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# ADJUSTMENT OF $K'$ FOR THE CREATINE KINASE, ADENYLATE KINASE AND ATP HYDROLYSIS EQUILIBRIA TO VARYING TEMPERATURE AND IONIC STRENGTH

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## Summary

**Comparative physiologists and biochemists working with tissues at varying temperatures and ionic strength are required to adjust apparent equilibrium constants ($K'$) of biochemical reactions to the experimental conditions prior to calculating cytosolic bioenergetic parameters (transformed Gibbs free energy of formation, $\Delta_f G'_{ATP}$; cytosolic phosphorylation ratio, [ATP]/[ADP][$P_i$]; [phosphocreatine]:[orthophosphate] ratio [PCr]/[$P_i$]) and kinetic parameters (free [ADP], [$P_i$] and [AMP]). The present study shows how to adjust both $K'$ and the equilibrium constants of reference reactions ($K_{ref}$) 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) to temperature and ionic strength. This information, together with our previous study showing how to adjust equilibria to varying pH and pMg, is vital for the quantification of organ and tissue bioenergetics of ectotherms and endotherms under physiological conditions.**

Key words: creatine kinase, ATP hydrolysis, adenylate kinase, pH, free $Mg^{2+}$, bioenergetics, metabolism, thermodynamics, enthalpy, ionic strength.

## Introduction

Temperature is directly linked to the metabolism and distribution of organisms because it affects reaction rates (Arrhenius, 1915) and thermodynamic equilibria (Van't Hoff, 1898; Teague and Dobson, 1992). In the polar regions, marine fish and invertebrate life are found at temperatures of −1.8 °C (Schmidt-Nielsen, 1991; Withers, 1992), whereas in the hot springs, some organisms survive water temperatures above 50 °C (Wickstrom and Castenholz, 1973). Some subarctic insects, intertidal marine bivalves, gastropods and barnacles, and at least four species of terrestrial hibernating frogs, survive temperatures below freezing by depressing the supercooling point of their body fluids (Storey and Storey, 1988). Other organisms have been found in hydrothermal vents and can survive temperatures in excess of 100 °C (Prosser, 1986).

In a previous study we showed (i) how to adjust a number of key near-equilibrium kinase reactions to pH and pMg at 38 °C and ionic strength of 0.25 mol $l^{-1}$, and (ii) how to use these expressions to calculate the cytosolic phosphorylation ratio ([ATP]/[ADP][$P_i$]), free cytosolic [ADP] and free cytosolic [AMP] (Golding *et al.* 1995). Because many organisms maintain body temperatures below 38 °C and because many are subjected to wide daily or seasonal temperature fluctuations (Schmidt-Nielsen, 1991), the aim of the present study is to extend the utility of the methods of adjustment of equilibria to include temperature ($T$) and ionic strength ($I$). We calculate, for example, that $K'$ of creatine kinase increases by a factor of nearly two as temperature decreases from 38 to 5 °C. If these temperature adjustments are not taken into account, in combination with the effects of pH and pMg (Golding *et al.* 1995), serious errors can enter our calculations and affect our understanding of the strategies of metabolic adaptation of vertebrates and invertebrates, including the bioenergetics of scaling (Dobson and Headrick, 1995).

## Theory and calculations

In order to adjust the $K'$ of a biochemical reaction to a new $T$ and $I$, it is important first to understand the difference between a $K'$ and a $K_{ref}$ (Alberty, 1994; Golding *et al.* 1995; Teague and Dobson, 1992). We will use the creatine kinase equilibrium as an example.

Biochemical equation:

$$PCr + ADP = ATP + Cr, \quad (1)$$

where

$$K'_{CK} = \frac{[ATP][Cr]}{[ADP][PCr]}, \quad (2)$$

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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. The chemical equation for the above reaction can now be written.

Chemical equation:

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

where

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

The mathematical relationship between $K'$ (which may be experimentally determined at specified values of pH, pMg, $T$ and $I$) and $K_{ref}$ (where each reactant represents a defined ionic species and the equilibrium constant must be calculated from a system of equations) is:

$$K'_{CK} = K_{ref}\frac{[H^{+}]\left\{1+\frac{[H^{+}]}{K_{aATP}}+(K_{bMgATP}[Mg^{2+}])+\frac{(K_{bMgHATP}[H^{+}][Mg^{2+}])}{K_{aATP}}\right\}}{\left\{1+\frac{[H^{+}]}{K_{aADP}}+(K_{bMgADP}[Mg^{2+}])+\frac{(K_{bMgHADP}[H^{+}][Mg^{2+}])}{K_{aADP}}\right\}\left\{1+\frac{[H^{+}]}{K_{aPCr}}+(K_{bMgPCr}[Mg^{2+}])\right\}}, \tag{5}$$

From the above equation, we can see that $K'$ equals $K_{ref}$ multiplied by an expression consisting of pH, pMg and all the appropriate acid-dissociation ($K_a$) and magnesium-binding constants ($K_b$) of the major ionic species in our equilibrium system. Thus, the effect of pH and pMg on $K'$ is exerted through their participation in the right-hand side of equation 5 (Golding *et al.* 1995). For our present calculations, $K_{ref}$ and the acid-dissociation constants ($K_a$ values) and magnesium-binding constants ($K_b$ values) must all be adjusted to $T$ and $I$ because it is through these chemical equations that temperature and ionic strength influence $K'$.

### *Method of adjusting* K′ *to a new* T *and* I *at specified pH and pMg*

Given $K_{ref}$ (at specified values of $T$ and $I$) from the published literature, the sequence of mathematical operations required to adjust $K'$ to new experimental $T$ and $I$ is as follows.

(1) Adjust $K_{ref}$ from ionic strength 0.25 mol $l^{-1}$ to the condition of $I$=0 mol $l^{-1}$ using the extended Debye–Hückel equation.

(2) Adjust $K_{ref}$ (now at $I$=0 mol $l^{-1}$) to the new temperature (18 °C) using the Van't Hoff equation.

(3) Adjust $K_{ref}$ to the new ionic strength ($I$=0.15 mol $l^{-1}$), again using the extended Debye–Hückel equation.

(4) Adjust all metal-binding and acid-dissociation constants in the same way and substitute them into the right-hand side of equation 5.

(5) $K'$ can now be calculated at specified pH, pMg, $T$, and $I$ (Golding *et al.* 1995).

### *Example calculation*

**Calculate $K'$ of creatine kinase (CK) at $T$=18 °C and $I$=0.15 mol $l^{-1}$ (pH 7.0, pMg 3.0)**

From published thermodynamic data on creatine kinase (Teague and Dobson, 1992): $K_{ref}$ for CK is $4.959\times10^{8}$ l $mol^{-1}$ (25 °C, $I$=0.25 mol $l^{-1}$) and enthalpy (ΔH) for $K_{ref}$ CK (equation 3) is $-16.73$ kJ $mol^{-1}$ at $I$=0.25 mol $l^{-1}$.

**Step 1: adjust $K_{ref}$ from $I$=0.25 mol $l^{-1}$ to $I$=0 mol $l^{-1}$**

Using the extended form of the Debye–Hückel equation (Clarke and Glew, 1980; Alberty and Goldberg, 1992), adjust $K_{ref}$ from $I$=0.25 mol $l^{-1}$ to $I$=0 mol $l^{-1}$. This adjustment is important because the ΔH° value used in the next calculation is given at $I$=0 mol $l^{-1}$ (Table 1).

$$K_{ref(I=0,\ T=\text{finite})} = K_{ref(I \text{ and } T \text{ both finite})}\Gamma, \tag{6}$$

where

$$\Gamma = \frac{\Pi\gamma_{(I=\text{finite})}\text{products}}{\Pi\gamma_{(I=\text{finite})}\text{reactants}}, \tag{7}$$

$$\ln\gamma = \frac{-A_m I^{1/2} z^2}{1 + BI^{1/2}}, \tag{8}$$

and $\gamma$ is the activity coefficient of each separate ionic species in the $K_{ref}$, $A_m$ is the Debye–Hückel constant ('ion-size parameter'), where $A_m$=3[−16.390+(261.337/$T$)+3.369ln$T$−1.437($T$/100)+0.112($T$/100)$^2$] with $T$ in K (Clarke and Glew, 1980; Alberty and Goldberg, 1992) and $T$(K)=273.15+$t$, $I$ is ionic strength (mol $l^{-1}$), $B$ is 1.6 $kg^{1/2}$ $mol^{-1/2}$, $z$ is charge; Π indicates the product of the specified values, and $t$ is temperature (°C).

Table 1. *Thermodynamic data for the creatine kinase, adenylate kinase and ATP hydrolysis equilibria required to adjust* K′ *and* $K_{ref}$ *to temperature and ionic strength*

| Reaction | Equilibrium constant, $K_{ref}$ | Γ | $K_{ref}$ for $I$=0, $T$=25 °C | ΔH° at $I$=0, $T$=25 °C (kJ mol$^{-1}$) |
|---|---|---|---|---|
| $HATP^{3-} \leftrightarrow H^+ + ATP^{4-}$ | $\frac{[H^+][ATP^{4-}]}{[HATP^{3-}]}$ | $\frac{\gamma(1)\gamma(4)}{\gamma(3)}$ | $2.512\times10^{-8}$ | −6.30 |
| $HADP^{2-} \leftrightarrow H^+ + ADP^{3-}$ | $\frac{[H^+][ADP^{3-}]}{[HADP^{2-}]}$ | $\frac{\gamma(1)\gamma(3)}{\gamma(2)}$ | $6.607\times10^{-8}$ | −5.60 |
| $HAMP^{1-} \leftrightarrow H^+ + AMP^{2-}$ | $\frac{[H^+][AMP^{2-}]}{[HAMP^{1-}]}$ | $\gamma(2)$ | $1.862\times10^{-7}$ | −5.40 |
| $HPCr^{1-} \leftrightarrow H^+ + PCr^{2-}$ | $\frac{[H^+][PCr^{2-}]}{[HPCr^{1-}]}$ | $\gamma(2)$ | $8.854\times10^{-6}$ | 2.66 |
| $H_2PO_4^{1-} \leftrightarrow H^+ + HPO_4^{2-}$ | $\frac{[H^+][HPO_4^{2-}]}{[H_2PO_4^{1-}]}$ | $\gamma(2)$ | $6.026\times10^{-8}$ | 3.60 |
| $Mg^{2+} + ATP^{4-} \leftrightarrow MgATP^{2-}$ | $\frac{[MgATP^{2-}]}{[Mg^{2+}][ATP^{4-}]}$ | $\frac{1}{\gamma(4)}$ | $1.514\times10^{6}$ | 22.90 |
| $Mg^{2+} + HATP^{3-} \leftrightarrow MgHATP^{1-}$ | $\frac{[MgHATP^{1-}]}{[Mg^{2+}][HATP^{3-}]}$ | $\frac{\gamma(1)}{\gamma(2)\gamma(3)}$ | $4.266\times10^{3}$ | 16.90 |
| $Mg^{2+} + ADP^{3-} \leftrightarrow MgADP^{1-}$ | $\frac{[MgADP^{1-}]}{[Mg^{2+}][ADP^{3-}]}$ | $\frac{\gamma(1)}{\gamma(2)\gamma(3)}$ | $4.466\times10^{4}$ | 19.0 |
| $Mg^{2+} + HADP^{2-} \leftrightarrow MgHADP$ | $\frac{[MgHADP]}{[Mg^{2+}][HADP^{2-}]}$ | $\frac{1}{\gamma(2)^2}$ | $3.163\times10^{2}$ | 12.50 |
| $Mg^{2+} + AMP^{2-} \leftrightarrow MgAMP$ | $\frac{[MgAMP]}{[Mg^{2+}][AMP^{2-}]}$ | $\frac{1}{\gamma(2)^2}$ | $6.165\times10^{2}$ | 11.30 |
| $Mg^{2+} + PCr^{2-} \leftrightarrow MgPCr$ | $\frac{[MgPCr]}{[Mg^{2+}][PCr^{2-}]}$ | $\frac{1}{\gamma(2)^2}$ | $2.320\times10^{2}$ | 8.19 |
| $Mg^{2+} + HPO_4^{2-} \leftrightarrow MgHPO_4$ | $\frac{[MgHPO_4]}{[Mg^{2+}][HPO_4^{2-}]}$ | $\frac{1}{\gamma(2)^2}$ | $5.128\times10^{2}$ | 12.20 |
| $PCr^{2-} + ADP^{3-} + H^+ \leftrightarrow ATP^{4-} + Cr$ | $\frac{[ATP^{4-}][Cr]}{[ADP^{3-}][PCr^{2-}][H^+]}$ | $\frac{\gamma(4)}{\gamma(1)\gamma(2)\gamma(3)}$ | $2.58\times10^{8}$ | −17.55 |
| $2ADP^{3-} \leftrightarrow ATP^{4-} + AMP^{2-}$ | $\frac{[ATP^{4-}][AMP^{2-}]}{[ADP^{3-}]^2}$ | $\frac{\gamma(4)\gamma(2)}{\gamma(3)^2}$ | $2.248\times10^{-1}$ | −1.50 |
| $ATP^{4-} + H_2O \leftrightarrow ADP^{3-} + HPO_4^{2-} + H^+$ | $\frac{[ADP^{3-}][HPO_4^{2-}][H^+]}{[ATP^{4-}][H_2O]}$ | $\frac{\gamma(3)\gamma(2)\gamma(1)}{\gamma(4)}$ | $2.946\times10^{-1}$ | −20.50 |

The ratio of activity coefficients ($\gamma$) is defined as Γ where $\Gamma=\Pi(\gamma_{products})/\Pi(\gamma_{reactants})$ and $K(I=0)=K(I)\Gamma$ (see text for details).

Thermodynamic values were obtained from Alberty and Goldberg (1992) with exception of the equilibria involving phosphocreatine (Teague and Dobson, 1992).

ΔHº, enthalpy; Π, product of specified values; $I$, ionic strength; $T$, absolute temperature.

Therefore, for the creatine kinase reaction:

$$K_{ref(I=0,T=25\,^{\circ}C)} = (4.959\times10^{8}\,\mathrm{l\,mol^{-1}})\left[\frac{(\gamma Cr_{I=0.25})(\gamma ATP^{4-}{}_{I=0.25})}{(\gamma PCr^{2-}{}_{I=0.25})(\gamma ADP^{3-}{}_{I=0.25})(\gamma H^{+}{}_{I=0.25})}\right] = 2.581\times10^{8}.$$

As a point of clarification, if the published ΔH is at an ionic strength other than $I$=0 mol l$^{-1}$, as with our values for creatine kinase which were at $I$=0.25 mol l$^{-1}$ (Teague and Dobson, 1992), ΔH must be adjusted to $I$=0 mol l$^{-1}$ before proceeding to step 2. The formula used for such an adjustment is as follows:

$$\Delta H(I=\text{finite}) = \Delta H^{\circ}(I=0) + \left(\frac{1.4775\sqrt{I}}{1+1.60\sqrt{I}}\right)\times(\sum z_{products}{}^2 - \sum z_{reactants}{}^2), \quad (9)$$

where 1.4775 and 1.60 are constants and $\sum z^2$ is the sum of the squared individual charges of the reactant species (Goldberg and Tewari, 1991).

The $\Delta H^{\circ}(I=0)$ for the creatine kinase reaction can now be calculated:

$$-16.73\,\mathrm{kJ\,mol^{-1}} = \Delta H^{\circ}(I=0) + \left(\frac{1.4775\sqrt{0.25}}{1 + 1.60\sqrt{0.25}}\right) \times 2\,,$$

yielding

$$\Delta H^{\circ}(I=0) = -17.55\,\mathrm{kJ\,mol^{-1}}\,.$$

**Step 2: adjust $K_{ref}$ (now at $I$=0 mol l$^{-1}$) to the new $T$=18 °C of the experiment**

Using the value of $\Delta H^{\circ}$ at $I$=0 mol l$^{-1}$, $K_{ref}$ is adjusted to a new temperature ($T_2$) using the Van't Hoff equation:

$$\log\left(\frac{K_2}{K_1}\right) = \frac{+\Delta H^{\circ}}{2.303\boldsymbol{R}}\left(\frac{T_2 - T_1}{T_1 T_2}\right), \qquad (10)$$

where $K_1$ is the value of the equilibrium constant at $I$=0 mol l$^{-1}$ and at the given temperature, $T_1$ is the temperature given for $K_1$ in Kelvin, and $K_2$ is the unknown value of the equilibrium constant at the new temperature $T_2$ ($\boldsymbol{R}$=8.3145 J K$^{-1}$ mol$^{-1}$, $\Delta H^{\circ}$ must be in J mol$^{-1}$).

For the $K_{ref}$ reaction (equation 3), $\Delta H^{\circ}$=−17.55 kJ mol$^{-1}$ at $I$=0 mol l$^{-1}$ (see Table 1). Substitution of $K_1$, $T_1$ and $T_2$ values in equation 10 yields a $K_{ref}$ of $5.332\times10^{8}$ at 18 °C ($I$=0 mol l$^{-1}$).

**Step 3: adjust $K_{ref}$ to the new ionic strength ($I$=0.15 mol l$^{-1}$)**

Once the $K_{ref}$ has been adjusted to the required temperature (in our example 18 °C), the $K_{ref}$ must then be adjusted from $I$=0 mol l$^{-1}$ to the desired ionic strength (in our example 0.15 mol l$^{-1}$). This is performed as described using the extended form of the Debye–Hückel equation (equation 6), ensuring that the value for $A_m$ in equation 8 is calculated for the new temperature by using the Clarke and Glew data (Clarke and Glew, 1980; Alberty and Goldberg, 1992).

Now that $K_{ref}$ is adjusted to $I$=0.15 mol l$^{-1}$, all of the magnesium-binding constants and acid-dissociation constants (a total of eight in the case of the creatine kinase reaction) must be adjusted in a similar manner. Following adjustment of all the constants, and having substituted these new values into equation 5 at specified pH and pMg, $K'$ may now be calculated. For our example using creatine kinase, $K'=3.127\times10^{2}$ ($T$=18 °C, $I$=0.15 mol l$^{-1}$, pH 7.0, pMg 3.0).

The thermodynamic data necessary to permit similar adjustment calculations for $K'$ of adenylate kinase and ATP hydrolase reactions have been tabulated in Table 1.

## Conclusions

A series of mathematical operations has been provided to facilitate adjustment of $K'$ of a biochemical equilibrium to $T$ and $I$ at specified pH and pMg. It was shown that temperature and ionic strength exert their effect on $K'$ through $K_{ref}$ and the values of $K_a$ and $K_b$ (equation 5). In order to ensure accurate bioenergetic comparisons between organs and tissues from a single species and across species spanning different vertebrate classes, the appropriate biochemical equilibria must be adjusted to the temperature, pH, pMg and ionic strength conditions of the cell. Assuming a constant value for any of these equilibrium constants can lead to errors which could conceivably show significant differences which did not really exist or, conversely, mask important differences that may affect conceptual advancement. We have provided information and theory in this study and in a companion paper dealing with pH and pMg (Golding *et al.* 1995) to make the necessary adjustments of $K'$, thereby permitting quantitative bioenergetic assessment.

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