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ADJUSTMENT OF K' TO VARYING pH AND pMg FOR THE CREATINE KINASE, ADENYLATE KINASE AND ATP HYDROLYSIS EQUILIBRIA PERMITTING QUANTITATIVE BIOENERGETIC ASSESSMENT

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Summary

Physiologists and biochemists frequently ignore the importance of adjusting equilibrium constants to the ionic conditions of the cell prior to calculating a number of bioenergetic and kinetic parameters. The present study examines the effect of pH and free magnesium levels (free $[Mg^{2+}]$) on the apparent equilibrium constants (K') of creatine kinase (ATP: creatine *N*-phosphotransferase; EC 2.7.3.2), adenylate kinase (ATP:AMP phosphotransferase; EC 2.7.4.3) and adenosinetriphosphatase (ATP phosphohydrolase; EC 3.6.1.3) reactions. We show how K' can be calculated using the equilibrium constant of a specified chemical reaction (K_{ref}) and the appropriate acid-dissociation and Mg^{2+}-binding constants at an ionic strength (I) of 0.25 mol l^{-1} and 38 °C. Substituting the experimentally determined intracellular pH and free $[Mg^{2+}]$ into the equation containing a known K_{ref} and two variables, pH and free $[Mg^{2+}]$, enables K' to be calculated at the experimental ionic conditions. Knowledge of K' permits calculation of cytosolic phosphorylation ratio ($[ATP]/[ADP][P_i]$), cytosolic free [ADP], free [AMP], standard transformed Gibbs energy of formation ($\Delta_f G'^{\circ}_{ATP}$) and the transformed Gibbs energy of the system ($\Delta_f G'_{ATP}$) for the biological system. Such information is vital for the quantification of organ and tissue bioenergetics under physiological and pathophysiological conditions.

Key words: creatine kinase, ATP hydrolysis, adenylate kinase, bioenergetics, metabolism, thermodynamics, free magnesium, pH.

Introduction

Knowledge of the thermodynamics of creatine kinase (EC 2.7.3.2), adenylate kinase (EC 2.7.4.3) and adenosinetriphosphatase (EC 3.6.1.3) reactions is central to studying the biochemical and physiological processes of the cell. The maintenance of near-equilibrium of the creatine kinase and adenylate kinase reactions *in vivo* (Lawson and Veech, 1979; Teague and Dobson, 1992; Veech *et al.* 1979) has led to their widespread use in estimating free cytosolic [ADP], free [AMP], cytosolic phosphorylation ratio ($[ATP]/[ADP][P_i]$) and $[PCr]/[P_i]$ (where PCr is phosphocreatine and P_i is orthophosphate) ratio (Chance *et al.* 1985, 1986; Gyulai *et al.* 1985; Veech *et al.* 1979). The cytosolic phosphorylation ratio provides an index of the energy status of the cell. Free [ADP] and $[P_i]$ have been implicated as the primary kinetic controllers of steady-state rates of oxygen consumption (Balaban, 1990; Chance *et al.* 1986; Chance and Williams, 1955; Headrick *et al.* 1994; Lardy and Wellman, 1952; Ugurbil *et al.* 1987), while free [AMP] has been shown to be involved in the regulation of a number of key glycogenolytic and glycolytic enzymes (Dobson *et al.* 1986; Matherne *et al.* 1993) and IMP levels (Matherne *et al.* 1993) and is possibly linked to cytosolic adenosine production (Headrick and Willis, 1990). The transformed Gibbs energy of the system ($\Delta_f G'_{ATP}$) can be calculated from knowledge of the standard transformed Gibbs energy of formation ($\Delta_f G'^{\circ}_{ATP}$) and from the phosphorylation ratio derived from the creatine kinase equilibrium and inorganic orthophosphate concentration. $\Delta_f G'_{ATP}$ may also be used to estimate the thermodynamic efficiency in forming 3 mol of ATP along the mitochondrial respiratory chain from NADH to O_2 for every 2 electrons cycled (Dobson and Headrick, 1995).

The aim of this study is to provide quantitative mathematical expressions for the adjustment of an equilibrium constant to varying pH and free $[Mg^{2+}]$, thereby permitting more accurate bioenergetic assessment of mammalian organs and tissues. It is argued that such parameters have little quantitative meaning

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without the K' of each reaction being adjusted to the intracellular pH, free [Mg^{2+}], temperature (T) and ionic strength (I) of the biological system under investigation (symbols are defined in Table 1). The importance of adjustment of the apparent equilibrium constant of creatine kinase (K'_{CK}) to the pH and pMg of the cell may be illustrated in the anaesthetized rat brain. The widely used value for the K'_{CK} constant for bioenergetic calculations is 166, which is specified at pH 7.0, free [Mg^{2+}], 1.0 mmol l^{-1}; I, 0.25 mol l^{-1} and T, 38 °C. However, the adjusted constant accounting for pH and pMg of anaesthetized rat brain is 122 (pH, 7.0; free [Mg^{2+}], 0.5 mmol l^{-1}; I, 0.25 mol l^{-1}; T, 38 °C). In this case, if K' is not adjusted there would be significant errors (26 %) in the calculation of free cytosolic [ADP] and the cytosolic phosphorylation ratio ([ATP]/[ADP][P_i]). Moreover, in muscle during vigorous exercise, pH may fall by up to 1 unit (from 7.2 to 6.2) (Fitts, 1994), which would require an adjustment of K'_{CK} by nearly an order of magnitude from 85 to 726.

Table 1. *Definitions of thermodynamic quantities, symbols and units*

Symbol	Definition	Units
c°	Standard state concentration, where $c^\circ = 1.0$ mol l^{-1}	mol l^{-1}
I	Ionic strength, $I=\frac{1}{2}\sum c_i z_i^2$, where c_i is the concentration of ions in mol l^{-1} and z_i is the charge of the ion	mol l^{-1}
K'	Apparent equilibrium constant of a biochemical reaction, where the reactant concentrations are the sum of all the species at specified pH and free [Mg^{2+}] (see Alberty, 1992). The meaning of K' cannot be interpreted unless accompanied by a biochemical equation and specification of the standard state of each of the reactants. K' has also been referred to in the literature as K_{obs} or K_{app}	Dependent on biochemical reaction
K_{ref}	Equilibrium constant of a chemical reaction in terms of species at a specified temperature, pressure and ionic strength. Strictly speaking, an equilibrium constant is made dimensionless by adding a term $c^\circ = 1.0$ mol l^{-1} to either the numerator or denominator of the expression.	Dimensionless
P	Pressure	Pa
$\boldsymbol{R}$	Gas constant from the ideal gas equation $PV=n\boldsymbol{R}T$, where P is the pressure, n is the amount in moles, V is the volume and T is the temperature. $\boldsymbol{R}$ is a constant = 8.3145 J K^{-1} mol^{-1} (1.9873 cal K^{-1} mol^{-1})	J K^{-1} mol^{-1}
T	Absolute temperature in Kelvin (K); T=273.15+t, where t is temperature in °C	K
$\Delta_f G'$	Transformed apparent Gibbs energy of a reaction under specified conditions of T, P, pH, pMg and I; $\Delta_f G' = \Delta_f G'^\circ - \boldsymbol{R}T \ln K'$	J mol^{-1}
$\Delta_f G^\circ$	Standard transformed Gibbs energy of a reaction under standard conditions of T, P and I	J mol^{-1}
$\Delta_f G'^\circ$	Standard apparent or transformed Gibbs energy of a reaction at specified T, P, pH, pMg and I; $\Delta_f G'^\circ = -\boldsymbol{R}T \ln K'$	J mol^{-1}
$\Delta_f H'^\circ$	Standard apparent enthalpy of a reaction at specified T, P, pH, pMg and I	J mol^{-1}
$\Delta_f S'^\circ$	Standard apparent entropy of a reaction at specified T, P, pH, pMg and I	J K^{-1} mol^{-1}

Table 2. *Acid-dissociation and magnesium-binding constants at* I=*0.25 mol l^{-1} and* T=*38 °C*

Acids		K_a	K_a value	Reference
$HATP^{3-} \leftrightarrow H^+ + ATP^{4-}$	K_{aATP}	$[ATP^{4-}]\,[H^+]/[HATP^{3-}]$	3.23×10^{-7}	Alberty (1992)
$HADP^{2-} \leftrightarrow H^+ + ADP^{3-}$	K_{aADP}	$[ADP^{3-}]\,[H^+]/[HADP^{2-}]$	4.45×10^{-7}	Alberty (1992)
$HAMP^{1-} \leftrightarrow H^+ + AMP^{2-}$	K_{aAMP}	$[AMP^{2-}]\,[H^+]/[HAMP^{1-}]$	6.28×10^{-7}	Teague and Dobson (1992)
$HPCr^{1-} \leftrightarrow H^+ + PCr^{2-}$	K_{aPCr}	$[PCr^{2-}]\,[H^+]/[HPCr^{1-}]$	3.53×10^{-5}	Alberty (1992)
$H_2PO_4^{1-} \leftrightarrow H^+ + HPO_4^{2-}$	K_{aHPO_4}	$[HPO_4^{2-}]\,[H^+]/[H_2PO_4^{1-}]$	2.41×10^{-7}	Alberty (1992)
Magnesium complexes		K_b	K_b value	Reference
$Mg^{2+} + ATP^{4-} \leftrightarrow MgATP^{2-}$	K_{bMgATP}	$[MgATP^{2-}]/[ATP^{4-}]\,[Mg^{2+}]$	9.90×10^{3}	Alberty (1992)
$Mg^{2+} + HATP^{3-} \leftrightarrow MgHATP^{1-}$	$K_{bMgHATP}$	$[MgATP^{1-}]/[HATP^{3-}]\,[Mg^{2+}]$	9.42×10^{1}	Alberty (1992)
$Mg^{2+} + ADP^{3-} \leftrightarrow MgADP^{1-}$	K_{bMgADP}	$[MgADP^{1-}]/[ADP^{3-}]\,[Mg^{2+}]$	1.11×10^{3}	Alberty (1992)
$Mg^{2+} + HADP^{2-} \leftrightarrow MgHADP$	$K_{bMgHADP}$	$[MgHADP]/[HADP^{2-}]\,[Mg^{2+}]$	2.62×10^{1}	Alberty (1992)
$Mg^{2+} + HPO_4^{2-} \leftrightarrow MgHPO_4$	K_{bMgHPO_4}	$[MgHPO_4]/[HPO_4^{2-}]\,[Mg^{2+}]$	4.34×10^{1}	Alberty (1992)
$Mg^{2+} + PCr^{2-} \leftrightarrow MgPCr$	K_{bMgPCr}	$[MgPCr]/[PCr^{2-}]\,[Mg^{2+}]$	1.84×10^{1}	Alberty (1992)
$Mg^{2+} + AMP^{2-} \leftrightarrow MgAMP$	K_{bMgAMP}	$[MgAMP]/[AMP^{2-}]\,[Mg^{2+}]$	5.47×10^{1}	Teague and Dobson (1992)

Theory and equations

Biochemical versus *chemical reactions*

There exists much confusion in the literature regarding the thermodynamics of a number of phosphotransferase reactions and their applicability to biological systems. For example, it is common to see the creatine kinase reaction written in the following way:

$$PCr + ADP + H^+ = ATP + Cr, \tag{1}$$

where each reactant represents the sum of all the ionic species and metal complexes. The problem with the above reaction is that it does not have an equilibrium constant, because it balances neither charge nor mass. It becomes particularly confusing when an apparent equilibrium constant (K_{obs} or K') follows the reaction at specified pH and pMg, temperature (T), ionic strength (I) and pressure (P). Having H^+ in the reaction, as above, and giving a value for K' at specified pH and other ionic conditions, is incompatible (Alberty, 1994*a*,*b*).

In order to clarify the situation, Alberty (1992, 1994*b*) has defined two equation types, a biochemical equation and a chemical equation. A biochemical equation is one describing an equilibrium mixture of total reactants followed by K' at specified pH, pMg, I, P and T. The value of K' may be analytically measured in the laboratory or calculated from *in vitro* experimental data using a system of equations as described by Teague and Dobson (1992). The point of emphasis here is that a biochemical equation deals with total concentrations and therefore does not balance charge, but it must balance elements, except for H^+ and Mg^{2+} when pH and free [Mg^{2+}] are specified. A chemical equation, in contrast, is defined as one comprising ionic species of reactants at specified I, P and T. By definition, a chemical reaction must balance charge and atoms of elements. Furthermore, because it is a reference equation, it may be part of a mathematical expression with the appropriate metal-binding and acid-dissociation constants with which to calculate K' with varying pH and pMg (Teague and Dobson, 1992). The equilibrium constant of a chemical equation is thus abbreviated K_{ref} and is dependent on I, P and T (Alberty and Goldberg, 1992). In contrast to a biochemical equation, there exist numerous chemical equations, each with specified ionic species and corresponding K_{ref} values. It is important to realize that chemical and biochemical equations are two separate systems and cannot be added or subtracted from one another to form one equation (Alberty and Goldberg, 1992).

This distinction between biochemical and chemical equations serves to promote understanding of complex physiological processes using precise thermodynamic language. What follows is the use of such nomenclature in the adjustment of K' with varying pH and free [Mg^{2+}] at I=0.25 mol l^{-1} and T=38 °C. Computations were performed using a Macintosh computer (Microsoft Excel software).

Calculation of K′ *for the creatine kinase reaction*

Biochemical equation:

$$PCr + ADP = ATP + Cr, \tag{2}$$

$$K'_{CK} = \frac{[ATP][Cr]}{[ADP][PCr]}, \tag{3}$$

where PCr is phosphocreatine, ADP is adenosine 5′-diphosphate, ATP is adenosine 5′-triphosphate, Cr is creatine and all concentrations are expressed in mol l^{-1}. Each reactant represents the sum of all the ionic and metal complex species.

Chemical equation:

$$PCr^{2-} + ADP^{3-} + H^+ = ATP^{4-} + Cr, \tag{4}$$

$$K_{ref} = \frac{[ATP^{4-}][Cr]}{[ADP^{3-}][PCr^{2-}][H^+]}. \tag{5}$$

To be more precise, the equilibrium constant K_{ref} (equation 5), should have the standard state concentration (c°), where c°=1.0 mol l^{-1}, in the numerator to make the constant dimensionless, but it has been omitted in all the K_{ref} expressions of this paper to simplify the equations (Alberty and Goldberg, 1992).

The total concentration of reactants in equation 2 are defined as:

$$[ATP] = [ATP^{4-}] + [HATP^{3-}] + [MgATP^{2-}] + [MgHATP^{1-}], \tag{6}$$

$$[ADP] = [ADP^{3-}] + [HADP^{2-}] + [MgADP^{1-}] + [MgHADP], \tag{7}$$

$$[PCr] = [PCr^{2-}] + [HPCr^{1-}] + [MgPCr]. \tag{8}$$

Equation 3 may be rearranged in terms of the ionic species and expressed as a function of the acid-dissociation constants (K_a values), magnesium-binding constants (K_b values) (see Table 2), pH and free [Mg^{2+}] to give the following equation:

$$K'_{CK} = K_{ref}[H^+]\frac{1 + \frac{[H^+]}{K_{aATP}} + (K_{bMgATP}[Mg^{2+}]) + \frac{(K_{bMgHATP}[H^+][Mg^{2+}])}{K_{aATP}}}{\left\{1 + \frac{[H^+]}{K_{aADP}} + (K_{bMgADP}[Mg^{2+}]) + \frac{(K_{bMgHADP}[H^+][Mg^{2+}])}{K_{aADP}}\right\}\left\{1 + \frac{[H^+]}{K_{aPCr}} + (K_{bMgPCr}[Mg^{2+}])\right\}}, \tag{9}$$

where K_{ref} is 3.77×10^8 at 38 °C (Teague and Dobson, 1992) and [Mg^{2+}] refers to the free [Mg^{2+}]. The adjustment of K'_{CK} with varying pH and free [Mg^{2+}] is shown in Table 3.

Calculation of K′ *for the adenylate kinase reaction*

Biochemical equation:

$$2\mathrm{ADP} = \mathrm{ATP} + \mathrm{AMP}, \tag{10}$$

$$K'_{\mathrm{AK}} = \frac{[\mathrm{ATP}][\mathrm{AMP}]}{[\mathrm{ADP}]^2}, \tag{11}$$

where K'_{AK} is the apparent equilibrium constant of adenylate kinase and all concentrations are expressed in mol l^{-1}. Each reactant represents the sum of all the ionic and metal complex species of the reactants.

Chemical equation:

$$2\mathrm{ADP}^{3-} = \mathrm{ATP}^{4-} + \mathrm{AMP}^{2-}, \tag{12}$$

$$K_{\mathrm{ref}} = \frac{[\mathrm{ATP}^{4-}][\mathrm{AMP}^{2-}]}{[\mathrm{ADP}^{3-}]^2}. \tag{13}$$

The total concentrations of reactants in equation 10 are defined as:

$$[\mathrm{ATP}] = [\mathrm{ATP}^{4-}] + [\mathrm{HATP}^{3-}] + [\mathrm{MgATP}^{2-}] + [\mathrm{MgHATP}^{1-}], \tag{14}$$

$$[\mathrm{ADP}] = [\mathrm{ADP}^{3-}] + [\mathrm{HADP}^{2-}] + [\mathrm{MgADP}^{1-}] + [\mathrm{MgHADP}], \tag{15}$$

$$[\mathrm{AMP}] = [\mathrm{AMP}^{2-}] + [\mathrm{HAMP}^{1-}] + [\mathrm{MgAMP}]. \tag{16}$$

Equation 11 may be rearranged as the sum of ionic species and expressed as a function of the acid-dissociation constants, magnesium-binding constants (Table 2), pH and free $[Mg^{2+}]$:

$$K'_{\mathrm{AK}} = K_{\mathrm{ref}} \frac{\left\{1 + \frac{[\mathrm{H}^+]}{K_{\mathrm{aATP}}} + (K_{\mathrm{bMgATP}}[\mathrm{Mg}^{2+}]) + \frac{(K_{\mathrm{bMgHATP}}[\mathrm{H}^+][\mathrm{Mg}^{2+}])}{K_{\mathrm{aATP}}}\right\}\left\{1 + \frac{[\mathrm{H}^+]}{K_{\mathrm{aAMP}}} + (K_{\mathrm{bMgAMP}}[\mathrm{Mg}^{2+}])\right\}}{\left\{1 + \frac{[\mathrm{H}^+]}{K_{\mathrm{aADP}}} + (K_{\mathrm{bMgADP}}[\mathrm{Mg}^{2+}]) + \frac{(K_{\mathrm{bMgHADP}}[\mathrm{H}^+][\mathrm{Mg}^{2+}])}{K_{\mathrm{aADP}}}\right\}^2}, \tag{17}$$

where K_{ref} is 3.74×10^{-1} at 38 °C and was calculated from the K' of 1.05 at pH=7.0, free $[Mg^{2+}]$=1.0 mmol l^{-1}, I=0.25 mol l^{-1}, T=38 °C (Lawson and Veech, 1979). The adjustment of K'_{AK} with varying pH and free $[Mg^{2+}]$ is given in Table 4.

Calculation of K′ *for the ATP hydrolysis reaction*

Biochemical equation:

$$\mathrm{ATP} + \mathrm{H_2O} = \mathrm{ADP} + \mathrm{P_i}, \tag{18}$$

$$K'_{\mathrm{ATP}} = \frac{[\mathrm{ADP}][\mathrm{P_i}]}{[\mathrm{ATP}]}, \tag{19}$$

where K'_{ATP} is the apparent equilibrium constant of ATP phosphohydrolase, P_i is orthophosphate and all concentrations are expressed in mol l^{-1}. By convention, H_2O concentration is unity and is omitted from equilibrium expressions. Each reactant represents the sum of all the ionic and metal complex species.

Chemical equation:

$$\mathrm{ATP}^{4-} + \mathrm{H_2O} = \mathrm{ADP}^{3-} + \mathrm{HPO_4}^{2-} + \mathrm{H}^+, \tag{20}$$

$$K_{\mathrm{ref}} = \frac{[\mathrm{ADP}^{3-}][\mathrm{HPO_4}^{2-}][\mathrm{H}^+]}{[\mathrm{ATP}^{4-}]}. \tag{21}$$

The total concentrations of reactants in equation 18 are defined as:

$$[\mathrm{ADP}] = [\mathrm{ADP}^{3-}] + [\mathrm{HADP}^{2-}] + [\mathrm{MgADP}^{1-}] + [\mathrm{MgHADP}], \tag{22}$$

$$[\mathrm{P_i}] = [\mathrm{HPO_4}^{2-}] + [\mathrm{H_2PO_4}^{1-}] + [\mathrm{MgHPO_4}], \tag{23}$$

$$[\mathrm{ATP}] = [\mathrm{ATP}^{4-}] + [\mathrm{HATP}^{3-}] + [\mathrm{MgATP}^{2-}] + [\mathrm{MgHATP}^{1-}]. \tag{24}$$

Equation 19 may be rearranged in terms of the speciated forms and expressed as a function of the acid-dissociation constants, magnesium-binding constants (Table 2), pH and free $[Mg^{2+}]$:

$$K'_{\mathrm{ATP}} = \frac{K_{\mathrm{ref}}}{[\mathrm{H}^+]} \frac{\left\{1 + \frac{[\mathrm{H}^+]}{K_{\mathrm{aADP}}} + (K_{\mathrm{bMgADP}}[\mathrm{Mg}^{2+}]) + \frac{(K_{\mathrm{bMgHADP}}[\mathrm{H}^+][\mathrm{Mg}^{2+}])}{K_{\mathrm{aADP}}}\right\}\left\{1 + \frac{[\mathrm{H}^+]}{K_{\mathrm{aHPO_4}}} + (K_{\mathrm{bMgHPO_4}}[\mathrm{Mg}^{2+}])\right\}}{\left\{1 + \frac{[\mathrm{H}^+]}{K_{\mathrm{aATP}}} + (K_{\mathrm{bMgATP}}[\mathrm{Mg}^{2+}]) + \frac{(K_{\mathrm{bMgHATP}}[\mathrm{H}^+][\mathrm{Mg}^{2+}])}{K_{\mathrm{aATP}}}\right\}}, \tag{25}$$

where K_{ref} is 7.22×10^{-2} at 38 °C and was calculated from the K' of 2.19×10^{5} mol l^{-1} at pH=7.0, free [Mg^{2+}]=1.0 mmol l^{-1}, I=0.25 mol l^{-1} and T=38 °C (Guynn and Veech, 1973). The adjustment of K'_{ATP} for a range of pH and free [Mg^{2+}] values is shown in Table 5.

Biochemical and physiological applications of thermodynamic data

The primary aim of this study is to provide biochemists and physiologists with a number of thermodynamic expressions that will enable them to adjust K' of key equilibria to the pH and free [Mg^{2+}] of their experimental system at I=0.25 mol l^{-1} and 38 °C. Such information is essential for quantifying the bioenergetic state of a tissue or organ. The reactant concentrations ATP and PCr and the parameters pH and pMg may be obtained by phosphorus magnetic resonance spectroscopy (^{31}P MRS) and the total creatine concentration by conventional metabolic analysis methodology (Chance *et al.* 1988; Conway and Radda, 1991; Gadian, 1982; Gadian and Radda, 1981; Ingwall, 1982; Kushmerick and Meyer, 1985; Meyer *et al.* 1982). It is important to convert the tissue enzymatic measurements from units of μmol g^{-1} wet mass to mol l^{-1}, which requires estimations of the total tissue water space and the intra- and extracellular water distribution of that total water space for the organ system under investigation (Dobson *et al.* 1992; Masuda *et al.* 1990).

Free cytosolic [ADP]

Free cytosolic [ADP] (in mol l^{-1}) can be calculated from rearrangement of the equilibrium expression (equation 3):

$$[ADP] = \frac{[ATP][Cr]}{[PCr]\,K'_{CK}}. \tag{26}$$

Before calculating [ADP], the apparent equilibrium constant, K'_{CK}, needs to be adjusted to the pH and free [Mg^{2+}] of the experimental conditions at I=0.25 mol l^{-1} and 38 °C using equation 9. The [ADP] calculated this way is often called the free cytosolic [ADP], which has been shown to be 20- to 50-fold less than the total measured tissue content (Bünger and Soboll, 1986; Seraydarian *et al.* 1962; Veech *et al.* 1979).

Cytosolic phosphorylation ratio

The phosphorylation ratio is often referred to as the phosphorylation potential, but it is not a potential with units of energy, but rather a ratio of the reactants of the ATP hydrolysis reaction in units of concentration (Slater, 1976). The phosphorylation ratio (in l mol^{-1}) is calculated from an arrangement of the creatine kinase equilibrium, and the free cytosolic orthophosphate concentration, [P_i], which must be determined independently:

$$\frac{[ATP]}{[ADP][P_i]} = \frac{[PCr]\,K'_{CK}}{[Cr]}\,\frac{1}{[P_i]}. \tag{27}$$

Before calculating the phosphorylation ratio, K'_{CK} needs to be adjusted to the pH and free [Mg^{2+}] of the experimental conditions at I=0.25 mol l^{-1} and 38 °C using equation 9.

Free cytosolic [AMP]

Free cytosolic [AMP] (in mol l^{-1}) can be calculated from rearrangement of the adenylate equilibrium expression (equation 11):

$$[AMP] = \frac{[ADP]^2\,K'_{AK}}{[ATP]}. \tag{28}$$

Prior to calculation of free [AMP], K'_{AK} needs to be adjusted to the pH and free [Mg^{2+}] of the experimental conditions at I=0.25 mol l^{-1} and 38 °C using equation 17. The [AMP] calculated in this way is often called the free cytosolic [AMP], which has been shown to be 20- to 50-fold less than the total measured tissue content (Bünger and Soboll, 1986).

Calculation of the $\Delta_f G'$ of ATP hydrolysis: relevance to biological systems

Since ATP is the primary energy currency of a cell, it is the chemical potential of its hydrolysis (equations 18, 19), as opposed to its synthesis, that drives the extent and direction of the energy transformations in living systems (Krebs and Kornberg, 1957). The transformed Gibbs energy of ATP hydrolysis, $\Delta_f G'_{ATP}$, can be determined from the following equation:

$$\Delta_f G'_{ATP} = \Delta_f G'^{\circ}_{ATP} + \boldsymbol{R}T \ln \frac{[ADP][P_i]}{[ATP]}, \tag{29}$$

where $\boldsymbol{R}$ is the gas constant, T is the temperature (in Kelvin; see Table 1) and $\Delta_f G'^{\circ}_{ATP}$ is the standard transformed Gibbs energy of ATP hydrolysis (ATP + H_2O = ADP + P_i) at a specified pH, free [Mg^{2+}], I, P and T (see below). The cytosolic phosphorylation ratio, [ATP]/([ADP][P_i]), is calculated from the creatine kinase equilibrium expression (equation 27). It should be noted that the phosphorus metabolite values in equations 26–29 represent their free concentrations as determined by ^{31}P magnetic resonance spectroscopy, rather than total tissue measurements.

Table 3. *Adjustment of* K'_{CK} *with varying pH and pMg for the creatine kinase equilibrium at* T=*38 °C,* P=*0.1 MPa and* I=*0.25 mol* l^{-1}

		$[Mg^{2+}]$ (mol l^{-1})													
pH	$[H^+]$ (mol l^{-1})	0	10^{-4}	2×10^{-4}	3×10^{-4}	4×10^{-4}	5×10^{-4}	6×10^{-4}	7×10^{-4}	8×10^{-4}	9×10^{-4}	10^{-3}	1.1×10^{-3}	1.2×10^{-3}	1.3×10^{-3}
6.4	3.98×10^{-7}	174.88	238.61	295.27	345.95	391.50	432.65	469.97	503.95	535.00	563.45	589.61	613.71	635.98	656.60
6.5	3.16×10^{-7}	136.70	192.64	241.83	285.40	324.23	359.01	390.34	418.67	444.40	467.84	489.28	508.94	527.02	543.69
6.6	2.51×10^{-7}	106.84	155.31	197.50	234.52	267.24	296.34	322.37	345.77	366.90	386.06	403.50	419.41	434.00	447.39
6.7	2.00×10^{-7}	83.54	125.04	160.81	191.93	219.23	243.35	264.80	283.97	301.20	316.75	330.84	343.66	355.36	366.06
6.8	1.58×10^{-7}	65.38	100.51	130.53	156.45	179.03	198.87	216.41	232.02	245.98	258.54	269.87	280.15	289.50	298.03
6.9	1.26×10^{-7}	51.23	80.67	105.64	127.05	145.59	161.79	176.06	188.70	199.96	210.06	219.14	227.36	234.81	241.59
7.0	1.00×10^{-7}	40.20	64.66	85.25	102.81	117.94	131.11	142.65	152.85	161.90	169.99	177.26	183.81	189.74	195.13
7.1	7.94×10^{-8}	31.59	51.75	68.62	82.94	95.23	105.88	115.18	123.38	130.64	137.11	142.90	148.12	152.83	157.11
7.2	6.31×10^{-8}	24.86	41.37	55.12	66.73	76.67	85.25	92.73	99.30	105.10	110.27	114.89	119.04	122.78	126.17
7.3	5.01×10^{-8}	19.59	33.03	44.18	53.57	61.57	68.47	74.46	79.72	84.36	88.48	92.15	95.45	98.43	101.12
7.4	3.98×10^{-8}	15.46	26.35	35.36	42.92	49.35	54.88	59.67	63.87	67.57	70.85	73.78	76.40	78.76	80.89

Table 4. *Adjustment of* K'_{AK} *with varying pH and pMg for the adenylate kinase equilibrium at* T=*38 °C,* P=*0.1 MPa and* I=*0.25 mol* l^{-1}

		$[Mg^{2+}]$ (mol l^{-1})													
pH	$[H^+]$ (mol l^{-1})	0	10^{-4}	2×10^{-4}	3×10^{-4}	4×10^{-4}	5×10^{-4}	6×10^{-4}	7×10^{-4}	8×10^{-4}	9×10^{-4}	10^{-3}	1.1×10^{-3}	1.2×10^{-3}	1.3×10^{-3}
6.4	3.98×10^{-7}	0.380	0.489	0.579	0.647	0.701	0.743	0.775	0.800	0.819	0.833	0.844	0.851	0.855	0.857
6.5	3.16×10^{-7}	0.380	0.503	0.601	0.674	0.729	0.772	0.804	0.828	0.845	0.857	0.865	0.870	0.873	0.873
6.6	2.51×10^{-7}	0.380	0.516	0.622	0.698	0.756	0.798	0.829	0.852	0.867	0.878	0.884	0.887	0.887	0.885
6.7	2.00×10^{-7}	0.380	0.528	0.641	0.721	0.780	0.822	0.852	0.873	0.887	0.895	0.900	0.900	0.899	0.895
6.8	1.58×10^{-7}	0.380	0.540	0.659	0.742	0.801	0.843	0.872	0.891	0.903	0.910	0.912	0.912	0.908	0.903
6.9	1.26×10^{-7}	0.379	0.550	0.674	0.760	0.820	0.861	0.889	0.907	0.917	0.922	0.923	0.920	0.916	0.909
7.0	1.00×10^{-7}	0.379	0.559	0.688	0.775	0.835	0.876	0.903	0.919	0.929	0.932	0.931	0.928	0.922	0.914
7.1	7.94×10^{-8}	0.378	0.566	0.700	0.789	0.849	0.889	0.915	0.930	0.938	0.940	0.938	0.933	0.926	0.918
7.2	6.31×10^{-8}	0.377	0.573	0.710	0.800	0.860	0.900	0.925	0.939	0.946	0.947	0.944	0.938	0.930	0.921
7.3	5.01×10^{-8}	0.377	0.579	0.718	0.809	0.869	0.909	0.933	0.946	0.952	0.952	0.948	0.942	0.933	0.923
7.4	3.98×10^{-8}	0.376	0.583	0.725	0.817	0.877	0.916	0.939	0.952	0.957	0.956	0.952	0.945	0.935	0.925

Table 5. *Adjustment of* K'_{ATP} *with varying pH and pMg for the ATP hydrolysis equilibrium at* T=*38 °C*, P=*0.1 MPa and* I=*0.25 mol l*$^{-1}$

pH	$[H^+]$ (mol l^{-1})	$[Mg^{2+}]$ (mol l^{-1})												
		0	10^{-4}	2×10^{-4}	3×10^{-4}	4×10^{-4}	5×10^{-4}	6×10^{-4}	7×10^{-4}	8×10^{-4}	9×10^{-4}	10^{-3}	1.1×10^{-3}	1.2×10^{-3}	1.3×10^{-3}
6.4	3.98×10^{-7}	4.08×10^{5}	2.99×10^{5}	2.41×10^{5}	2.06×10^{5}	1.82×10^{5}	1.65×10^{5}	1.52×10^{5}	1.41×10^{5}	1.33×10^{5}	1.26×10^{5}	1.21×10^{5}	1.16×10^{5}	1.12×10^{5}	1.08×10^{5}
6.5	3.16×10^{-7}	4.56×10^{5}	3.24×10^{5}	2.57×10^{5}	2.19×10^{5}	1.92×10^{5}	1.74×10^{5}	1.60×10^{5}	1.49×10^{5}	1.40×10^{5}	1.33×10^{5}	1.28×10^{5}	1.23×10^{5}	1.18×10^{5}	1.15×10^{5}
6.6	2.51×10^{-7}	5.16×10^{5}	3.55×10^{5}	2.79×10^{5}	2.35×10^{5}	2.07×10^{5}	1.86×10^{5}	1.71×10^{5}	1.60×10^{5}	1.51×10^{5}	1.43×10^{5}	1.37×10^{5}	1.32×10^{5}	1.28×10^{5}	1.24×10^{5}
6.7	2.00×10^{-7}	5.92×10^{5}	3.96×10^{5}	3.07×10^{5}	2.58×10^{5}	2.26×10^{5}	2.04×10^{5}	1.87×10^{5}	1.75×10^{5}	1.65×10^{5}	1.57×10^{5}	1.50×10^{5}	1.45×10^{5}	1.40×10^{5}	1.36×10^{5}
6.8	1.58×10^{-7}	6.87×10^{5}	4.47×10^{5}	3.44×10^{5}	2.88×10^{5}	2.52×10^{5}	2.27×10^{5}	2.08×10^{5}	1.95×10^{5}	1.84×10^{5}	1.75×10^{5}	1.68×10^{5}	1.62×10^{5}	1.57×10^{5}	1.52×10^{5}
6.9	1.26×10^{-7}	8.06×10^{5}	5.12×10^{5}	3.91×10^{5}	3.26×10^{5}	2.85×10^{5}	2.56×10^{5}	2.36×10^{5}	2.20×10^{5}	2.08×10^{5}	1.98×10^{5}	1.90×10^{5}	1.84×10^{5}	1.78×10^{5}	1.73×10^{5}
7.0	1.00×10^{-7}	9.55×10^{5}	5.94×10^{5}	4.51×10^{5}	3.75×10^{5}	3.27×10^{5}	2.95×10^{5}	2.71×10^{5}	2.53×10^{5}	2.39×10^{5}	2.28×10^{5}	2.19×10^{5}	2.12×10^{5}	2.05×10^{5}	2.00×10^{5}
7.1	7.94×10^{-8}	1.14×10^{6}	6.98×10^{5}	5.27×10^{5}	4.37×10^{5}	3.81×10^{5}	3.43×10^{5}	3.16×10^{5}	2.95×10^{5}	2.79×10^{5}	2.67×10^{5}	2.56×10^{5}	2.47×10^{5}	2.40×10^{5}	2.34×10^{5}
7.2	6.31×10^{-8}	1.38×10^{6}	8.30×10^{5}	6.23×10^{5}	5.16×10^{5}	4.50×10^{5}	4.05×10^{5}	3.73×10^{5}	3.49×10^{5}	3.30×10^{5}	3.15×10^{5}	3.03×10^{5}	2.93×10^{5}	2.84×10^{5}	2.77×10^{5}
7.3	5.01×10^{-8}	1.68×10^{6}	9.95×10^{5}	7.45×10^{5}	6.16×10^{5}	5.37×10^{5}	4.84×10^{5}	4.45×10^{5}	4.17×10^{5}	3.94×10^{5}	3.77×10^{5}	3.62×10^{5}	3.50×10^{5}	3.40×10^{5}	3.32×10^{5}
7.4	3.98×10^{-8}	2.05×10^{6}	1.20×10^{6}	8.99×10^{5}	7.42×10^{5}	6.47×10^{5}	5.82×10^{5}	5.37×10^{5}	5.02×10^{5}	4.76×10^{5}	4.54×10^{5}	4.37×10^{5}	4.23×10^{5}	4.11×10^{5}	4.01×10^{5}

Calculation of the $\Delta_f G'^{\circ}$ *of ATP hydrolysis with varying free* $[Mg^{2+}]$ *and pH at* I=*0.25 mol l*$^{-1}$ *and* T=*38 °C*

The standard apparent Gibbs energy of ATP hydrolysis ($ATP + H_2O = ADP + P_i$) provides a quantitative measure of the chemical potential for phosphate group transfer between the reactants ATP, ADP and P_i for specifed ionic conditions, *I*, *P* and *T*. This is in contrast to the ATP 'high-energy phosphate' concept, which refers to bond energy and not to the free energy difference between the reacting components of a specified reaction (Lipmann, 1941). $\Delta_f G'^{\circ}_{ATP}$ is calculated by equating the apparent transformed Gibbs energy ($\Delta_f G'$) to zero and solving using the following equation:

$$\Delta_f G'^{\circ}_{ATP} = -\boldsymbol{R}T\ln K'_{ATP}, \qquad (30)$$

where K'_{ATP} is the apparent equilibrium constant of the ATP hydrolysis reaction (equation 19), $\boldsymbol{R}$ is the gas constant and T is the temperature (in Kelvin; Table 1). $\Delta_f G'^{\circ}_{ATP}$ can also be related to the standard transformed enthalpy and standard transformed entropy of reaction where $\Delta_f G'^{\circ} = \Delta_f H'^{\circ} - T\Delta_f S'^{\circ}$ (symbols defined in Table 1).

Conclusion

The present study has provided mathematical expressions for calculating the apparent equilibrium constant (K') of the creatine kinase, adenylate kinase and ATP hydrolysis reactions in terms of K_{ref} and the appropriate acid-dissociation and magnesium-binding constants. We have calculated K_{ref} for each reaction and demonstrated how K' can be adjusted to varying levels of experimental pH and free $[Mg^{2+}]$. Tables of K' as a function of pH and free $[Mg^{2+}]$ at I=0.25 mol l^{-1} and T=38 °C have also been provided for convenience. Finally, we have indicated some of the biochemical applications for using the equilibrium constants in assessing cellular bioenergetics taking place under physiological and pathophysiological conditions.

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