Regulatory Properties of Skeletal-Muscle Pyruvate Kinase

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Hitherto it has been generally supposed that pyruvate kinase (EC 2.7.1.40) has no significant role in the regulation in vivo of glycolytic flux in skeletal muscle (Carbonell et al., 1973). This supposition, apart from providing an explanation for the apparent lack of potentially regulatory allosteric interactions by skeletal-muscle (M-type) pyruvate kinase, is in agreement with the idea that glycolytic flux in skeletal muscle is regulated primarily, if not exclusively, at the level of phosphofructokinase. However, the concept of the phosphofructokinase–fructose 1,6-bisphosphatase/fructose 6-phosphate–fructose 1,6-bisphosphate substrate cycle (Newsholme, 1976; Katz & Rognstad, 1976) and the postulated pathway of amino acid oxidation and alanine formation in skeletal muscle (Goldstein & Newsholme, 1976) suggest that pyruvate kinase in skeletal muscle could indeed have an important regulatory function in vivo. This line of reasoning has led us to re-examine the kinetic properties of pyruvate kinase purified from rabbit skeletal muscle.

1. Pyruvate kinase: adenylates- and magnesium-dependence

Kinetic investigations of skeletal-muscle pyruvate kinase invariably use an excess concentration of Mg²⁺. In vivo, the magnesium concentration may be quasi-limiting, although this is open to serious dispute (Seeley et al., 1976; Ainsworth & Phillips, 1976). Skeletal-muscle pyruvate kinase activity was found to be Mg²⁺-dependent at concentrations of magnesium, ATP, ADP and phosphoenolpyruvate that occur in vivo. However, in agreement with Ainsworth & Phillips (1976), our results suggest that the changes in Mg²⁺ concentration (total, complexed and free) in vivo are of insufficient magnitude to account for the known alterations in glycolytic flux through the pyruvate kinase reaction.

2. Pyruvate kinase: phenylalanine and alanine inhibition

Phenylalanine is a known inhibitor of skeletal-muscle pyruvate kinase (Carminatti et al., 1971) at alkaline pH, maximally above pH 8.0. Inhibition by phenylalanine at pH 8.2 was shown to be Mg²⁺-dependent and competitive with respect to phosphoenolpyruvate. Inhibition was greatest at low Mg²⁺ concentrations. At concentrations of ATP, ADP and phosphoenolpyruvate occurring in vivo, and at 1.0 mM-total Mg²⁺, 1 mM-phenylalanine resulted in excess of 80% inhibition of activity. The magnesium-dependence of phenylalanine inhibition would appear not to be a function of Mg²⁺-binding capacity.

Alanine reverses phenylalanine inhibition of pyruvate kinase at pH 8.2 (Carminatti et al., 1971). However, alanine alone has a slight inhibitory effect on the enzyme. This inhibition was Mg²⁺-dependent and competitive with respect to phosphoenolpyruvate.

The pH-dependence, Mg²⁺-dependence and the concentrations of phenylalanine required to inhibit pyruvate kinase activity to any significant extent are such that it would appear unlikely that the phenylalanine (and alanine) inhibition is of physiological significance. However, it remains to be established whether such effects might be important in the phase of skeletal-muscle proteolysis and alanine mobilization that accompany starvation.

3. Pyruvate kinase: ATP and creatine phosphate inhibition

In broad agreement with Kemp (1973), creatine phosphate has been shown to be a potent inhibitor of skeletal-muscle pyruvate kinase. At pH 7.2, inhibition with respect to phosphoenolpyruvate was competitive ($K_i$ 2.2 mM).

ATP, itself a known inhibitor of pyruvate kinase (competitive with respect to both ADP and phosphoenolpyruvate), increased the inhibition by creatine phosphate on a
synergistic basis. The synergism of ATP–creatine phosphate inhibition may provide the basis for a theory for the control of pyruvate kinase activity in vivo, although this is purely speculative.

Discussion

From the standpoint of attempting to elucidate the mechanisms whereby pyruvate kinase activity might be regulated in vivo, the results obtained to date, and summarized above, are largely inconclusive. Whereas the enzyme in vitro has been shown to be inhibited by ATP, limiting Mg²⁺, creatine phosphate, etc., the inhibition is probably insufficient in magnitude to account per se for the presumed flux through pyruvate kinase that occurs in vivo. It is concluded that skeletal-muscle pyruvate kinase is probably severely inhibited at concentrations of substrates and effectors in vivo, and this is consistent with the results of previous studies.


Enzymic Activities of the Smooth Body-Wall Muscle of the Onychophoran Peripatopsis mosleyi

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The onychophoran Peripatopsis mosleyi is of considerable interest to biologists because it forms a link between the Arthropoda and the segmented worms, the Annelida. The onychophorans are rare and sluggish animals capable of great changes in body dimensions. All of their body muscles are smooth, with the exception of the jaw muscles, which are striated. Lavallard (1966), by using optical and electron microscopy, observed that onychophoran smooth muscle had an extensively developed sarcoplasmic reticulum incorporating many large cisternae, and an abundance of mitochondria and glycogen granules, suggesting a high activity and a relatively high metabolic rate. The presence of an abundant and vesicular sarcoplasmic reticulum in onychophoran smooth muscle has been confirmed (Heffron et al., 1976). These findings are unexpected for a smooth muscle, especially considering the sluggish nature of the animal. The abundance of glycogen in the muscle suggests an active glycolytic pathway, and the mitochondrial density suggests an active tricarboxylic acid cycle and possibly an active fatty acid-oxidation pathway. We therefore measured the maximal activities of one enzyme of each of these metabolic pathways to determine the maximal rate of the pathway in the intact muscle. In addition, we measured the ATPase (adenosine triphosphatase) activity of actomyosin and sarcoplasmic reticulum, since this would provide information on the activity and speed of this seemingly anomalous muscle.

Both sexes of Peripatopsis mosleyi (0.4–1.4g body wt.) were used. The smooth body-wall muscle was obtained by careful dissection, weighed, and an appropriate amount and type of homogenizing medium was added. The muscle was minced finely and homogen-