THE COUPLING OF THE ADENYLATE KINASE AND CREATINE KINASE EQUILIBRIA. CALCULATION OF SUBSTRATE AND FEEDBACK SIGNAL LEVELS IN MUSCLE

Angela VINCENT and J.McD.BLAIR
Department of Biochemistry, University College London

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1. Introduction

It has been estimated that the ratio of glycolytic flux in fully active muscle to that in resting muscle is in the region of 1,000 : 1 [1]. The flux is controlled partly by adrenaline, which acts through 3′,5′-cyclic AMP, and partly by feedback signals from the adenine nucleotide pool, the control in both cases being exerted by modifying the activity of glycogen phosphorylase (EC 2.4.1.1) [2, 3] and phosphofructokinase (EC 2.7.1.11) [4, 5]. The calcium release initiating muscular contraction may also play a part in activating glycogen phosphorylase [6].

One way in which the substrate and feedback signal levels have been investigated in muscle is by measurement of the total tissue levels of the various species under suitable physiological conditions; however, this method suffers from the disadvantage that it is difficult to take into account compartmentation of enzymes and substrates and binding of particular substrate species to proteins and other cellular components, and as a consequence, misleading conclusions may be drawn [7].

This paper is concerned with an alternative approach to the study of substrate availability and feedback signals in muscle, namely the calculation of the concentrations of the various species during the progressive utilisation of creatine phosphate and ATP, assuming that the adenine nucleotides, creatine, and creatine phosphate are in their equilibrium concentrations as catalysed by the enzymes adenylate kinase (EC 2.7.4.3) and creatine kinase (EC 2.7.3.2). That this is a reasonable assumption is supported by the fact that when a muscle is stimulated, changes in adenine nucleotide levels are undetectable, but the levels of creatine phosphate and creatine can be observed to change [7]. Both enzymes have high activity in skeletal muscle, especially in the 'white' variety [8] which has a greater capacity for rapid anaerobic glycolysis for short periods of intense muscular activity. Both enzymes are localised in the supernatant fractions of muscle [9], although adenylate kinase has been reported to be absent from rat skeletal muscle supernatant [10]. The glycolytic enzymes are also recovered in the supernatant from tissue homogenates, and so, we can probably assume that they are in a medium *in vivo* where the adenine nucleotides, creatine, and creatine phosphate are equilibrated by adenylate kinase and creatine kinase.

There is evidence to suggest that muscle remains relaxed with a concentration of 10^{-7} M free calcium ions, that 10^{-6} M Ca^{2+} is sufficient to cause a contraction, and that at about 10^{-5} M Ca^{2+} muscle is maximally contracted [11]. Calcium is chelated by the adenine nucleotides to nearly the same extent as magnesium [12], so that the total calcium concentration required to cause a contraction must be considerably greater than 10^{-6} M, and as ATP is hydrolysed the total level of calcium required to reach 10^{-6} M Ca^{2+} decreases.

2. Conditions

For the purposes of the calculations a homogeneous closed system is considered, with starting concentrations of substrates approximating those found *in vivo* under resting conditions. The initial pH is taken as 7 [13, 14], and a buffer is prescribed to limit the pH

change. As a high potassium concentration is found in muscle, potassium is also included in the system. A value of 0.311 has been specified for the 'true' equilibrium constant of adenylate kinase

$$(K = \frac{[AMP^{2-}]\,[ATP^{4-}]}{[ADP^{3-}]^2})$$ [15] and one of 3×10^{-10} M for the 'true' equilibrium constant of creatine kinase

$$(K' = \frac{[ADP^{3-}]\,[CrP^{2-}]\,[H^+]}{[ATP^{4-}]\,[Cr]})$$ [16]. Table 1 lists the other constants and initial concentrations used in the calculations. Most of the constants were measured at 25° and at an ionic strength near 0.1, which may result in some error.

3. Equations and computer programme

An equation which describes the effects of the ions of hydrogen, potassium and magnesium on the equilibrium catalysed by adenylate kinase, and a programme which calculates the changing levels of adenine nucleotide species equilibrated by adenylate kinase, during the complete hydrolysis of ATP at constant pH, are published elsewhere [15].

An outline of the computer programme used for the present calculations is appended; some specific points require comment.

It is necessary to solve a 6th power equation to obtain the free magnesium ion concentration $[Mg^{2+}]$ as ATP, ADP, AMP, creatine phosphate, and phosphate measurably chelate magnesium.

In each cycle representing an increment of ATPase activity, the adenylate kinase equilibrium is satisfied initially. With $[MgADP^-]$ and $[MgATP^{2-}]$ established, the concentrations of creatine and creatine phosphate conforming to the creatine kinase equilibrium can be calculated, allowance being made for magnesium - creatine phosphate complex formation.

On the basis that there is no generation of ionised + ionisable hydrogen during the hydrolysis of creatine phosphate*.

$$CrP^{2-} + H_2O \rightarrow Cr + Pi^{2-}$$

and that one equivalent of ionised + ionisable hydrogen is generated when both ATP and ADP are hydrolysed

$$ATP^{4-} + H_2O \rightarrow ADP^{3-} + Pi^{2-} + H^+$$
$$ADP^{3-} + H_2O \rightarrow AMP^{2-} + Pi^{2-} + H^+,$$

the net generation of ionised + ionisable hydrogen for each ATPase increment can be calculated. By considering only the buffering capacity of the buffer itself and of phosphate, the hydrogen ion concentration is obtained by solution of a quadratic equation. By using small increments of ATPase activity the hydrogen ion concentration changes are small, obviating the necessity to readjust the adenylate kinase and creatine kinase equilibria. The small errors are not cumulative.

For the purposes of approximation the stability constants of the calcium complexes of the adenine nucleotides were assumed to have the same values as those of the magnesium complexes. The total calcium concentration required to obtain 10^{-6} M Ca^{2+} is then given by the expression $Ca_t \approx 10^{-6} Mg_t / Mg^{2+}$ M.

4. Results and discussion

The results of the calculations are shown in figs. 1 and 2. Fig. 1 shows the variation of the total concentrations of ATP, ADP, AMP, creatine phosphate and creatine as a function of phosphate release from creatine phosphate and ATP. The changes in concentration of $MgATP^{2-}$, $MgADP^-$ and AMP^{2-} are also shown. Fig. 2 shows the concomitant change in hydrogen ion concentration and magnesium ion concentration, together with the total calcium concentration required to reach 10^{-6} M free Ca^{2+} in the system. Fig. 1 illustrates clearly the effective buffering of ATP by creatine phosphate. During this buffering period the only signals available for feedback from the pool as an isolated system are those of creatine phosphate, creatine, phosphate and hydrogen ion. Recently creatine phosphate has been confirmed as an inhibitor of phosphofructokinase [20]. The phosphate concentration change *in vivo* will be something less than is indicated in the figures as phosphate esters accumulate to some extent during accelerated glycolysis [21]; phosphate relieves the ATP^{4-} inhibition of fructose-6-phosphate binding by phosphofructokinase [22], and is a substrate for phosphorylase with a suitable K_m for it to control the activity of that enzyme [3]. Measurements

* The third H of H_3PO_4 is considered unionisable under physiological conditions.

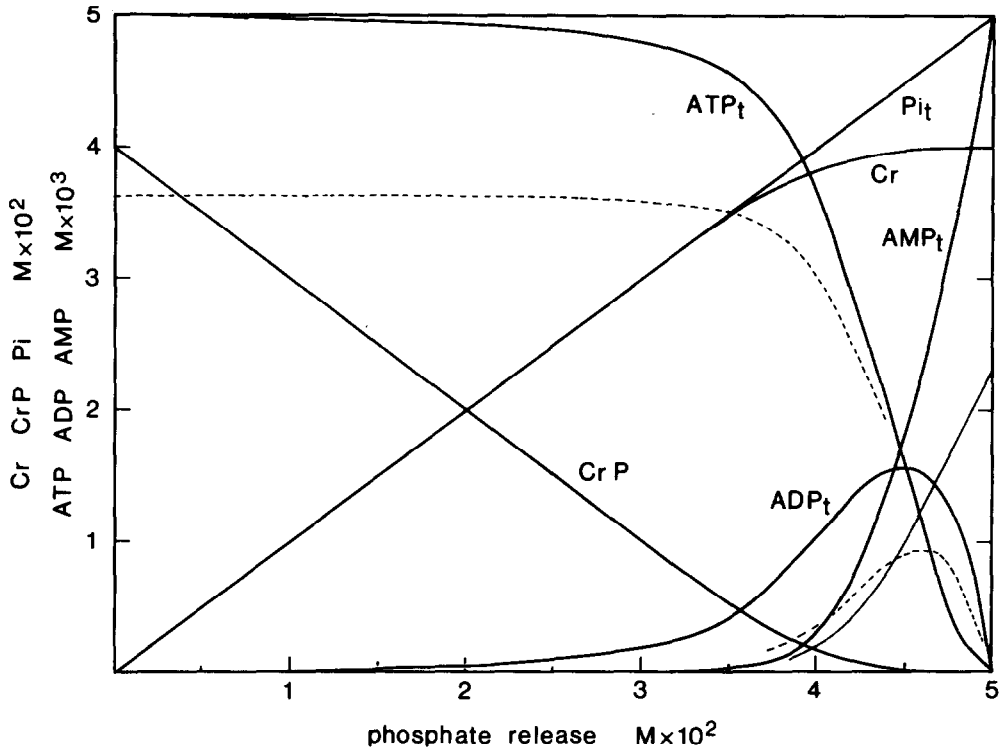


Fig. 1. The variation of the total concentrations of ATP, ADP, AMP, creatine phosphate, and creatine as a function of phosphate release from creatine phosphate and ATP (thick lines). The concentrations of $MgATP^{2-}$ and $MgADP^{-}$ (discontinuous lines) and of AMP^{2-} (thin line) are also shown. The concentrations were calculated as described in the text.

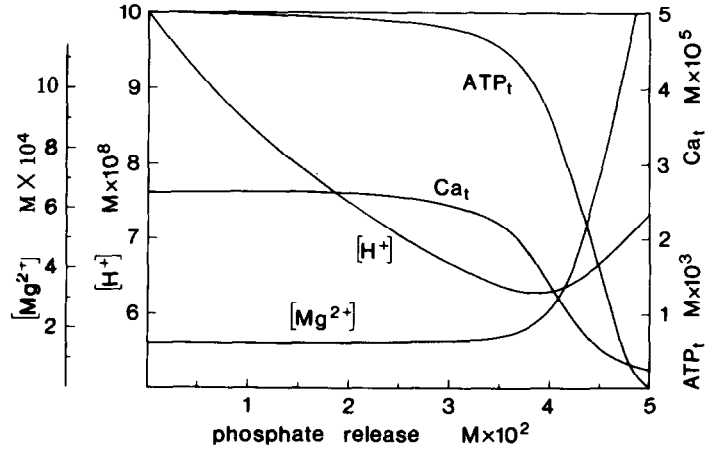
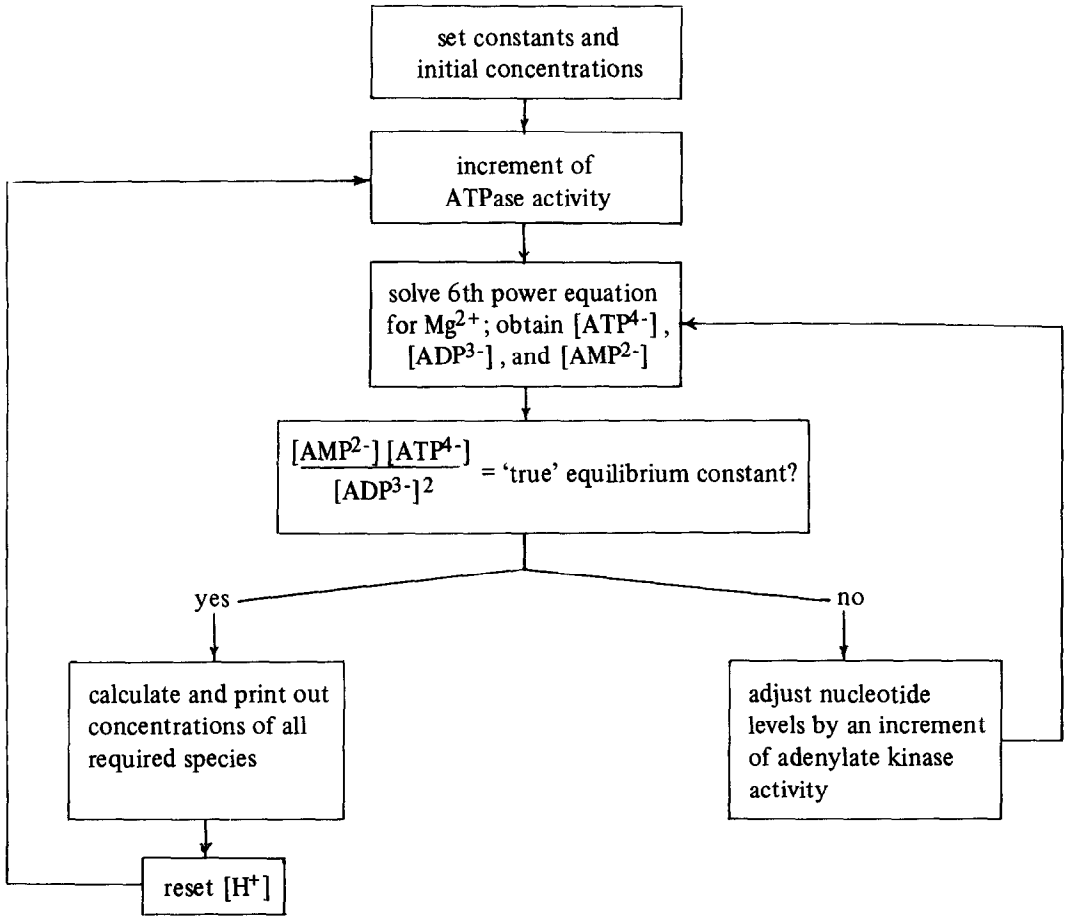


Fig. 2. The changes in hydrogen and magnesium ion concentrations accompanying the changes shown in fig. 1. Also shown is the total calcium ion concentration required to obtain 10^{-6} M free Ca^{2+} ion.



Outline of computer programme

Table 1
Constants and initial concentrations used in the calculations.

Substance	pK	Stability constant (M^{-1})		Initial concentration (M)
		Magnesium complex	Potassium complex	
ATP	7.05 [17]	73,300 [15]	15.6 [15]	5×10^{-3}
ADP	6.78 [17]	4,290 [15]	5.9 [15]	
AMP	6.47 [17]	90 [13]	4.3 [15]	
Creatine phosphate	4.5 [18]	40 [19]		4×10^{-2}
Phosphate	6.7 [13]	60 [13]		
'Buffer'	7.0			1×10^{-1}
Magnesium				4×10^{-3}
Potassium				1.4×10^{-1}

of the pH change in muscle accompanying stimulation indicate a probable initial rise in pH to the extent of 0.1 unit [13], followed by a lowering of the pH probably due to lactate formation; lactate production only becomes appreciable when creatine phosphate is almost depleted [23]. Phosphofructokinase is extraordinarily sensitive to pH, a change of 0.1 unit changing the activity by about 2 orders of magnitude under certain conditions [24]; glycogen phosphorylase is also sensitive to pH [23], a lowering of pH favouring the conversion of phosphorylase b to phosphorylase a. It would therefore appear that while ATP is buffered by creatine phosphate, the changing concentrations of phosphate, creatine phosphate and hydrogen ion are the feedback signals for the control of glycolysis. Once the ATP level starts to fall there is clearly no shortage of feedback signals from the pool, decreasing levels of ATP^{4-}, $MgATP^{2-}$, and increasing levels of Mg^{2+} and ADP and AMP species all being effective [2, 5, 15], and presumably this transfer of control corresponds with the onset of a greatly increased glycolytic flux.

The calculated concentration changes discussed in this paper are not in agreement with whole tissue measurements [21]; but it is possible, as has been suggested by Cori [25], that large concentration changes in a small pool may be obscured in whole tissue measurements; it is also possible, as has been pointed out by Srere [26], that significant fractions of some substrates may be protein bound, and while protein bound substrates are included in whole tissue measurements, only the unbound substrate concentrations are kinetically significant.

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