ACTIVATION OF MUSCLE GLYCOLYSIS: A ROLE FOR CREATINE PHOSPHATE IN PHOSPHOFRUCTOKINASE REGULATION

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1. Introduction

When vertebrate skeletal muscle is electrically stimulated under anoxic conditions, the glycolytic rate increases about 100-fold in a manner directly proportional to the stimulation frequency, and it can increase up to 600-fold during tetanus [1,2]. Phosphofructokinase (EC 2.7.1.11) undoubtedly plays a pivotal role in this extreme swing from a low to a high rate of glycolysis, and because the percent activation required during intense muscle work is so large, it is clear that this enzyme's regulatory properties must be geared to allow for essentially 'complete on–complete off' catalytic behaviour [3]. How this may occur is not yet clear because the concentration of no single regulatory metabolite thus far studied falls or rises by a large enough factor to account for phosphofructokinase activation [1,2]. One possible mechanism of activation calls for synergistic effects by various metabolites [4,5]. Another possibility calls for mechanisms that amplify the small concentration changes in ATP** and AMP. A third possibility for phosphofructokinase regulation is the depletion of creatine phosphate known to occur in actively working muscle [6].

Creatine phosphate displays a number of characteristics that warrant serious consideration in regard to glycolytic control. During large (100-fold) glycolytic activation in anoxic muscle, creatine phosphate concentrations drop from about 30 mM to 1 mM [2]; at the same time, the concentration changes of other potentially important regulatory metabolites are very modest. ATP levels drop from 7.2 mM by only 15–30%; ADP concentrations increase from about 2.3 mM to 5.0 mM; AMP levels rise from 0.4 to 0.8 mM; inorganic phosphate levels rise from about 4 to 8 mM [1]. Because both the percentage change and the absolute change in creatine phosphate concentrations far surpass those of other regulatory metabolites, any effect on a key enzyme such as phosphofructokinase would be reflected in large changes in glycolytic rate. Other investigators have postulated a role for creatine phosphate in glycolytic activation in relation to its inhibitory effects on glyceraldehyde-3-phosphate dehydrogenase [7] and pyruvate kinase [8]. However, no attempts have been made to: a) look at the effect of creatine phosphate on the kinetic parameters of phosphofructokinase, or, b) quantitate the physiological importance of the creatine phosphate inhibition by evaluating its effects in concert with other known effectors of the enzyme. For this reason, and because creatine phosphate plays an important role in phosphofructokinase control in the diving turtle [9], we were prompted to examine its effects on mammalian muscle phosphofructokinase.

We have found creatine phosphate inhibits muscle phosphofructokinase in a manner competitive with both substrates. The inhibition can be fully reversed with adenosine monophosphate and inorganic phosphate. This regulatory effect, in concert with known concentration changes in several key metabolites, can account for the 'complete off-complete on' catalytic behaviour that seems to occur at this enzyme step during activation of muscle glycolysis.

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** Abbreviations used: ATP, ADP, AMP, adenosine tri-, di-, mono-phosphate; P_i, inorganic phosphate; G6P, glucose-6-phosphate; F6P, fructose-6-phosphate; CrP, creatine phosphate.

2. Materials and methods

Crystalline rabbit muscle reagents, phosphofructokinase, and coupling enzymes were obtained through Sigma Chemical Co., St. Louis. The phosphofructokinase was supplied as a suspension containing 1.3 M $(NH_4)_2SO_4$, 50 mM glycerophosphate, 4 mM adenosine phosphate, and 1 mM dithiothreitol. The enzyme was diluted 40-fold prior to assay in a mixture of 50 mM Tris–HCl (pH 8.0), 2 mM EDTA, 50 mM β-mercaptoethanol and 2 mM Mg^{2+}. Under these conditions the enzyme is stable for several hours. In all experiments 5 μl of the diluted enzyme was used to start the reaction.

Standard assays for the determination of kinetic constants contained the following in a final vol of 1 ml: 50 mM Tris–HCl (pH 7.0), Mg^{2+}, K^+, fructose-6-phosphate, ATP, *P*-enolpyruvate, NADH, and excess dialyzed Sigma pyruvate kinase and lactate dehydrogenase.

Assays measuring the effects of simulated in vivo conditions contained in a final vol of 3 ml: 50 mM Tris–HCl (pH 7.2), various substrates and effectors as listed in table 1, NADH and excess dialyzed Sigma alphaglycerophosphate dehydrogenase, triosephosphate isomerase, and aldolase. All assays were performed at 25°C.

The following experiments were performed to rule out indirect effects of creatine phosphate:

(1) In both assay methods, creatine phosphate may inhibit one of the coupling enzymes and give rise to apparent phosphofructokinase inhibition. The fructose–diphosphate sampling method [10] was used to measure phosphofructokinase activity in the presence and absence of creatine phosphate. These studies showed that under the assay conditions employed the effect of creatine phosphate is on phosphofructokinase alone.

(2) If the commercial enzymes contain significant activities of creatine phosphokinase, this will have the effect of raising the ATP concentration (due to ADP contamination of reagents). The levels of creatine phosphokinase was measured and found to be 0.0003 units per min per assay tube. This very low activity would have no significant effects on the ATP concentrations over the assay time periods used.

3. Results and discussion

In agreement with previous studies [9,11], we found creatine phosphate to be a potent inhibitor of rabbit muscle phosphofructokinase. The inhibition is competitive with respect to both substrates, fructose-6-phosphate and ATP. Dixon plots of 1/velocity vs. creatine phosphate concentration indicate an inhibition constant of about 13 mM with respect to fructose-6-phosphate. The effect of creatine phosphate plus a number of other phosphofructokinase modulators is shown in fig. 1. In common with other phosphofructokinases [12], fructose-6-phosphate saturation curves are strongly sigmoidal. Creatine phosphate greatly decreases the apparent enzyme–substrate affinity but has essentially no effect on the maximum velocity of the reaction. Most significantly, all creatine phosphate inhibitory effects can be fully reversed by AMP and

Table 1
An attempt to assess the relative activities of phosphofructokinase under conditions simulating muscle at rest and muscle at work

Rate relative to control[1]	Muscle metabolite concentrations[2], mM										Physiological state of muscle[2]
	CrP	ATP	ADP	AMP	P_i	G6P	F6P	NH_4^+	Mg^{2+}	K^+	
1	30	7.2	3.3	0.4	4.0	0.2	0.05	0.3	11	100	resting
100	30	4.0	4.0	0.8	8.0	1.9	1.0	1.0	11	100	creatine phosphate inhibited
200	1	4.0	4.0	0.8	8.0	1.9	1.0	1.0	11	100	contracting

[1] Control: The rate of reaction (0.0021 $O.D._{340}$/min) with the concentration of effectors and substrates observed in resting muscle.
[2] These values taken from [2,5].

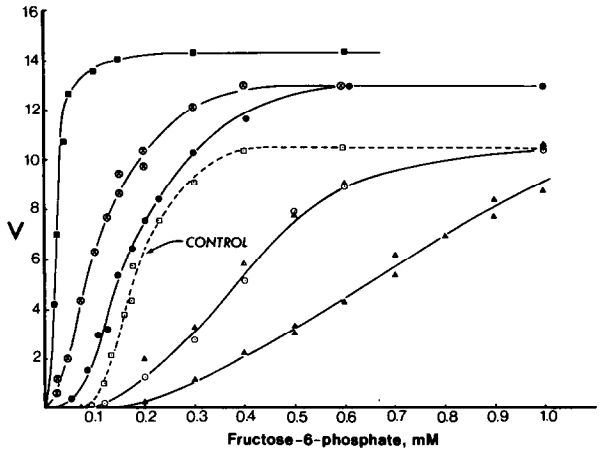

Fig. 1. Effects of metabolites on the velocity *(v)* of rabbit skeletal muscle phosphofructokinase at pH 7.0. Reaction conditions: 50 mM Tris–HCl buffer, 5 mM Mg^{2+}, 100 mM K^+, 0.15 mM NADH, varying concentrations of fructose-6-phosphate, 1 mM *P*-enolpyruvate, excess Sigma dialyzed pyruvate kinase and lactate dehydrogenase. The symbols represent the following conditions: (□) control with 0.5 mM ATP; (△) control plus 1 mM ATP; (○) control plus 20 mM creatine phosphate; (▲) control plus 1 mM ATP and 20 mM creatine phosphate; (●) control plus 0.5 mM AMP with 1 mM ATP and 20 mM creatine phosphate; (⊕) control plus 0.5 mM AMP with 1 mM ATP or control plus 0.5 mM AMP with 20 mM creatine phosphate; (■) control plus 7 mM inorganic phosphate and 20 mM creatine phosphate.

inorganic phosphate, but the latter is clearly more effective. Thus in the presence of both inorganic phosphate and creatine phosphate, the substrate saturation curve becomes hyperbolic and there is a large (40-fold) increase in the apparent enzyme–substrate affinity as well as in the maximum velocity of the reaction. The combined regulatory effects of these metabolites raises the potential for sensitive control of this key step in glycolysis, but just how large might the role of creatine phosphate be?

To answer the latter question we measured the activity of muscle phosphofructokinase under conditions simulating muscle at rest and muscle at work (see [4,5] for similar attempts by others). From these experiments (table 1), it is clear that the summed effects of increasing substrate availability, increasing positive modulator concentrations, and decreasing ATP lead to about a 100-fold activation of phosphofructokinase. When creatine phosphate concentrations are also dropped (from 30 to 1 mM), the overall phosphofructokinase activity rises by about 200-fold (table 1). The magnitude of these effects therefore would appear to be large enough to account for the known percentage changes that occur in glycolysis during intense muscle work [1,2], but they would not appear to be large enough to account for the level of glycolytic activation noted during extreme situations such as tetanus.

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