PHOSPHOFRUCTOKINASE AND THE PASTEUR EFFECT*

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Several recent studies, based on measurement of substrate levels, indicate that the enzyme reaction primarily responsible for the Pasteur effect is phosphofructokinase (PFK) in yeast (1), ascites tumor cells (2), heart (3), and diaphragm (4). The last confirms earlier less clearcut evidence for skeletal muscle (5). The rapid increase in fructose diphosphate (FDP) following onset of ischemia in brain (6,7) may be interpreted in the same way, and this is substantiated by in vitro studies with supernatant fluid from brain homogenates supplemented with liver mitochondria (8,9).

Bücher (10) concludes that in insect wing muscles PFK is activated when the metabolism is increased during muscular activity. He stated the PFK could be activated in vitro by changes in concentrations of ATP, Mg and fructose-6-P (F6P).

Mansour and Menard found that glycolysis in liver flukes is controlled by PFK (11). When glycolysis was stimulated by serotonin or 3',5'-cycloadenylate (3',5'-AMP), the concentrations of glucose-6-P (G6P) and F6P fell and FDP rose (12). In addition it was shown that partially purified PFK could be activated in vitro by ATP, Mg and 3',5'-AMP, and that activation was characterized by decrease in the concentration of F6P required for activity in the presence of high levels of ATP (13). Dr. Mansour has recently found a similar phenomenon in heart muscle (personal communication).

In studies to be reported elsewhere, every member of the glycolytic cycle was measured in mouse brain at short intervals after decapitation.

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Within 3 seconds (10 day old mice) G6P and F6P fell to half and FDP nearly doubled. Clearly F6P was being phosphorylated at a more rapid rate than before decapitation. All other substrate and cofactor changes were those expected from the increased rate of glycolysis and eventual deficit in ATP. There was no sign that any other step except that of glucose phosphorylation was stimulated by the anoxia.

This communication presents evidence that the peculiar kinetic properties of PFK may explain the Pasteur effect.

EXPERIMENTAL

PFK activity was measured at 26 to 29°. In most experiments the FDP generated was led enzymatically to glycerol-P, and the disappearance of DPNH (initially .01 mM) was followed fluorometrically. The substrate was F6P in studies with purified PFK, or a mixture of G6P and F6P, kept near equilibrium with P-glucoisomerase, in the case of crude enzyme preparations.

In a few experiments ADP, rather than FDP, was measured with added lactic dehydrogenase, DPNH, P-enolpyruvate and its kinase. Again the decrease in DPNH was followed fluorometrically.

In all cases, in an effort to partly simulate conditions *in vivo*, the studies were conducted in 0.02 M imidazole buffer, pH 7.0, with 0.15 M potassium acetate, 0.01% bovine plasma albumin, and, unless noted, 5 mM $MgCl_2$. Rabbit muscle PFK was purified 20 fold according to Ling et al. (14).

In the presence of 2.3 mM ATP and 0.1 mM F6P, activity was increased 10 fold by either 0.09 mM 3',5'-AMP, 0.22 mM AMP or 4.5 mM P_i (estimated from Fig. 1) whereas ADP was comparatively inactive. If, however, all samples contained 2 mM P_i, activity was increased by much lower concentrations of 3',5'-AMP and AMP; ADP also became quite effective (Table I). To simulate the changes which occur early in anoxia, P_i was raised, ATP lowered, and AMP and ADP added at low levels. Activity increased 30 fold (Table I), which is 4 times the sum of the individual increments when these components were changed one at a time.

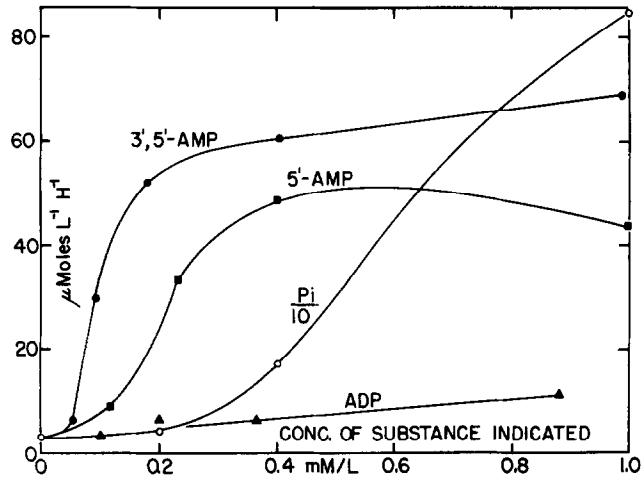

Figure 1. Effect of P_i and three nucleotides on activity of rabbit muscle PFK. The other concentrations of reactants were ATP, 2.3 mM; Mg, 5 mM; F6P, 0.1 mM. The P_i concentrations, as indicated, are drawn to a 10 fold smaller scale.

TABLE I

Activation of Rabbit Muscle Phosphofructokinase

Except where noted, concentrations were ATP, 2.3 mM; Mg, 5 mM; F6P, 0.1 mM; P_i, 2 mM. The enzyme preparation was used at a concentration of 0.1 μg per ml and preincubated 5 minutes with all components present, except F6P, which was added to start the reaction. Velocity is expressed as micromoles per liter per hour.

ADDITION		VELOCITY
0		2
ADP	.058 mM	7
ADP	.144 "	12
5'-AMP	.02 "	5
5'-AMP	.12 "	61
3',5'-AMP	.007 "	4

ADDITION OR CHANGE		VELOCITY
3',5'-AMP	.047 mM	71
ATP	1.8 "	3
ATP	1.8 "	84
5'-AMP	.02 "	
ADP	.058 "	
P_i	4 "	

The PFK stimulators appear to exert their action chiefly by lowering, as much as 25 fold, the concentration of F6P required for activity (Fig. 2), whereas increasing ATP concentration has the opposite effect.

Mg concentration is not critical. With low levels of F6P and high ATP, both P_i and AMP stimulated markedly whether Mg was 1, 2, 5 or 10 mM. However, absolute activity was decreased at the low Mg levels in agreement with Lardy and Parks (15).

Whole mouse brain homogenate and supernatant fluid were studied extensively and the PFK behavior was very similar to that of purified muscle PFK. Whole homogenates from four rabbit tissues behaved in a comparable manner as far as they were tested (Table II).

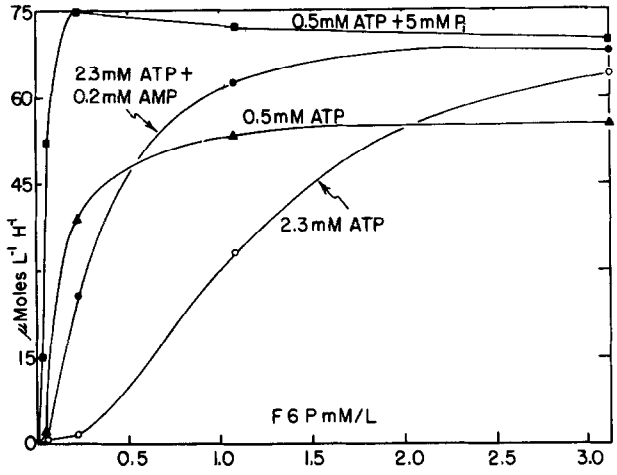

Figure 2. Effect of F6P concentration on PFK activity under four different conditions.

TABLE II

Phosphofructokinase Activity of Whole Homogenates of Rabbit Tissues

The concentrations were ATP, 2.5 mM; Mg, 5 mM; F6P, 0.25 mM; (G6P + F6P = 1 mM). The velocity is expressed as millimoles per kilo of tissue per hour.

Tissue	Dilution during assay	ACTIVITY		
		No addition	+5 mM P_i	+.2 mM 5'-AMP
Muscle	93,000	304	3580	
Muscle	55,000			2580
Brain	8,600	8	328	
Kidney	8,800	9	107	125
Liver	12,800	12	128	74

The following compounds had little or no stimulating activity for muscle PFK at concentrations equal to or greater than those likely to be found in the cell: glucose, 6-P-gluconate, glucose-1-P, 3-P-glycerate, 2-P-glycerate, P-enolpyruvate, pyruvate, lactate, α-glycerol-P, creatine, P-creatine, 5'-mono-P or di-P derivatives of cytosine, guanosine, uridine or inosine, 5'-TMP, 3'-AMP or adenosine tetraphosphate. G6P was about a fifth to a tenth as effective as F6P as a stimulator.

The most active stimulator of all so far discovered is the product FDP itself. At a concentration of 0.01 mM it was as active as 0.2 mM AMP in the presence of 2.5 mM ATP, and 0.1 mM F6P. As little as 0.001 mM FDP tripled activity with 1 mM ATP. With ATP raised to 4 mM, FDP was ineffective even at 0.1 mM concentration.

In addition to these kinetic effects, it should be noted that PFK is exceedingly unstable at pH 7 to 8 unless some stabilizing substance is present. Under the conditions of assay, ATP (1 to 2.5 mM) or P_i (10 mM or greater) preserve activity unchanged for 10 minutes at least. Without either of these, activity is almost completely lost in 2 minutes. This stability behavior is not involved in the phenomena described, although it may of course be related.

DISCUSSION

There are some striking similarities between the kinetic behavior of PFK from muscle and that from the liver fluke (13). The activity of each is increased by 3',5'-AMP, provided that ATP is high and F6P is low. However, Mansour and Mansour report that 5'-AMP is inactive for the fluke enzyme and it also appears that a preliminary activation or solubilization by ATP plus Mg is involved (13). How closely the kinetic findings of Bücher for insect wing muscles resemble those reported here, is not clear from the preliminary report (10).

Clearly the kinetics of mammalian PFK are very complex, and it may be premature to try to explain them. However, the following hypothesis would account for most of the results to date: 1) that there are two ATP sites, a primary active site and a second inhibitory one, and 2) that P_i, AMP, F6P, FDP and the other stimulators all can compete with ATP for the second site and when these occupy the second site they are not inhibitory.

The results if they can be carried over to the cell, indicate that glycolysis can be turned on if either ATP falls, or P_i, ADP or AMP rises. This would provide multiple protection in the case of emergency. The fact that FDP also stimulates sets the stage for a trigger phenomenon. It seems a truly unique situation to have inhibition by one substrate, and stimulation by the other substrate and both products. Lardy and Parks (15) as well as Mansour and Mansour (13) have already suggested that the increase in PFK activity which occurs when ATP decreases may have significance for control of glycolysis.

The question of whether or not hexokinase is in turn controlled by PFK through variation in G6P levels, as suggested by several authors, is beyond the scope of this note.

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