THE ROLE OF FRUCTOSE-1,6-DIPHOSPHATASE IN THE REGULATION OF GLYCOLYSIS IN SKELETAL MUSCLE

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1. Introduction

The maximum activities of fructose-1,6-diphosphatase (FDPase) (EC 3.1.3.11) and mitochondrial glycerol-1-phosphate dehydrogenase (EC 1.1.2.1) have been measured in a wide variety of different muscles [1] and the distribution of these two enzymes fails to support the two previous hypotheses concerning the role of FDPase in muscle: the removal of glycerol-1-phosphate after exercise [2] and the control of the glycerol-1-phosphate cycle [3]. Therefore another role for FDPase was sought and to this end the maximum activities of phosphofructokinase (PFK) (EC 2.7.1.11) and FDPase were measured in a variety of muscles from different animals. Some of these activities are presented in this paper and a hypothesis is advanced which explains both the relationship between the activities of PFK and FDPase and the surprising distribution of FDPase in muscle tissue. The paper examines theoretically the problem of controlling the activity of an enzyme through the equilibrium-binding of a regulatory molecule. It is proposed that FDPase is present in skeletal muscle to provide cycling between fructose-6-phosphate and fructose disphosphate (catalysed by PFK and FDPase) when the muscle is at rest, and this markedly increases the sensitivity of fructose-6-phosphate phosphorylation to changes in the concentration of AMP.

2. Methods

Animals were obtained from sources described previously [4]. Muscle tissue was extracted and PFK and FDPase activities were assayed as described previously [3], except that the incubation buffer for the latter enzyme contained 4 mM creatine phosphate, 0.16 mg/ml creatine phosphokinase and 1 mM Mn^{2+}: these additions were made to reduce as much as possible the AMP inhibition of FDPase. The specifity of FDPase was tested by showing inhibition upon addition of AMP (1 mM) or fructose diphosphate (5 mM). In some insect flight muscles PFK was found to be very unstable and therefore its maximum activity has been assessed as the sum of phosphorylase and hexokinase activities [1]. The enzyme activities reported in this paper are the means of at least two separate determinations (with two animals or two separate pools of muscle from a larger number of animals) with a variation of less than 30% between individual determinations.

3. Results

On the basis of the maximal activities of PFK and FDPase, muscles can be divided into two groups: firstly those in which FDPase activity is very low (<0.05 μmole/min/g fresh muscle); and secondly those in which the activity of FDPase varies between approximately 2–10% of that of PFK (table 1). The highest activities of FDPase were obtained in two completely different types of muscle: aerobic muscles of some insects and the anaerobic pectoral muscles of some birds (e.g. pheasant) (table 1). However very low activities have been observed in some aerobic vertebrate

Table 1
The activities of PFK and FDPase in various muscles from different animals.

Animal	Muscle	Enzyme activities (μmoles/min/g fresh muscle)		FDPase as % of PFK
		PFK	FDPase	
Locust *(Schistocerca gregaria)*	Flight	20.0*	0.9	4.5
Cockroach *(Periplaneta americana)*	Flight	19.0	1.2	6.3
Honey bee *(Apis mellifera)*	Flight	33.0*	0.9	2.7
Fresh fly *(Sarcophaga barbata)*	Flight	74.0*	1.3	1.8
Hawk moth *(Laothoe populi)*	Flight	9.0	<0.05	–
Frog *(Rana temporaria)*	Sartorius	22.0	0.6	2.9
Domestic flowl	Pectoral	105.7	2.5	2.4
Pheasant *(Phasianus colchicas)*	Pectoral	143.3	4.4	3.1
Cat	Gastrocnemius	19.4	1.3	6.7

* These values represent the sum of hexokinase and phosphorylase activities.

muscles [2, 3] and in insect flight muscles that have low glycolytic capacities (table 1).

4. Discussion

In relation to the provision of energy for mechanical activity in skeletal muscle the activity of PFK (and therefore glycolysis) is regulated primarily by changes in the concentration of AMP [5, 6], which through the adenylate kinase reaction magnifies changes in the concentration ratio ATP/ADP [7]. However the magnitude of the change in the latter is restricted because of the role that these nucleotides play in the provision of biological energy; the free energy of hydrolysis of ATP will vary as its concentration and large variations in this could interfere in the many reactions which are dependent upon a relatively constant supply of energy.

For an enzyme whose activity is regulated by equilibrium-binding of a regulatory molecule, the sensitivity of this regulation can be analysed theoretically by application of the allosteric model of Monod, Wyman and Changeux [8]. Although this model (and other similar models) increase the sensitivity of an enzyme to a change in concentration of the regulatory molecule above that obtained from a hyperbolic response, an increase in PFK activity from 10% to 90% requires at least a fourfold change in AMP concentration (tables 2 and 3). From studies on intact muscle preparations a fourfold change in AMP concentration is the largest that has been reported [9]. However, in some muscle tissues in which the demand for energy is extremely high during contraction the increase in catalytic activity of PFK when the muscle is stimulated from rest may extend over a range greater than 10% to 90% of the maximum activity. For example in pheasant pectoral muscle the maximum activity of PFK is 143 μmole/g muscle/min and this represents the capacity of glycolysis to supply energy during flight; it can be estimated that the maximum glycolytic capacity during rest is approximately 2 μmole/g muscle/min.

In such muscles the sensitivity of the control mecha-

Table 2

The change in concentration of regulator molecule that is necessary to increase the fractional saturation of an allosteric enzyme from 0.1 to 0.9 according to the model of Monod, Wyman and Changeux [8].

Allosteric constant	Concentration of regulator providing fractional saturation of		Increase in concentration of regulator necessary to increase fractional saturation from 0.1 to 0.9
	0.1	0.9	
1	0.18	8.0	44.4
100	1.2	9.8	9.2
500	2.0	11.5	5.7
1,000	2.6	12.6	4.8
10,000	5.0	19.6	3.9

The enzyme is assumed to be tetrameric ($n = 4$), $c = 0$ and L is the allosteric constant T/R. The simplified model of Monod et al., has been used to calculate the fractional saturation $\left(\text{i.e. } Y = \frac{L(1+\alpha)^{n-1}}{L+(1+\alpha)^n}\right)$

Table 3

Theoretical effects of changes in the AMP concentration on the activities of PFK and FDPase and on the net rate of fructose-6-phosphate phosphorylation.

Concn. AMP	Fractional saturation of enzyme with AMP	PFK activity (maximum = 100)	FDPase activity (maximum = 10)	Net F6P phosphorylation (i.e. PFK–FDPase)
0.0	0.000	0.0	10.0	0.0
2.0	0.050	5.0	9.5	0.0
2.5	0.093	9.3	9.1	0.2
3.0	0.150	15.0	8.5	6.5
4.0	0.310	31.0	6.9	24.1
6.0	0.600	60.0	4.0	56.0
12.0	0.890	89.0	1.1	87.9
20.0	0.950	95.0	0.5	94.5

PFK activity is increased and that of FDPase is inhibited by AMP. It is assumed that the maximum activity of PFK is 100 units whereas that of FDPase is 10 units. Also at an AMP concentration approaching zero PFK is completely inhibited and FDPase is maximally active. The fractional saturation of the enzyme with AMP is calculated as in table 2; $L = 10^3$ and $n = 4$. It is assumed that there is strict proportional relationship between the binding of AMP and its activating or inhibitory effects on the two enzymes.

nism can be markedly increased by the operation of a cycle between fructose-6-phosphate and fructose diphosphate catalysed by the simultaneous activities of PFK and FDPase. A comparison of the sensitivities of the control mechanisms involving PFK alone and PFK in combination with FDPase is shown in table 3. The effect of cycling is to provide a threshold response of the regulatory system to a change in AMP concentration, so that the concentration range over which AMP is an effective regulatory molecule is reduced and sensitivity is increased. As muscle FDPase is inhibited by AMP, an increase in the AMP concentration, which increases the activity of PFK, will depress that of FDPase and ensure that, when energy is required for muscular contraction, loss of energy and restriction of the rate of glycolysis at the stage of fructose-6-phosphate phosphorylation is reduced to a minimum (table 3).

This hypothesis explains why the activity of FDPase is only 2–10% of that of PFK in muscle; it also explains the observed variation in distribution of FDPase in different muscles. For example, in both the aerobic insect flight muscles and the anaerobic pectoral muscles of birds the demand for energy varies tremendously depending whether the muscle is working or at rest, so that the sensitivity of the glycolytic control mechanism would not be sufficient without cycling. However, in vertebrate smooth muscle, in heart muscle and in postural muscles (e.g. semitendinosus of the rabbit), in which the activities of FDPase are very low there is almost always some mechanical activity and therefore a demand for glycolysis; and for this reason variation in the demand will not be excessive and cycling is not required. Similarly is not detectable in those insect flight muscles in which the rate of glycolysis is low, i.e. where the muscle relies mainly on fat for energy production.

It is possible to reduce glycolysis to very low rates in the isolated perfused rat heart by inhibition of PFK [10]. This is brought about not by changes in the mechanical activity of the heart muscle, but by the oxidation of fatty acids (or ketone bodies) which increase the intracellular concentration of an inhibitor of PFK, citrate; the measured increase in the content of citrate in heart under these conditions is about five-fold [11], whereas equilibrium-binding data (table 2) would predict a much larger change in citrate in order to produce substantial inhibition of PFK. Such a large change (approximately twenty-fold) is however possible as citrate is produced in the mitochondria and PFK is extramitochondrial, so that the specific change in citrate in the latter compartment might be larger than the measured change in the whole tissue. It has been shown that the increase in citrate in the perfused heart on provision of ketone bodies requires approximately ten minutes to reach a maximum [12]; a large extramitochondrial accumulation could explain this delay. It should be re-emphasised that equilibrium-binding can produce a satisfactory response in enzyme activity modulation provided the change in concentration of the regulator is sufficiently large; this is not possible with AMP because it is related to the ATP/ADP concentration ratio of the cell and this must remain relatively constant.

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References

[1] B.Crabtree and D.Phil. Thesis: University of Oxford (1970).
[2] H.A.Krebs and M.Woodford, Biochem. J. 94 (1965) 436.
[3] L.H.Opie and E.A.Newsholme, Biochem. J. 103 (1967) 391.
[4] E.A.Newsholme and K.Taylor, Biochem. J. 112 (1969) 465.
[5] J.V.Passonneau and O.H.Lowry, Biochem. Biophys. Res. Commun. 7 (1962) 10.
[6] B.Sacktor and E.C.Hurlbut, J. Biol. Chem. 241 (1966) 632.
[7] H.A.Krebs, Proc. Roy. Soc. B 159 (1964) 545.
[8] J.Monod, J.Wyman and J-P.Changeux, J. Mol. Biol. 12 (1965) 88.
[9] H.E.Morgan and A.Parmeggiani, J. Biol. Chem. 239 (1964) 2435.
[10] P.J.Randle, E.A.Newsholme and P.B.Garland, Biochem. J. 93 (1964) 652.
[11] P.B.Garland and P.J.Randle, Biochem. J. 93 (1964) 678.
[12] P.J.Randle, R.M.Denton and P.J.England, Metabolic roles of citrate, ed. T.W.Goodwin (Academic Press, New York, London, 1968) p. 87.