substrate for PFK is  $\text{Mg}^{2+}$ -ATP chelate which activates the enzyme in a hyperbolic manner. Excess of ATP over  $\text{Mg}^{2+}$  is inhibitory. The enzyme needs  $\text{Mg}^{2+}$  for full activity. Compounds known to be positive or negative heterotrophic modifiers of PFK in other organisms are without effect. It is concluded that PFK and HK probably do not play a regulatory role in glycolysis in *T. brucei*.

**Key words:** *Trypanosoma brucei*; hexokinase; phosphofructokinase; regulation of glycolysis.

### Introduction

Studies by several authors have shown that the “long slender” bloodstream form of the *Trypanosoma brucei* group depends exclusively upon glycolysis

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<sup>2</sup> Abbreviations: HK = hexokinase (EC 2.7.1.1); PFK = phosphofructokinase (EC 2.7.1.11); F-6-P = fructose-6-phosphate

(Bowman and Flynn, 1976). Exogenous carbohydrate is the only energy source since there is no accumulation of storage polysaccharides (Von Brand, 1973) and a functional Krebs' cycle and mitochondrial oxidative phosphorylation are absent (Bowman and Flynn, 1976). Glycolysis in *T. brucei* proceeds at an extremely high rate: 50% of its own weight in glucose is consumed per hour (Von Brand, 1973) and as much as 98% of the glucose-carbon is released as pyruvate in the medium (Grant and Fulton, 1957; Opperdoes et al., 1976a; Brohn and Clarkson, 1978). It has been suggested that entry of D-glucose into the cell might be the rate-limiting step in glycolysis (Gruenberg et al., 1978). If true, the three enzymes that normally have a regulatory function in glycolysis in eukaryotes, i.e. hexokinase (HK), phosphofructokinase (PFK) and pyruvate kinase, cannot have such a role in *T. brucei* bloodstream forms. This is supported by the observation that under anaerobic conditions intracellular ATP levels drop by 50%, resulting in a drastic decrease of the phosphate potential ( $[ATP]/[ADP] \times [P_i]$ ) (Visser and Opperdoes, 1980), whereas the rate of glucose consumption is not affected at all.

Recently it was discovered that part of the glycolytic pathway which involves the conversion of glucose into 3-phosphoglycerate is localized in organelles called "glycosomes" (Opperdoes and Borst, 1977) which seem to be unique to trypanosomes (Taylor et al., 1980). Such an exceptional subcellular organization of the glycolytic enzymes led us to believe that the properties of these enzymes could be strikingly different from those present in other eukaryotic cells, where they are localized in the cell-sap. This might render the *T. brucei* enzymes good targets for future chemotherapeutic attack.

Marr and collaborators (Ozanich and Marr, 1976; Berens and Marr, 1977; Marr and Berens, 1977) have extensively studied the properties of one of the regulatory enzymes of glycolysis, PFK, from a number of trypanosomatids like *Crithidia* and *Leishmania* spp. The data obtained indicated that PFK, although allosteric with regard to its substrate fructose-6-phosphate (F-6-P), did not respond to any of the known heterotrophic modifiers other than AMP and probably had little or no regulatory function in glycolysis.

In this paper we describe the properties of HK and PFK from *T. brucei*. We found that HK has an extremely high affinity for glucose, but no indications were found for any regulation of its activity. Also PFK does not respond to heterotrophic modifiers although it behaves as an allosteric protein with regard to substrate activation by F-6-P. In this respect the *T. brucei* enzyme resembles the PFK from *Crithidia* and *Leishmania* spp.

## Materials and Methods

*Trypanosoma brucei* stock 427 was used in all experiments. Trypanosomes were grown in 300-g male Wistar rats, isolated as described previously (Opperdoes et al., 1976b) and obtained free of blood elements by DEAE-cellulose chromatography (Lanham and Godfrey, 1970). Subcellular fractions enriched in glycosomes were prepared as described previously (Opperdoes et al., 1977).

and stored in liquid nitrogen until use. Triton X-100 was added to the samples immediately before use to a final concentration of 0.1%.

Hexokinase was assayed in a reaction mixture (1 ml volume) in which formation of glucose-6-phosphate was coupled to reduction of NADP by glucose-6-phosphate dehydrogenase (Slein et al., 1950; Fromm and Zewe, 1962). The standard assay mixture contained 100 mM Tris-HCl buffer, pH 7.8, 100 mM glucose, 10 mM  $\text{MgCl}_2$ , 4.8 mM ATP, 0.5 mM NADP, 3 units glucose-6-phosphate dehydrogenase and subcellular fraction, containing approximately 10  $\mu\text{g}$  of protein. The reaction was started with ATP and the blank rate subtracted. Control experiments showed that the concentration of glucose-6-phosphate dehydrogenase was in excess of that required for maximal reaction rate.

Phosphofructokinase activity was determined by measuring the rate of formation of fructose-1,6-diphosphate or ADP (Uyeda and Kurooka, 1970; Bloxham and Lardy, 1973; Uyeda, 1979). The standard assay mixture (1 ml volume) contained 100 mM Tris-HCl, pH 7.7, 5 mM  $\text{MgSO}_4$ , 1 mM EDTA, 1 mM ATP, 3 mM F-6-P, 0.1 mM NADH and subcellular fraction, containing 6 to 18  $\mu\text{g}$  of protein. The auxiliary enzymes used in the assay, namely, aldolase (2 units), triose phosphate isomerase (3 units), and glycerol-3-phosphate dehydrogenase (3 units) on the one hand and lactate dehydrogenase (2 units) and pyruvate kinase (2 units) on the other hand, were freed of  $(\text{NH}_4)_2\text{SO}_4$  by dialysis against 10 mM Tris-HCl, pH 7.7, for 18 h. The incubation mixture for assaying the effect of fructose-1,6-diphosphate on phosphofructokinase activity included the auxiliary enzymes, pyruvate kinase and lactate dehydrogenase; in all other experiments, the auxiliary enzymes, aldolase, triosephosphate isomerase and glycerol-3-phosphate dehydrogenase were used. Control experiments showed that the concentrations of the auxiliary enzymes did not limit the reaction rate, i.e., they were in excess of those required to ensure maximal rates. This was also checked at the pH values used to construct the activity-pH profiles for hexokinase and phosphofructokinase.

Protein was determined fluorometrically (Stein et al., 1973). All standard metabolites, substrates and enzymes were products of Boehringer, GmbH, Mannheim. All chemicals were of analytical grade.

## Results and Discussion

### *Hexokinase activity*

Hexokinase was active over a broad pH range with a peak at pH 7.8; no activity was detectable at pH 4.5 (Fig. 1). Approximately 80% of the maximal activity was still present at pH 10. However, Risby and Seed (1969) reported a pH optimum between pH 6.5 and 7.0 for a number of hexokinases from African trypanosomes including *T. brucei*.

Hexokinase from *T. brucei* showed a high affinity for its substrate, glucose, with an apparent  $K_m$  of 17  $\mu\text{M}$  (Fig. 2); the apparent  $K_m$  for ATP was 116  $\mu\text{M}$  (Fig. 3). The  $V_{\text{max}}$  of the enzyme preparation for glucose as well as for ATP as calculated from Figs. 2 and 3 was 5 units per mg protein.

Four isoenzymes of mammalian hexokinase having different  $K_m$  values for glucose are present in different proportions in such tissues as brain, liver, muscle and kidney (Colowick, 1973). Only mammalian hexokinase III has an affinity for glucose ( $K_m = 7 \mu\text{M}$ ) (Colowick, 1973) comparable to that of the trypanosome enzyme. Mammalian hexokinase I and II have much higher  $K_m$  values of 50  $\mu\text{M}$  and 200  $\mu\text{M}$ , respectively. Type IV mammalian hexokinase (glucokinase) has a  $K_m$  value of 12 mM (Colowick, 1973).

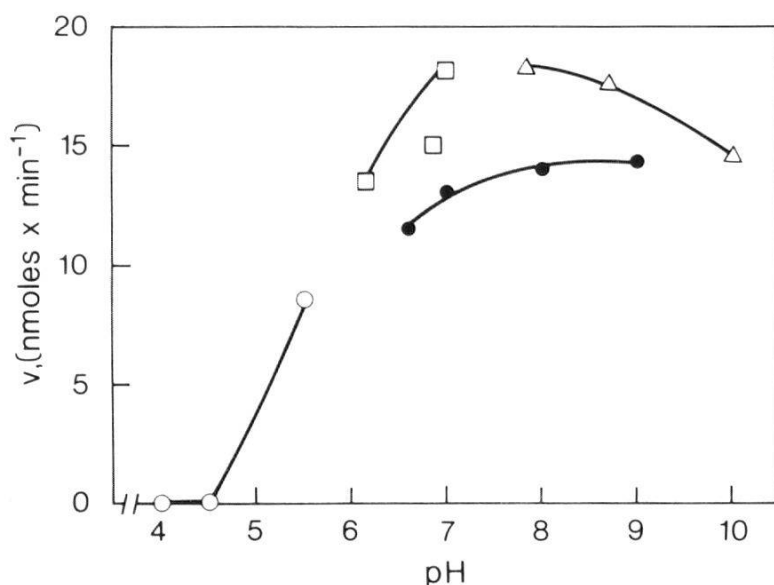


Fig. 1. Hexokinase: effect of  $H^+$ . The experiment was carried out as described under "Materials and Methods", except that in order to cover the pH range, different buffers of 100 mM each were used; ○—○ = acetate (pH 4 to 5.5); □—□ = cacodylate (pH 6.2 to 6.9); ●—● = phosphate (pH 6.5 to 9) and △—△ = Tris-HCl (pH 7 to 10). 10  $\mu$ g of protein was added.

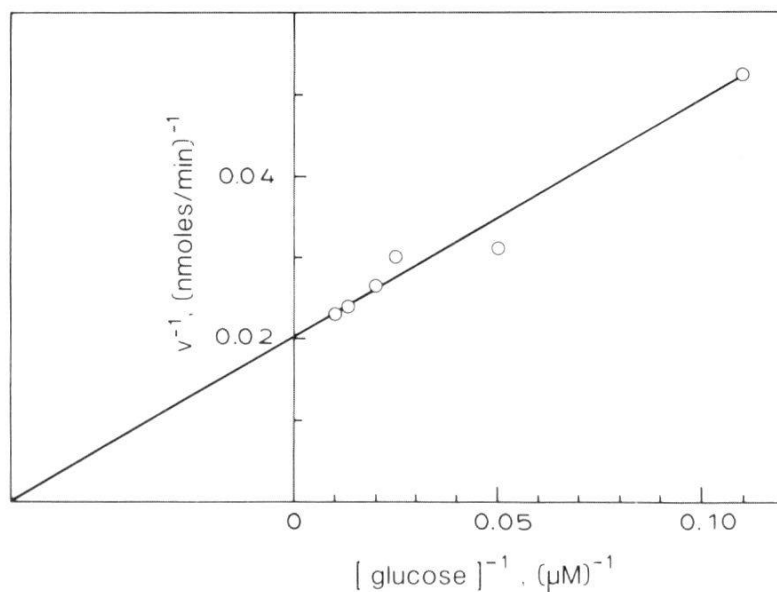


Fig. 2. Hexokinase: Lineweaver-Burk plot for glucose. The sample was prepared in glucose-free medium (280 mM mannitol, 25 mM Tris-HCl, 1 mM EDTA) pH 7.8. The mannitol was previously analyzed and found to be free of glucose. Sample containing 12.5  $\mu$ g of protein was added to the assay. All other conditions were as given under "Materials and Methods".

Hexokinase showed an absolute requirement for  $Mg^{2+}$ . Maximum activity was obtained with 4 mM ATP, 10 mM  $Mg^{2+}$  and 1 mM EDTA. With 5 mM  $Mg^{2+}$ , 4 mM ATP and 1 mM EDTA, approximately 89% of the optimal activity was still present, suggesting that a  $Mg^{2+}$ /ATP ratio of 1 is required for optimal HK activity. In the presence of 4 mM ATP, high  $Mg^{2+}$  concentrations up to 50

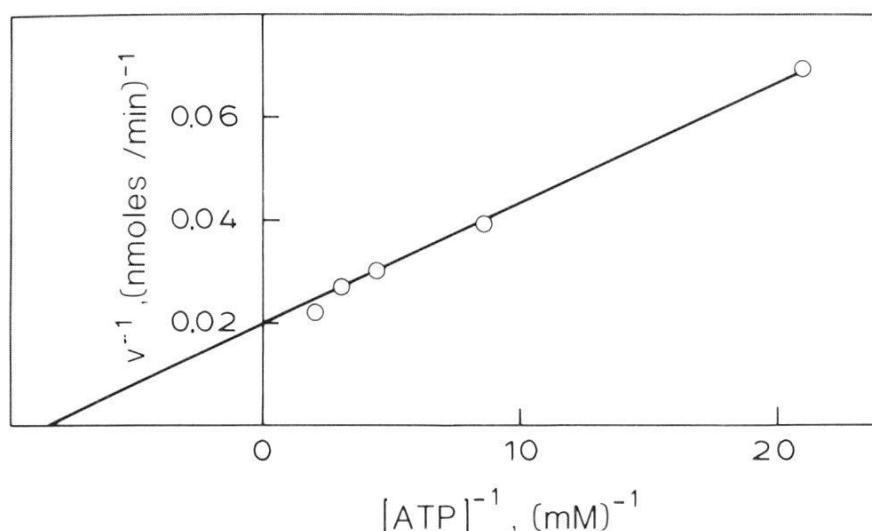


Fig. 3. Hexokinase: Lineweaver-Burk plot for ATP. The experiment was carried out with sample containing 10  $\mu$ g of protein.

mM did not affect HK activity.  $\text{Mn}^{2+}$  could replace  $\text{Mg}^{2+}$  but only 65% of the maximum activity was obtained.

Glucose-6-phosphate, a potent inhibitor of mammalian hexokinase, had no effect on the enzyme from *T. brucei*, even at a concentration as high as 17 mM. Adenosine diphosphate (4.5 mM) inhibited the enzyme activity by 50%. This inhibition cannot be due to chelation of  $\text{Mg}^{2+}$  by ADP, since the experiment was performed under optimal conditions (4 mM ATP, 10 mM  $\text{Mg}^{2+}$  and 1 mM EDTA), thus leaving enough  $\text{Mg}^{2+}$  (4 mM) to maintain a  $\text{Mg}^{2+}$ /ATP ratio of 1. The following glycolytic intermediates, up to a concentration of 5 mM, did not inhibit hexokinase activity: pyruvate, fructose-1,6-diphosphate, glyceraldehyde phosphate, 3-phosphoglycerate, 2-phosphoglycerate and phosphoenol pyruvate. Glucose-1,6-diphosphate, which is a potent inhibitor of mammalian hexokinase types I and II (Beitner, 1979), had, at 2 mM, no effect on the trypanosomal hexokinase activity.

#### Phosphofructokinase activity

Phosphofructokinase activity was high over a broad pH range with an optimum at pH 7.7; approximately 68% of the maximum activity was measurable at pH 10 (Fig. 4). This is in agreement with the results which show a pH optimum around pH 8.0 for the PFK from *Crithidia fasciculata* (Ozanich and Marr, 1976). At pH 6.2 only 13% of the maximum activity was detected (Fig. 4). This should be contrasted with the observation by Jaffe et al. (1970) that the activity of PFK from *T. rhodesiense* at pH 6 was twice that at pH 8.

Phosphofructokinase reacted with its substrate F-6-P in an apparently sigmoidal manner (Fig. 5). In a wide variety of organisms PFK shows a sigmoidal rate dependence on the concentration of F-6-P (Bloxham and Lardy, 1973; Uyeda, 1979). The enzyme from *T. brucei* behaved in this respect similar-



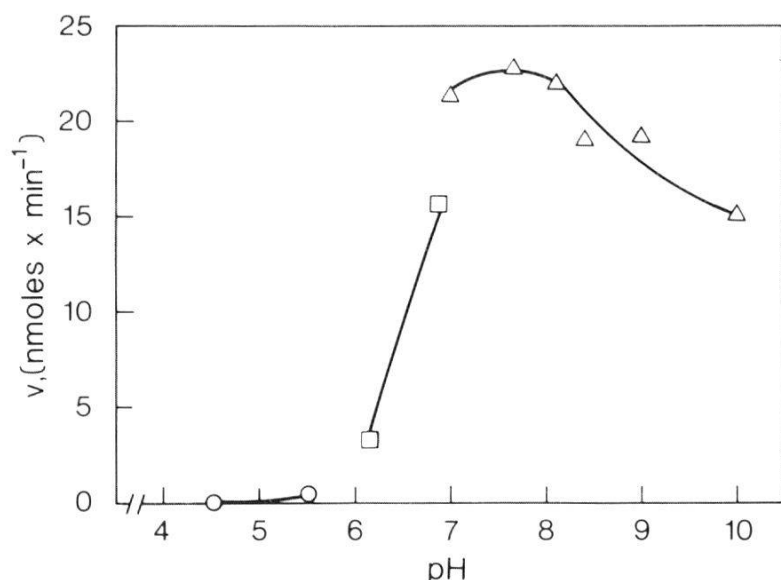


Fig. 4. Phosphofructokinase: effect of  $H^+$ . The experiment was carried out as described in the legend to Fig. 1, except that phosphate buffer was omitted.  $\circ$ — $\circ$  = acetate buffer;  $\square$ — $\square$  = cacodylate buffer;  $\triangle$ — $\triangle$  = Tris/HCl buffer. Each assay contained  $7 \mu\text{g}$  of protein and the auxiliary enzymes, aldolase, triosephosphate isomerase and glycerol-3-phosphate dehydrogenase. The velocity ( $V$ ) is given in nmoles fructose-1,6-diphosphate formed per minute. In this and subsequent figures (Figs. 5–7), the dialyzed auxiliary enzymes used in the assay were aldolase, triosephosphate isomerase and glycerol-3-phosphate dehydrogenase.

ly to the mammalian PFK. Hill plots gave straight lines with  $n$  values between 2.1 and 1.5 indicating that the number of substrate-binding sites is greater than 1.

Marr and co-workers (Ozanich and Marr, 1976; Berens and Marr, 1977; Marr and Berens, 1977) have reported  $n$  values of between 2.1 and 3 for PFK from *C. fasciculata* and a  $n$  value of 3.2 for the enzyme from *Leishmania braziliensis*. They observed that upon ageing the  $n$  value decreased indicating a weakening of the interactions between the sub-units of the enzyme. It is therefore quite possible that in situ the  $n$  value for PFK from *T. brucei* is higher than 2.

The  $S_{50}$  value of PFK for its substrate, F-6-P of 0.5 mM (Fig. 5) is in good agreement with the value of 0.3 mM, for the enzyme from *T. rhodesiense* (Jaffe et al., 1970). In contrast the  $S_{50}$  for F-6-P of PFK from *C. fasciculata* is 12.8 mM and ranges from 10 mM to 1.4 mM for the enzyme from *Leishmania* spp. (Ozanich and Marr, 1976; Berens and Marr, 1977). Thus, PFK from *T. brucei* and *T. rhodesiense* have a much higher affinity for F-6-P than the enzyme from *C. fasciculata* and *Leishmania* spp.

Adenosine monophosphate, AMP (0.32 mM) activated the enzyme at sub-optimal F-6-P concentrations (up to 1 mM) in such a way that the activity versus substrate curve changed to a hyperbolic one (Fig. 5). At higher substrate concentrations (above 1 mM), AMP had no effect on PFK activity; AMP (0.32 mM) reduced the apparent  $n$  value from 2 to 1, with a concomitant decrease in

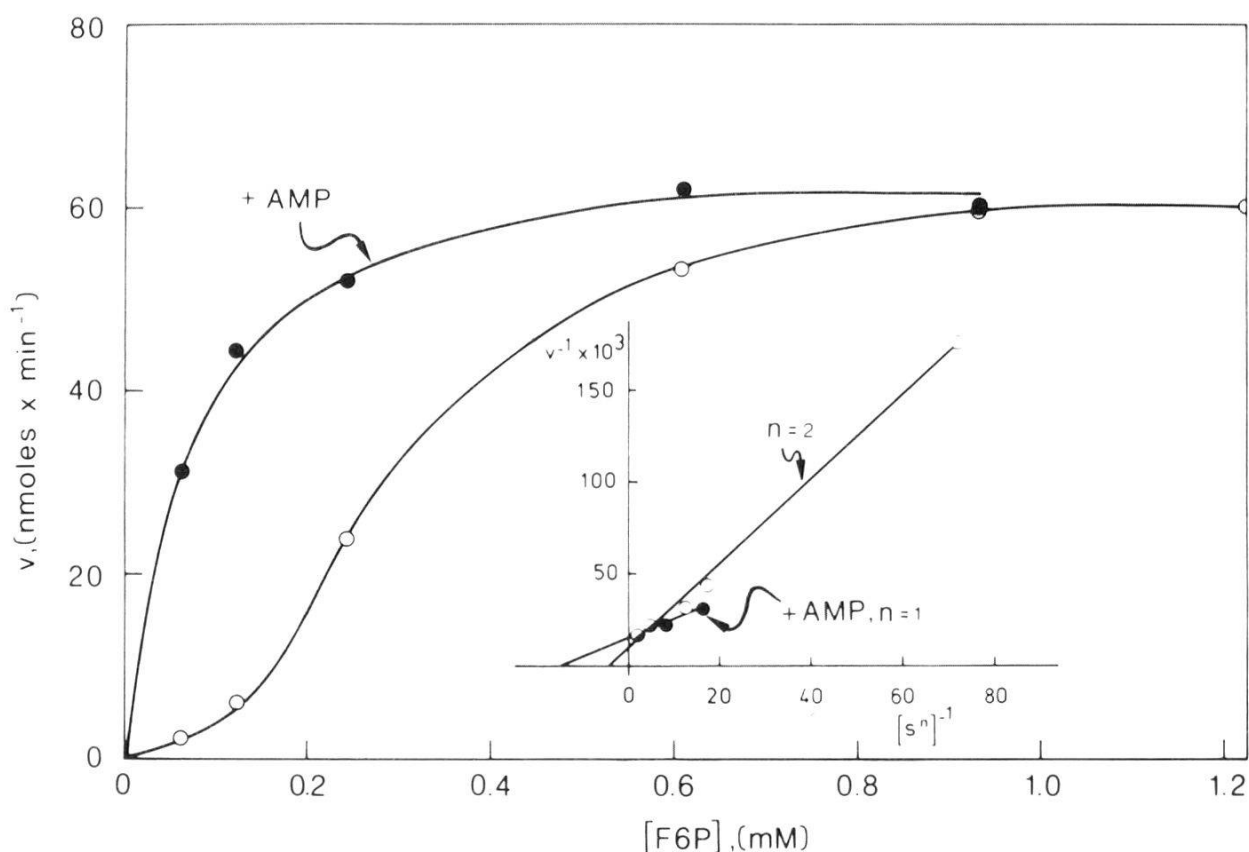


Fig. 5. Phosphofructokinase: reaction of enzyme with substrate, fructose-6-phosphate, in the absence (○—○), and presence (●—●) of AMP (0.32 mM). The reaction mixture included sample containing 18  $\mu\text{g}$  of protein. The velocity,  $V$ , is given in nmoles fructose-1,6-diphosphate formed per minute. Other conditions were as given under "Materials and Methods" and in the legend to Fig. 4. Inset: Plot of reciprocal velocity vs reciprocal of the concentration of F-6-P raised to a computed  $n$  value ( $S^n$ ) in the absence (○—○) and presence (●—●) of AMP. The  $V_{\text{max}}$  for the reaction in the absence of AMP is 5.6  $\mu\text{moles}$  fructose-1,6-diphosphate per mg of protein per minute.

the  $S_{50}$  value for F-6-P from 0.5 mM to 0.07 mM (Fig. 5). It should, however, be stressed that increasing the concentration of AMP caused a decrease in the  $S_{50}$  and a decrease in the cooperativity of F-6-P. Thus, the enzyme from *T. brucei* behaved similarly to its mammalian counterpart (Bloxham and Lardy, 1973; Uyeda, 1979) and the enzyme from *Crithidia* and *Leishmania* (Ozanich and Marr, 1976; Berens and Marr, 1977; Marr and Berens, 1977). At low F-6-P and optimal ATP concentrations (0.24 mM and 1 mM, respectively) PFK from *T. brucei* responded to AMP in a hyperbolic manner with an apparent  $K_m$  value of 7  $\mu\text{M}$  (Fig. 6).

Phosphofructokinase showed an absolute requirement for ATP and  $\text{Mg}^{2+}$ . When the ratio of  $\text{Mg}^{2+}$ :ATP exceeded 1, the enzyme responded in a hyperbolic manner to increasing ATP concentrations, with an apparent  $K_m$  value of 60  $\mu\text{M}$  for ATP (not shown). When the concentration of ATP exceeded that of the metal ion, however, ATP behaved as an inhibitor (Fig. 7), suggesting that free, non-chelated ATP acts as an inhibitor of PFK. It must be stressed that since we have not determined the actual concentrations of free and chelated ATP, we



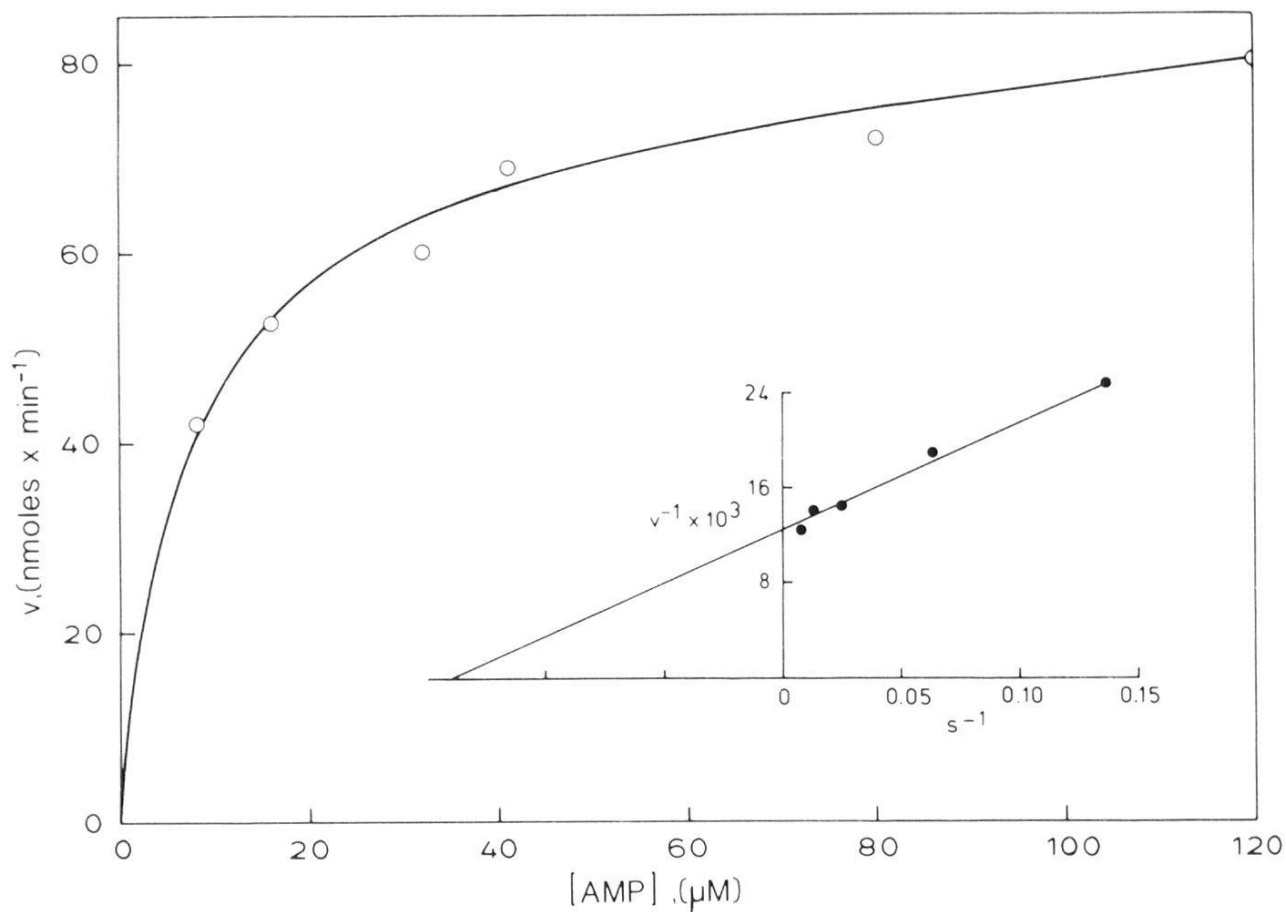


Fig. 6. Phosphofructokinase: The effect of AMP. The apparent  $K_m$  for AMP was determined in the presence of a low concentration of fructose-6-phosphate (0.24 mM) and optimal ATP concentration (1 mM).

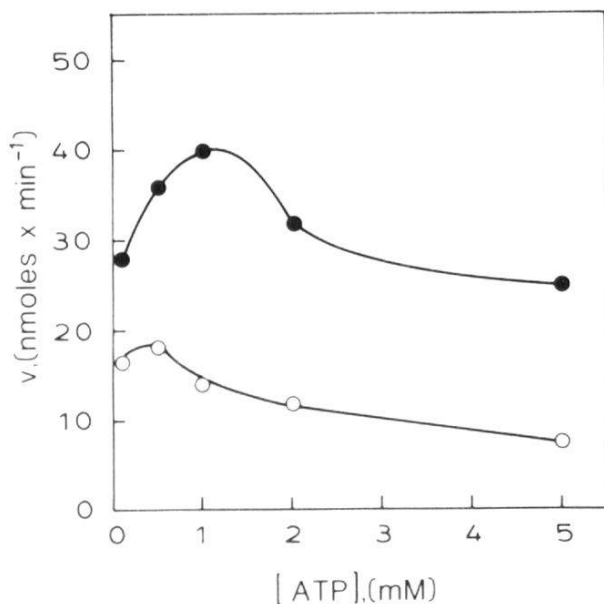


Fig. 7. Phosphofructokinase: effect of the  $\text{Mg}^{2+}/\text{ATP}$  ratio. The reaction mixture included sample containing 18  $\mu\text{g}$  of protein. The velocity ( $V$ ) is given in nmoles fructose-1,6-diphosphate formed per minute. Other conditions were as given under "Materials and Methods" and in the legend to Fig. 4. ( $\bullet$ — $\bullet$ ) = 1 mM  $\text{Mg}^{2+}$ ; ( $\circ$ — $\circ$ ) = 0.1 mM  $\text{Mg}^{2+}$ .

cannot definitely state whether or not free ATP is the inhibitor of PFK of *T. brucei*. In agreement with these results, free ATP also inhibited PFK from *Crithidia* and *Leishmania* spp. (Ozanich and Marr, 1976; Berens and Marr, 1977; Marr and Berens, 1977).

One of the most potent activators of rabbit muscle PFK is one of the products of the reaction, fructose-1,6-diphosphate which, at a concentration as low as 10  $\mu$ M, is twice as active as 200  $\mu$ M AMP (Passonneau and Lowry, 1962). In contrast, fructose-1,6-diphosphate (5 mM) had no effect on PFK from *T. brucei* either at low (0.2 mM) or high (3 mM) F-6-P concentration. Besides AMP, ADP also behaved as a positive effector, since at 1 mM and at sub-optimal F-6-P concentrations (0.2 mM) it stimulated the enzyme of *T. brucei* by threefold. At high F-6-P concentrations (3 mM), however, ADP had no effect on PFK. Bowman et al. (1977) have also reported that PFK from *T. brucei* is stimulated by ADP. It is not clear whether these authors tested the effect of ADP under conditions of high or low F-6-P concentration. In contrast, ADP (1 mM) had no effect on PFK from *Leishmania* (Berens and Marr, 1977).

Inorganic phosphate ( $P_i$ , 5 mM) did not affect PFK from *T. brucei*, unlike the condition in mammalian cells in which PFK is activated by  $P_i$  (Bloxham and Lardy, 1973). Ozanich and Marr (1976) and Berens and Marr (1977) also found that  $P_i$  (1 mM) had no effect on PFK from *Leishmania* and *Crithidia* spp.

Bowman et al. (1977) have reported that PFK from *T. brucei* is inhibited by phosphoenol pyruvate (PEP). We found that PEP (1 mM) did not affect PFK; however, at a concentration of 5 mM which is well above the physiological concentration (0.3 mM) (Visser and Oppendoes, 1980), PEP inhibited PFK by 50%. Berens and Marr (1977) also found that PEP at 1 mM did not affect PFK from *Leishmania* spp.

A number of other compounds producing a negative or positive effect on PFK of mammalian tissues was tested. The behaviour of the enzyme towards its substrate F-6-P was modified by  $NH_4^+$ . At a concentration of 5 mM,  $NH_4^+$  changed the kinetics from sigmoidal to hyperbolic and decreased the Hill coefficient from 1.5 to 1. However, at this concentration,  $NH_4^+$  had no other effect on PFK: the  $S_{50}$  for F-6-P and the  $V_{max}$  were still 0.5 mM and 5 units/mg protein, respectively.  $NH_4^+$  (15 mM) doubled both the  $S_{50}$  for F-6-P and the  $V_{max}$  to 1 mM and 10 units/mg protein, respectively, while also changing the kinetics from sigmoidal to hyperbolic and decreasing the Hill coefficient from 1.5 to 1. Since such a high  $NH_4^+$  concentration is unlikely to be present in vivo in the glycosome, this activation may be essentially a laboratory phenomenon.

When PFK was pre-incubated with  $Ca^{2+}$  (1 mM) for 2 minutes, the enzyme activity was decreased by approximately 50%. However, when  $Ca^{2+}$  (1 mM) was added after the reaction had started, PFK activity was not affected; a further addition of 1 mM  $Ca^{2+}$ , bringing the total to 2 mM, depressed the activity by 60%.

It has been shown that certain phosphorylated intermediates of glycolysis

such as 2-phosphoglycerate and 3-phosphoglycerate inhibit PFK (Uyeda, 1979). We did not observe any significant inhibition of PFK by these two compounds at concentrations up to 5 mM. Glucose-1,6-diphosphate activates PFK from a number of organisms (Beitner, 1979), but at both low (0.2 mM) and high (3 mM) F-6-P concentrations this compound had no effect on PFK from *T. brucei*, suggesting that it plays no regulatory role in vivo.

### *Regulation of glycolysis*

Hexokinase and phosphofructokinase have been implicated as regulators of glycolysis in a number of organisms. In mammalian cells the level of glucose-6-phosphate is determined by PFK via glucosephosphate isomerase which operates at equilibrium. An acceleration of PFK activity decreases glucose-6-phosphate concentration, which deinhibits HK with a concomitant increase in glucose consumption.

In *T. brucei* there are no indications, so far, of the existence of any regulation by either glucose-6-phosphate or any other metabolite of glycolysis.

Because the activity of PFK is subject to multiple control by a series of positive and negative effectors, in addition to its pronounced cooperative properties, PFK possesses the highest effector strength of all glycolytic enzymes (Rapoport et al., 1974). Mammalian PFK is regulated in a number of ways: (1) cooperativity with respect to binding of F-6-P; (2) ATP is a negative effector of phosphofructokinase whereas ADP and AMP are positive effectors and increasing fructose-6-phosphate concentrations reduce the inhibition by ATP; (3) citrate is an inhibitor and may serve to intensify the inhibitory effect of ATP.

In Trypanosomatids no indications have been found for the existence of such important regulatory mechanisms in glycolysis (Ozanich and Marr, 1976; Berens and Marr, 1977; Marr and Berens, 1977) and it is concluded that PFK of *Crithidia* and *Leishmania* spp. does not function as a critical regulator of glycolysis (Marr and Berens, 1977). A possible regulator of phosphofructokinase of *T. brucei* is AMP which, at sub-optimal concentrations of F-6-P, activated PFK with an apparent  $K_m$  of 7  $\mu$ M. Though the concentration of AMP within the glycosome is not yet determined, it is likely that AMP would be available at a sufficiently high concentration to maximally activate PFK at all times.

We therefore suggest that PFK is not under regulation by AMP. If, however, it is shown that the AMP concentration in the glycosome is much lower than 7  $\mu$ M and also that the F-6-P concentration is sub-optimal, then availability of AMP would greatly influence PFK activity.

Other factors also need to be considered. In *T. brucei* bloodstream forms the aerobic/anaerobic transition does not change the glycolytic rate at all (Visser, 1981). Cross-over analysis of the levels of glycolytic metabolites in *T. brucei* at aerobic/anaerobic transitions revealed that there is no cross-over point in the main pathway of glycolysis between glucose-6-phosphate and phosphoenolpyruvate; instead the level of all metabolites is decreased upon anaerobiosis

(Visser and Oppendoes, 1980). In Organisms in which PFK is a strong regulator of glycolysis, the enzyme is responsible for the classical Pasteur effect, that is an enhanced rate of glycolysis under anaerobic conditions. That such an effect is not observed in *T. brucei* indicates the absence of regulation at the level of PFK.

Bloodstream forms of *T. brucei* consume glucose at a rate which is fifty times that of mammalian tissues. Glucose has to pass the plasma membrane, the cell sap and the glycosomal membrane before it can be phosphorylated by hexokinase. Even though *T. brucei* lives in blood, an environment relatively high in glucose concentration (5 mM), it is probably the combination of a high glycolytic flux and the unique compartmentation of glycolysis which dictates a high affinity of hexokinase for glucose. Recent findings suggest that hexokinase and phosphofructokinase exist as, or are a part of, a dense macromolecular complex in the core of the glycosome (Oppendoes and Nwagwu, 1980). Accessibility of glucose to hexokinase in such a complex may, therefore, be rate-limiting in the hexokinase reaction. A high affinity of hexokinase for glucose would enable the trypanosome to cope with a steep concentration gradient of glucose between the plasma membrane and the glycosome's interior and so allow for a fast glycolytic rate.

We conclude that glycolysis in *T. brucei* is not regulated by any mechanism other than a rate-limiting first step of glycolysis. This could be glucose transport into the cell as suggested by Gruenberg et al. (1978) or the accessibility of glucose to hexokinase and thereby phosphorylation of glucose itself.

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