The Effect of pH and Free Mg^{2+} on ATP Linked Enzymes and the Calculation of Gibbs Free Energy of ATP Hydrolysis†

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The apparent equilibrium constants, K', of biochemical reactions containing substrates which bind $[Mg^{2+}]$ unequally can be significantly altered by changes in free intracellular $[Mg^{2+}]$. Intracellular free $[Mg^{2+}]$ can be estimated by measurements of [citrate]/[isocitrate], a ratio known to vary with tissue free $[Mg^{2+}]$. The combined equilibrium constant for glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate kinase, and triose phosphate isomerase for the three reactions ($K'_{GG\text{-}TPI}$) was corrected using new binding constants for dihydroxyacetone-phosphate and 3-phosphoglycerate. The result of this calculation is demonstrated in the calculation of the free energy of ATP hydrolysis. In addition, the dependence of the equilibrium constant for the glutamine synthetase reaction on pH and free $[Mg^{2+}]$ was demonstrated. Furthermore, a theory linking the $\Delta G'$ value of mitochondrial complex I−II and the cytosolic $\Delta G'$ value of ATP hydrolysis is discussed with evidence from previous publications.

Introduction

In the early part of the twentieth century, it was generally believed that living systems exhibited special "vital forces" or were too complex to be described by classical thermodynamics. It took several decades and the work of dozens of scientists around the world to demonstrate the usefulness of thermodynamic principles in describing biochemical relationships within living, complex systems. The journey began with the elucidation of basic metabolic pathways and has now expanded to include complex mathematical relationships allowing the calculation of the energetics of living organisms.

In 1939, Krebs' student, W. A. Johnson, measured the equilibrium constant of the aconitase reaction (EC 4.2.1.3),[1] and in 1940, Krebs determined the equilibrium constant of the fumarase reaction (EC 4.2.1.2).[2] Both of these reactions were part of the citric acid cycle described three years earlier by Krebs and Johnson.[3] By the early 1950s, many biochemists, recognizing the power of classical thermodynamics, began to determine the equilibrium constants of other important biochemical reactions. In 1953, Krebs[4] determined the equilibrium constant of the transaminase reactions[5] and repeated the determination of the equilibrium constant of the fumarase reaction, as did Robert Alberty.[6] A powerful impetus to the advance of the use of classical thermodynamics in the description of living systems came in 1953 from two publications[7,8] by Ken Burton, a technician in Krebs' lab in Sheffield with a thorough understanding of thermodynamics who later became professor of biochemistry at the University of Sheffield.

The inference that classical thermodynamics could indeed answer important questions about the actual energetics of living systems came in the form of reports from Feodor Lynen and Helmut Holzer and from Theodore Bücher and Martin Klingenburg. They demonstrated, for the first time, that measurements of near-equilibrium metabolite couples [acetaldehyde]/[ethanol][9] in yeast and [lactate]/[pyruvate][10] in rat liver represented an accurate estimate of the cytosolic redox state, free $[NAD_{ox}]/[NAD_{red}]$, in living tissues. The agreement between the calculated free $[NAD_{ox}]/[NAD_{red}]$ ratio obtained by three different NAD linked cytosolic reactions was taken as proof of the validity of this approach.

In 1967, Krebs' lab published an article titled "The redox state of free nicotinamide-adenine dinucleotide in the cytoplasm and mitochondria of rat liver" by D. H. Williamson, P. Lund, and H. A. Krebs.[11] This paper showed that the measured ratio of the reactants of D-β-hydroxybutyrate dehydrogenase (EC 1.1.1.30), located on the mitochondrial inner membrane, gave the same value for the free mitochondrial $[NAD_{ox}]/[NAD_{red}]$ as the reactants of the glutamate dehydrogenase reaction (EC 1.4.1.2), located within the mitochondrial matrix. Other laboratory members showed[12] that the free cytosolic $[NADP_{ox}]/[NADP_{red}]$ ratio could also be determined by the same principles and had a much lower redox potential, −0.42 V, as opposed to the potential of the free cytosolic NAD couple, −0.19 V. The lower redox potential of the NADP system explains why it is used in reductive synthesis, whereas the NAD couple is used to accept electrons from energy producing substrates.

Upon examination of the metabolite couples in near-equilibrium with the NADP and NAD systems, it became apparent that the various redox states with their differing potentials were algebraically related to one another and to the energy of ATP hydrolysis.[13] Armed with the ability to measure cytosolic $[NAD_{ox}]/[NAD_{red}]$ (Table 1), Krebs' lab set out to determine the phosphorylation potential and the

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TABLE 1: The Enzyme Systems Used to Calculate the Cytosolic and Mitochondrial [NAD_{ox}]/[NAD_{red}] Ratio at pH 7[a]

The Enzyme Systems in Near Equilibrium with Free Cytosolic [NAD_{ox}]/[NAD_{red}]		
lactate dehydrogenase (EC 1.1.1.27)	L-lactate$^-$ + NAD_{ox}^- ↔ pyruvate$^-$ + NAD_{red}^{2-} + H^+ $\frac{[NAD_{ox}]_{cyto}}{[NAD_{red}]_{cyto}} = \frac{[pyruvate]}{[lactate]} \times \frac{1}{K'_{LDH}}$	$K'_{LDH} = 1.11 \times 10^{-4}$ at pH 7[11]
malate dehydrogenase (EC 1.1.1.37)	malate^{2-} + NAD_{ox}^- ↔ oxaloacetate^{2-} + NAD_{red}^{2-} + H^+ $\frac{[NAD_{ox}]_{cyto}}{[NAD_{red}]_{cyto}} = \frac{[oxaloacetate]}{[malate]} \times \frac{1}{K'_{MDH}}$	$K'_{MDH} = 2.86 \times 10^{-5}$ at pH 7[50]
α-glycerophosphate dehydrogenase (EC 1.1.1.8)	α-GP^{2-} + NAD_{ox}^- ↔ $DHAP^{2-}$ + NAD_{red}^{2-} + H^+ $\frac{[NAD_{ox}]_{cyto}}{[NAD_{red}]_{cyto}} = \frac{[DHAP]}{[\alpha\text{-GP}]} \times \frac{1}{K'_{\alpha GPDH}}$	$K'_{\alpha GPDH} = 1.35 \times 10^{-4}$ at pH 7[51]
The Enzyme Systems in Near Equilibrium with Free Mitochondrial [NAD_{ox}]/[NAD_{red}]		
β-hydroxybutyrate dehydrogenase (EC 1.1.1.30)	β-hydroxybutyrate$^-$ + NAD_{ox}^- ↔ acetoacetate$^-$ + NAD_{red}^{2-} + H^+ $\frac{[NAD_{ox}]_{mito}}{[NAD_{red}]_{mito}} = \frac{[acetoacetate]}{[\beta\text{-hydroxybutyrate}]} \times \frac{1}{K'_{\beta HBDH}}$	$K'_{\beta HBDH} = 4.93 \times 10^{-2}$ at pH 7[11]
glutamate dehydrogenase (EC 1.4.1.2)	glutamate$^-$ + NAD_{ox}^- ↔ α-ketoglutarate^{2-} + NH_4^+ + NAD_{red}^{2-} + H^+ $\frac{[NAD_{ox}]_{mito}}{[NAD_{red}]_{mito}} = \frac{[\alpha\text{-ketoglutarate}][ammonium]}{[glutamate]} \times \frac{1}{K'_{GLDH}}$	$K'_{GLDH} = 3.87 \times 10^{-6}$ M at pH 7[52]

[a] K' is defined at $T = 38$ °C, pH = 7, and $I = 0.25$.

free energy of ATP hydrolysis in various mammalian tissues. The development of these advances has been recently reviewed.[14]

In order to calculate the phosphorylation potential [ATP]/([ADP][P_i]) and subsequent $\Delta G'$ of ATP hydrolysis in the cytosol, Krebs' lab used the relationship between the reactions of glyceraldehyde-3-phosphate dehydrogenase (GAPDH, E.C. 1.2.1.12), 3-phosphoglycerate kinase (PGK, E.C. 2.7.2.3), and triose phosphate isomerase (TPI, E.C. 5.3.1.1). The phosporylation potential cannot be obtained by measuring each metabolite because ADP is mainly located at mitochondria. Therefore, it became necessary to evaluate the relationship between phosphorylation potential and measurable metabolites.

$$\text{GAPDH reaction}: GAP^{2-} + NAD_{ox}^- + P_i^{2-} \leftrightarrow 1,3DPG^{4-} + NAD_{red}^{2-} + H^+ \quad (1)$$

$$\text{PGK reaction}: ADP^{3-} + 1,3DPG^{4-} \leftrightarrow ATP^{4-} + 3PG^{3-} \quad (2)$$

$$\text{TPI reaction}: GAP^{2-} \leftrightarrow DHAP^{2-} \quad (3)$$

GAPDH + PGK − TPI reaction :

$$DHAP^{2-} + NAD_{ox}^- + ADP^{3-} + P_i^{2-} \leftrightarrow 3PG^{3-} + NAD_{red}^{2-} + H^+ + ATP^{4-} \quad (4a)$$

$$DHAP + NAD_{ox} + ADP + P_i \leftrightarrow 3PG + NAD_{red} + ATP \quad (4b)$$

The combined equilibrium constant ($K'_{GG\text{-}TPI}$) can be expressed from eq 4b as

$$K'_{GG\text{-}TPI} = \frac{[\sum 3PG]}{[\sum DHAP]} \times \frac{[NAD_{red}]}{[NAD_{ox}]} \times \frac{[\sum ATP]}{[\sum ADP][\sum P_i]} \quad (5)$$

It is important to note that [NAD_{ox}]/[NAD_{red}] is sometimes referenced as [NAD^+]/[NADH]. In order to measure the combined equilibrium constant at various conditions, Veech and Krebs[11] employed the use of the lactate dehydrogenase reaction (Table 1) to link [NAD_{red}]/[NAD_{ox}] to [L-lactate]/[pyruvate] using K'_{LDH}. The new equilibrium constant ($K'_{GG\text{-}(TPI+LDH)}$) was used to calculate the phosphorylation potential in the cytosol.

$$K'_{GG\text{-}(TPI+LDH)} = \frac{[\sum 3PG]}{[\sum DHAP]} \times \frac{[\text{L-lactate}]}{[pyruvate]} \times \frac{[\sum ATP]}{[\sum ADP][\sum P_i]} \quad (6)$$

For the reactions involving dehydrogenases and iosomerases, ignoring the effects of changes in free [Mg^{2+}] caused no major problems. However, in measuring the equilibrium constant of the combined GAPDH−PGK system (eq 6), ignoring the effects from

$[Mg^{2+}]$ led to major errors of several orders of magnitude and an erroneous conclusion about the magnitude of the phosphorylation potential as well as totally missing the compartmentalization of ADP within the mitochondria of mitochondrial containing tissue.[15] Had attention been paid to the paper of Alberty,[16] which defined the ionization constants of ATP, this error would have been avoided. It would take 10 years to correct this error.

The problem in 1969 was that the free intracellular $[Mg^{2+}]$ was unknown, except for the assumption that it was lower than the approximately 10 mM total magnesium measured in tissues.[17] In the early 1970's, Walser estimated the free serum $[Mg^{2+}]$ to be about 0.5 mM.[18] Shortly after Walser's publication, Veech undertook a project to measure the free intracellular $[Mg^{2+}]$ by comparing total magnesium binding to the components of cell homogenates to the variations in the observed equilibrium constant of the aconitase reaction along with variations in free $[Mg^{2+}]$.[17] The ratio of [citrate]/[isocitrate] varied free $[Mg^{2+}]$ within the physiological range, 0.1 mM to over 1 mM, depending upon tissue and metabolic conditions.

To illustrate the variation in the combined equilibrium constant ($K'_{GG\text{-}(TPI+LDH)}$) system with pH and free $[Mg^{2+}]$, Cornell measured the constant and found it to vary by a factor of 500 depending upon free $[Mg^{2+}]$.[19] To further demonstrate the importance of magnesium binding, Lawson[20] measured the equilibrium constant of the creatine kinase reaction (K'_{CK}, EC 2.7.3.2, eq 7b) at pH 7, free $[Mg^{2+}] = 1$ and 0 mM, $T = 38$ °C, and $I = 0.25$.

$$ADP^{3-} + \text{phosphocreatine}^{2-} + H^+ \leftrightarrow ATP^{4-} + \text{creatine} \quad (7a)$$

$$K'_{CK} = \frac{[\sum \text{ATP}][\sum \text{creatine}]}{[\sum \text{ADP}][\sum \text{phospocreatine}]} \quad (7b)$$

$K'_{CK} = 1.66 \times 10^2$ at free $[Mg^{2+}] = 1$ mM and 0.378×10^2 at free $[Mg^{2+}] = 0$ mM.

The calculated $\Delta G'$ value of ATP hydrolysis was estimated to be about −57.3 kJ/mol in red cells and about −58.5 kJ/mol in other tissues (liver, brain, and muscle).[21] The values obtained for the $\Delta G'$ value of ATP hydrolysis from the GAPDH−PGK combined enzyme system and the metabolites of the creatine kinase couple agreed well with one another. This finding became the basis for energetic estimations of the phosphorylation potential widely used in ^{31}P NMR spectroscopy.[22]

The equilibrium constant of the phosphoglycerate kinase reaction (reaction 2) varied from very low values of around 700 at 0.01 mM free $[Mg^{2+}]$ to over 4000 at 1.2 mM free $[Mg^{2+}]$[19] and presumably would have been even lower at 0 mM free $[Mg^{2+}]$, conditions Krebs' lab had used when the combined constant was first measured in 1968.[23] On repeated, more careful determination, the value of the GAPDH reaction (reaction 1) was found to be independent of variations in free $[Mg^{2+}]$ but dependent upon variation in ionic strength[19] which in tissue was taken to be about 0.25.

Following Alberty's model of defining a K_{ionic} value for the most ionized form of an enzymatic reactant, it was possible to calculate the variation in equilibrium constants for a number of important intracellular reactions.[24,25] At the time these calculations were performed, there was no Mg^{2+} binding constant for 3-phosphoglycerate (3PG) or dihydroxyacetone-phosphate (DHAP) and therefore it was not possible to make the magnesium binding correction for these substrates. The constants have now been calculated, and for the first time, the calculation that demonstrates the variation in equilibrium constant of the GAPDH−PGK combined enzyme system and other significant intracellular reactions, including glutamine synthetase, has been correctly performed.

Methods

Calculating K_{ionic} and K'. In order to calculate the apparent equilibrium constant of various biochemical reactions at specified pH and free $[Mg^{2+}]$, Alberty defined the concept of K_{ionic}.[16]

$$K_{ionicGG\text{-}(TPI+LDH)} = \frac{[3PG^{3-}]}{[DHAP^{2-}]} \times \frac{[ATP^{4-}]}{[ADP^{3-}][P_i^{2-}]} \times \frac{[\text{L-lactate}^-]}{[\text{pyruvate}^-]} M^{-1} \quad (8)$$

K_{ionic} is the equilibrium constant of the reaction written for the most ionized species present and represents a value independent of pH and free $[Mg^{2+}]$. Therefore, it can be used to calculate the apparent equilibrium constant, K', using f functions defined with acid dissociation and magnesium binding constants at various pH and free $[Mg^{2+}]$ conditions. It is important to note that K_{ionic} cannot be determined directly, since there is no measurement specific for the ionic species of each reactant in either tissue or cuvette. As done previously, K_{ionic} was calculated from experimental data with known pH and free $[Mg^{2+}]$.[19,25]

Furthermore, it is important to note the distinction between measured values and subsequent species present. As listed (eqs 9−13), the actual measured component is expressed as a sum of species, e.g., [∑ATP] rather than $[ATP^{4-}]$. The amount of the species present is dependent on the f function defined for that compound, e.g., $[ATP^{4-}] = [\sum ATP]/f ATP$.

$$[\sum \text{ATP}] = [ATP^{4-}] + [HATP^{3-}] + [H_2ATP^{2-}] + [MgATP^{2-}] + [MgHATP^-] = [ATP^{4-}] \times f\text{ATP} \quad (9)$$

$$[\sum \text{ADP}] = [ADP^{3-}] + [HADP^{2-}] + [H_2ADP^-] + [MgADP^-] + [MgHADP] = [ADP^{3-}] \times f\text{ADP} \quad (10)$$

$$[\sum P_i] = [P_i^{2-}] + [HP_i^-] + [MgP_i] = [P_i^{2-}] \times fP_i \quad (11)$$

$$[\sum 3PG] = [3PG^{3-}] + [H3PG^{2-}] + [H_23PG^-] + [Mg3PG^-] + [MgH3PG] = [3PG^{3-}] \times f3PG \quad (12)$$

$$[\sum \text{DHAP}] = [DHAP^{2-}] + [HDHAP^-] + [MgDHAP] = [DHAP^{2-}] \times f\text{DHAP} \quad (13)$$

The ionic equilibrium constant for the combined GAPDH−PGK reaction, $K_{ionicGG\text{-}(TPI+LDH)}$, was calculated from a combined equilibrium constant, $K'_{GG\text{-}(TPI+LDH)}$, with defined pH, temperature, ionic strength, and free $[Mg^{2+}]$ values using the f functions as listed below (eq 14).

$$K_{\text{ionicGG-(TPI+LDH)}} = K'_{\text{GG-(TPI+LDH)}} \times \frac{f\text{DHAP}}{f3\text{PG}} \times \frac{f\text{ADP} \times f\text{P}_\text{i}}{f\text{ATP}} \text{M}^{-1} \quad (14)$$

The f functions (eqs 15− 19) are only defined within physiological pH because of differential speciation at lower or higher pH values. The current calculations rely on acid dissociation constants that have been carefully defined.

$$f\text{ATP} = 1 + \frac{[\text{H}^+]}{K_{\text{aATP}}} + \frac{[\text{H}^+]^2}{K_{\text{aATP}} \times K_{\text{aHATP}}} + [\text{Mg}^{2+}] \times K_{\text{bMgATP}} + \frac{[\text{Mg}^{2+}][\text{H}^+] \times K_{\text{bMgHATP}}}{K_{\text{aATP}}} \quad (15)$$

$$f\text{ADP} = 1 + \frac{[\text{H}^+]}{K_{\text{aADP}}} + \frac{[\text{H}^+]^2}{K_{\text{aADP}} \times K_{\text{aHADP}}} + [\text{Mg}^{2+}] \times K_{\text{bMgADP}} + \frac{[\text{Mg}^{2+}][\text{H}^+] \times K_{\text{bMgHADP}}}{K_{\text{aADP}}} \quad (16)$$

$$f\text{Pi} = 1 + \frac{[\text{H}^+]}{K_{\text{aPi}}} + [\text{Mg}^{2+}] \times K_{\text{bMgPi}} \quad (17)$$

$$f3\text{PG} = 1 + \frac{[\text{H}^+]}{K_{\text{a3PG}}} + [\text{Mg}^{2+}] \times K_{\text{bMg3PG}} \quad (18)$$

$$f\text{DHAP} = 1 + \frac{[\text{H}^+]}{K_{\text{aDHAP}}} + [\text{Mg}^{2+}] \times K_{\text{bMgDHAP}} \quad (19)$$

L-lactate and pyruvate do not significantly bind Mg^{2+} and do not differ in their weak dissociation constant with the result that corrections for changes in free $[Mg^{2+}]$ for that couple are not required. Acid dissociation and Mg^{2+} binding constants for ATP, ADP, P_i, 3PG, and DHAP were taken from previous publications (Table 2).[25−27] All values are listed or calculated at pH 7, $T = 38$ °C, and $I = 0.25$ using the Debye−Hückel theory (see the Supporting Information) for ionic strength correction and the Van't Hoff equation for temperature correction.

The apparent equilibrium constant for the combined enzyme system, $K'_{\text{GG-(TPI+LDH)}}$, can be calculated for approximate in vivo conditions by rearranging eq 14 as follows:

$$K'_{\text{GG-(TPI+LDH)}} = K_{\text{ionicGG-(TPI+LDH)}} \times \frac{f3\text{PG}}{f\text{DHAP}} \times \frac{f\text{ATP}}{f\text{ADP} \times f\text{P}_\text{i}} \text{M}^{-1} \quad (20)$$

Calculation of K_{ionic} and K' for Glutamine Synthetase. The effect of pH and free $[Mg^{2+}]$ changes on the equilibrium constant for glutamine synthetase (GS, E.C. 6.3.1.2) was evaluated in a similar fashion as $K'_{\text{GG-(TPI+LDH)}}$. The reaction for glutamine synthetase is listed below:

$$\text{ATP}^{4-} + \text{L-glutamate}^{2-} + \text{NH}_3 \leftrightarrow \text{ADP}^{3-} + \text{P}_\text{i}^{2-} + \text{L-glutamine}^- \quad (21)$$

TABLE 2: Acid Dissociation Constants and Magnesium Binding Constants at pH 7, $T = 38$ °C, and $I = 0.25$

acid dissociation constants, K_a	magnesium binding constants, K_{bMg}
$K_{aATP} = 3.13 \times 10^{-7}$	$K_{bMgATP} = 9.40 \times 10^3$
$K_{aHATP} = 1.91 \times 10^{-4}$	$K_{bMgHATP} = 1.08 \times 10^2$
$K_{aADP} = 4.26 \times 10^{-7}$	$K_{bMgADP} = 1.20 \times 10^3$
$K_{aHADP} = 2.19 \times 10^{-4}$	$K_{bMgHADP} = 2.82 \times 10^1$
$K_{aPi} = 1.96 \times 10^{-7}$	$K_{bMgPi} = 7.48 \times 10^1$
$K_{a3PG} = 1.95 \times 10^{-7}$	$K_{bMg3PG} = 2.39 \times 10^2$ [a]
$K_{aDHAP} = 1.73 \times 10^{-6}$ [a]	$K_{bMgDHAP} = 1.96 \times 10^1$ [a]
$K_{aCit} = 2.31 \times 10^{-6}$	$K_{bMgCit} = 1.73 \times 10^3$ [a]
$K_{aIsocit} = 2.84 \times 10^{-6}$	$K_{bMgHCit} = 1.96 \times 10^1$ [a]
	$K_{bMgIsocit} = 1.83 \times 10^2$ [a]
	$K_{bMgHIsocit} = 1.97 \times 10^0$ [a]

[a] These values are calculated for $I = 0.25$ and $T = 25$ °C. The correction for temperature could not be performed, since ΔH is unknown. The correction for ionic strength and temperature was performed as done previously using the Debye−Hückel theory and the Van't Hoff equation[25] (see the Supporting Information).

The equilibrium constant, K'_{GS}, is defined as

$$K'_{\text{GS}} = \frac{[\sum \text{ADP}][\sum \text{P}_\text{i}][\text{L-glutamine}]}{[\sum \text{ATP}][\text{L-glutamine}][\text{ammonia}]} \quad (22)$$

$K'_{\text{GS}} = 1.2 \times 10^3$ at pH 7, total $[Mg^{2+}] = 50$ μM, $T = 37$ °C, and $I = 0.2$.[28,29]

The ionic strength of the imidazole buffer was about 0.2 (Benzinger, T. Personal communication). Therefore, in order to calculate K_{ionicGS}, all acid dissociation and magnesium constants have been corrected for $I = 0.2$ with a previous method.[25] In addition, since the free $[Mg^{2+}]$ was unknown, it was calculated as 49.2 μM by solving five simultaneous equations for free $[Mg^{2+}]$ using Mathematica (see the Supporting Information). Finally, K_{ionicGS} was calculated using f functions defined at pH 7, $T = 38$ °C, $I = 0.2$, and free $[Mg^{2+}] = 49$ μM according to eq 23.

$$K_{\text{ionicGS}} = K'_{\text{GS}} \times \frac{f\text{ATP}}{f\text{ADP} \times f\text{P}_\text{i}} \quad (23)$$

The apparent equilibrium constant, K'_{GS}, is calculated like eq 20 by using K_{ionicGS} and defining the pH and free $[Mg^{2+}]$ in the f functions as follows:

$$K'_{\text{GS}} = K_{\text{ionicGS}} \times \frac{f\text{ADP} \times f\text{P}_\text{i}}{f\text{ATP}} \quad (24)$$

Calculation of Free Intracellular Mg^{2+}. Following Alberty's method of defining an ionic species of a substrate at some defined pH and free $[Mg^{2+}]$,[30] free intracellular $[Mg^{2+}]$ can be calculated in tissue using K_{ionic} for the aconitase reaction and Γ_{Aconi} as follows:[17,31]

$$[Mg^{2+}] = \frac{K_{\text{ionicAconi}}\left(1 + \frac{[H^+]}{K_{\text{aCit}}}\right) - \Gamma_{\text{Aconi}}\left(1 + \frac{[H^+]}{K_{\text{aIsoCit}}}\right)}{\Gamma_{\text{Aconi}}\left(K_{\text{bMgIsocit}} + \frac{[H^+] \times K_{\text{bMgHIsocit}}}{K_{\text{aIsocit}}}\right) - K_{\text{ionicAconi}}\left(K_{\text{bMgCit}} + \frac{[H^+] \times K_{\text{bMgHCit}}}{K_{\text{aCit}}}\right)} \quad (25)$$

Γ_{Aconi} is defined as the ratio of [citrate]/[isocitrate] measured in a tissue sample. $K_{\text{ionicAconi}}$ was calculated as 8.78 from the in vitro measured equilibrium, [citrate]/[isocitrate], value of 20 at pH 7 and free $[Mg^{2+}] = 1$ mM.[17]

Calculation of Phosphorylation Potential and $\Delta G'$ of ATP Hydrolysis. Following the relationship in eq 6, it becomes possible to calculate the phosphorylation potential, $[\sum ATP]/([\sum ADP][\sum P_i])$, using the apparent equilibrium constant, $K'_{\text{GG-(TPI+LDH)}}$, at tissue specified pH and free $[Mg^{2+}]$ conditions.

$$\frac{[\sum \text{ATP}]}{[\sum \text{ADP}][\sum \text{P}_\text{i}]} = K'_{\text{GG-(TPI+LDH)}} \frac{[\text{pyruvate}^-]}{[\text{L-lactate}^-]} \times \frac{[\sum \text{DHAP}]}{[\sum \text{3PG}]} \text{M}^{-1} \quad (26)$$

The Gibbs free energy of ATP hydrolysis can be calculated using the inverse of the phosphorylation potential as listed in eq 27 below:

$$\Delta G'_{\text{ATP hydrolysis}} = \Delta G' + RT \ln \frac{[\sum \text{ADP}][\sum \text{P}_\text{i}]}{[\sum \text{ATP}]} \quad (27)$$

To calculate the Gibbs free energy of ATP hydrolysis, the standard Gibbs free energy of ATP hydrolysis, $\Delta G'$, was adjusted for correct pH and free $[Mg^{2+}]$ through eqs 28 and 29.[32]

$$K'_{\text{ATP hydrolysis}} = \frac{[\sum \text{ADP}][\sum \text{P}_\text{i}]}{[\sum \text{ATP}]} = 2.19 \times 10^5 \text{ M} \quad \text{at } T = 38\ ^\circ\text{C}, I = 0.25, \text{free } [Mg^{2+}] = 1 \text{ mM}, \text{pH } 7 \quad (28)$$

$$K_{\text{ionic ATP hydrolysis}} = \frac{[ADP^{3-}][P_i^{2-}][H^+]}{[ATP^{4-}]} = K'_{\text{ATP hydrolysis}} \times \frac{[H^+] \times f\text{ATP}}{f\text{ADP} \times f\text{P}_\text{i}} = 6.09 \times 10^{-2} \text{ M}^2 \quad (29)$$

For eq 29, the *f* functions are defined at pH 7 and free $[Mg^{2+}] = 1$ mM. For in vivo tissue conditions, the apparent equilibrium constant, $K'_{\text{ATP hydrolysis}}$, can be calculated from K_{ionic} as described in eq 30.

$$K'_{\text{ATP hydrolysis}} = K_{\text{ionic ATP hydrolysis}} \times \frac{f\text{ADP} \times f\text{P}_\text{i}}{[H^+] \times f\text{ATP}} \quad \text{under in vivo tissue conditions} \quad (30)$$

From eq 30, it becomes possible to calculate $\Delta G'_{\text{ATP hydrolysis}}$, as illustrated by eq 31.

$$\Delta G'^{\circ}_{\text{ATP hydrolysis}} = -RT \ln(K'_{\text{ATP hydrolysis}}) \quad R = 8.31451 \text{ J/mol} \times \text{K}; T = 311.15 \text{ K} \quad (31)$$

Calculation of Energy between free Mitochondrial NAD and Free Coenzyme Q Couples. A calculation of the change in Gibbs free energy for the first two complexes in the mitochondrial electron transport chain was calculated using standard electrode potentials with a physiologically relevant adjustment to pH 7.2 (Table 3). This energy is referred to as the Gibbs free energy of mitochondrial complex I−II. Also, it was demonstrated that the energy of complex I−II can be calculated independently of the reduction potential of the coenzyme Q system. This can be justified by the equilibration between succinate/fumarate and the coenzyme Q couples established by succinate dehydrogenase (complex II).[33]

$$\text{Complex I (NADH dehydrogen ase)} : NAD^{2-}_{red} + H^+ + CoQ \leftrightarrow NAD^-_{ox} + CoQH_2 \quad (32)$$

$$\text{Complex II (succinate dehydrogen ase)} : \text{succinate}^{2-} + CoQ \leftrightarrow \text{fumarate}^{2-} + CoQH_2 \quad (33)$$

$$\text{NET Reaction (Complex I - Complex II)} : NAD^{2-}_{red} + H^+ + \text{fumarate}^{2-} \leftrightarrow NAD^-_{ox} + \text{succinate}^{2-} \quad (34)$$

TABLE 3: Half Cell Reaction in Electron Transport Chain Complex I and Complex II

reduction reaction	E'° at pH 7, $T = 25$ °C	E'° at pH 7.2, $T = 38$ °C
$NAD_{ox} + H^+ + 2e^- \rightarrow NAD_{red}$	$E'^{\circ}_{NAD/NADH} = -0.32$ V	$E'^{\circ}_{NAD/NADH} = -0.326$ V
$fumarate^{2-} + 2H^+ + 2e^- \rightarrow succinate^{2-}$	$E'^{\circ}_{fum/succ} = 0.031$ V	$E'^{\circ}_{fum/succ} = 0.019$ V
$CoQ + 2H^+ + 2e^- \rightarrow CoQH_2$	$E'^{\circ}_{Q/QH2} = 0.10$ V	$E'^{\circ}_{Q/QH2} = 0.088$ V

The adjustment of the standard reduction potential from pH 7, $T = 25$ °C to pH 7.2, $T = 38$ °C was done using the Nernst equation as follows:[34]

$$E'_{pH7.2} = E'_{pH7} + \frac{RTh}{nF} \ln\frac{(1 \times 10^{-7.2})}{(1 \times 10^{-7.0})} \quad (35)$$

where $R = 8.3145$ J/mol × K, $T = 311.15$ K, $F = 96.485$ J/mol × V, n = number of electrons, and h = number of protons.

In order to calculate the free energy between the redox potentials of complex I and complex II of the electron transport chain, the following calculations were performed:[35]

$$\Delta G'^{\circ} = -nFE'^{\circ} \quad (37)$$

$$\Delta G'_{\text{mito compI}-\text{II}} = \Delta G'^{\circ} + RT \ln(\Gamma_{\text{mito compI}-\text{II}}) \quad (36)$$

In eq 36, $\Gamma_{\text{mito compI}-\text{II}}$ is defined as the ratio of products/reactants in reaction 34. Using eqs 36 and 37, reaction 34 and omitting $[H^+]$, since it is not active in half-cell reactions, eq 36 can be rearranged as follows:

$$\Delta G'_{\text{mito compI}-\text{II}} = -nF(E'^{\circ}_{fum/succ} - E'^{\circ}_{NAD/NADH}) + RT \ln\left(\frac{[succinate^{2-}][NAD_{ox}^{-}]}{[fumarate^{2-}][NAD_{red}^{2-}]}\right) \quad (38)$$

Since the mitochondrial free $[NAD_{ox}]/[NAD_{red}]$ ratio cannot be measured directly, the concentration of the metabolites of the glutamate dehydrogenase reaction was used to determine the free mitochondrial $[NAD_{ox}]/[NAD_{red}]$ ratio.[11] Alternatively, the reactants of the D-β-hydroxybutyrate dehydrogenase reaction may be used instead of those of the glutamate dehydrogenase reaction to calculate the free $[NAD_{ox}]/[NAD_{red}]$ ratio in mitochondria. Substituting K'_{GLDH} from Table 1, eq 38 is rearranged to

$$\Delta G'_{\text{mito compI}-\text{II}} = -nF(E'^{\circ}_{fum/succ} - E'^{\circ}_{NAD/NADH}) + RT \ln\left(\frac{[succinate^{2-}][\alpha\text{-ketoglutarate}^{2-}][NH_4^{+}]}{[fumarate^{2-}][glutamate^{-}] \times K'_{GLDH}}\right) \quad (39)$$

It is important to note that this reaction can be calculated at pH 7.2 using standard electrode potentials corrected for pH as above (Table 3). Also, note that the calculation does not depend on the electrode potential of the coenzyme Q system.

Results and Discussion

The effect of pH and free $[Mg^{2+}]$ on apparent equilibrium constants in biochemical and thermodynamic applications has been discussed extensively previously.[25,30,36−38] However, in the present publication, the history of enzymatic analysis as it pertains to the determination of Gibbs free energy of ATP hydrolysis was presented. In addition, an emphasis was placed on the importance of magnesium binding constants and for the first time correct constants for 3PG and DHAP were presented. With these constants, the apparent equilibrium constant of the combined GAPDH−PGK enzyme system was calculated. On the basis of this constant, the Gibbs free energy of ATP hydrolysis was calculated and compared to the Gibbs free energy of mitochondrial complex I−II. Furthermore, a hypothesis linking these values was discussed. In addition, free $[Mg^{2+}]$ concentrations from previously measured substrates was calculated and the dependence of free $[Mg^{2+}]$ and $\Delta G'$ of ATP hydrolysis on the state of the animal, starved vs fed, was presented for the first time.

Effect of Free $[Mg^{2+}]$ and pH on $K'_{GG\text{-}(TPI+LDH)}$. The apparent equilibrium constant can be calculated at various pH and free $[Mg^{2+}]$ values using eq 20 and the calculated value for $K_{ionicGG\text{-}(TPI+LDH)}$ of 1.657×10^5 M^{-1}. The specific in vivo

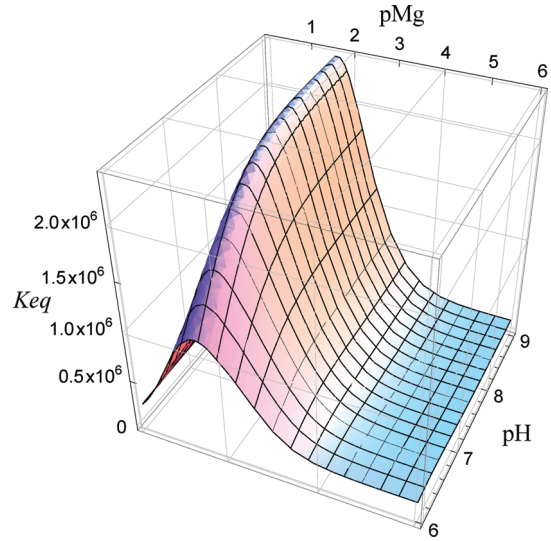

Figure 1. The variation of $K'_{GG\text{-}(TPI+LDH)}$ with pH and free $[Mg^{2+}]$.

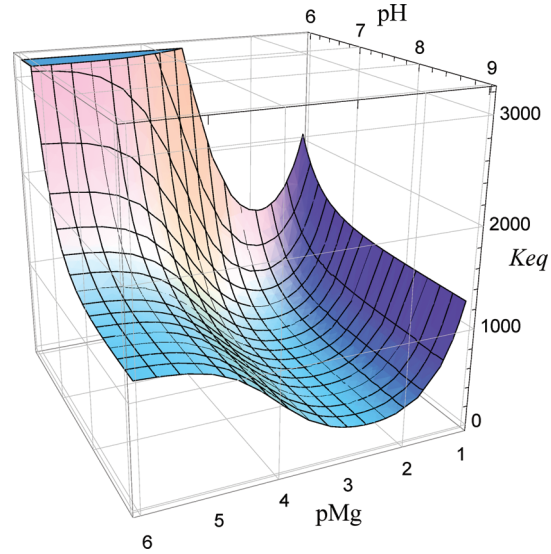

Figure 2. The variation of K'_{GS} with pH and free $[Mg^{2+}]$.

TABLE 4: The Value of the Apparent Equilibrium Constant, $K'_{GG\text{-}(TPI+LDH)}$, with Varying pH and Free [Mg^{2+}] at $T = 38$ °C and $I = 0.25$

	free [Mg^{2+}], mM															
pH	0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1	1.1	1.2	1.3	1.4	1.5
6.8	1.67×10^5	2.52×10^5	3.25×10^5	3.89×10^5	4.46×10^5	4.96×10^5	5.42×10^5	5.84×10^5	6.22×10^5	6.57×10^5	6.89×10^5	7.20×10^5	7.48×10^5	7.74×10^5	7.99×10^5	8.22×10^5
6.9	1.68×10^5	2.58×10^5	3.37×10^5	4.05×10^5	4.65×10^5	5.18×10^5	5.67×10^5	6.10×10^5	6.50×10^5	6.87×10^5	7.21×10^5	7.53×10^5	7.82×10^5	8.10×10^5	8.36×10^5	8.60×10^5
7	1.68×10^5	2.64×10^5	3.47×10^5	4.19×10^5	4.82×10^5	5.38×10^5	5.88×10^5	6.34×10^5	6.75×10^5	7.13×10^5	7.49×10^5	7.82×10^5	8.12×10^5	8.41×10^5	8.68×10^5	8.93×10^5
7.1	1.68×10^5	2.69×10^5	3.56×10^5	4.30×10^5	4.96×10^5	5.54×10^5	6.06×10^5	6.53×10^5	6.96×10^5	7.36×10^5	7.73×10^5	8.06×10^5	8.38×10^5	8.68×10^5	8.96×10^5	9.22×10^5
7.2	1.68×10^5	2.73×10^5	3.63×10^5	4.40×10^5	5.08×10^5	5.68×10^5	6.22×10^5	6.70×10^5	7.15×10^5	7.55×10^5	7.93×10^5	8.28×10^5	8.60×10^5	8.91×10^5	9.19×10^5	9.46×10^5
7.3	1.67×10^5	2.77×10^5	3.69×10^5	4.49×10^5	5.18×10^5	5.80×10^5	6.35×10^5	6.84×10^5	7.30×10^5	7.71×10^5	8.10×10^5	8.45×10^5	8.79×10^5	9.10×10^5	9.39×10^5	9.67×10^5
7.4	1.67×10^5	2.79×10^5	3.74×10^5	4.56×10^5	5.27×10^5	5.89×10^5	6.45×10^5	6.96×10^5	7.42×10^5	7.85×10^5	8.24×10^5	8.60×10^5	8.94×10^5	9.26×10^5	9.56×10^5	9.84×10^5

TABLE 5: The Value of the Apparent Equilibrium Constant, K'_{GS}, with Varying pH and Free [Mg^{2+}] at $T = 38$ °C and $I = 0.25$

	free [Mg^{2+}], mM															
pH	0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1	1.1	1.2	1.3	1.4	1.5
6.8	1.91×10^3	1.28×10^3	1.01×10^3	8.49×10^2	7.49×10^2	6.80×10^2	6.29×10^2	5.90×10^2	5.60×10^2	5.35×10^2	5.15×10^2	4.98×10^2	4.84×10^2	4.72×10^2	4.62×10^2	4.53×10^2
6.9	1.76×10^3	1.16×10^3	8.98×10^2	7.56×10^2	6.66×10^2	6.05×10^2	5.60×10^2	5.26×10^2	4.99×10^2	4.77×10^2	4.60×10^2	4.46×10^2	4.33×10^2	4.23×10^2	4.14×10^2	4.06×10^2
7	1.64×10^3	1.05×10^3	8.15×10^2	6.84×10^2	6.03×10^2	5.47×10^2	5.06×10^2	4.76×10^2	4.52×10^2	4.33×10^2	4.18×10^2	4.05×10^2	3.94×10^2	3.85×10^2	3.77×10^2	3.71×10^2
7.1	1.54×10^3	9.75×10^2	7.49×10^2	6.28×10^2	5.53×10^2	5.02×10^2	4.65×10^2	4.37×10^2	4.16×10^2	3.99×10^2	3.85×10^2	3.73×10^2	3.63×10^2	3.55×10^2	3.48×10^2	3.42×10^2
7.2	1.47×10^3	9.13×10^2	6.98×10^2	5.85×10^2	5.14×10^2	4.67×10^2	4.33×10^2	4.07×10^2	3.87×10^2	3.72×10^2	3.59×10^2	3.48×10^2	3.40×10^2	3.32×10^2	3.26×10^2	3.21×10^2
7.3	1.40×10^3	8.63×10^2	6.58×10^2	5.50×10^2	4.84×10^2	4.40×10^2	4.08×10^2	3.84×10^2	3.65×10^2	3.51×10^2	3.39×10^2	3.29×10^2	3.21×10^2	3.14×10^2	3.08×10^2	3.03×10^2
7.4	1.35×10^3	8.24×10^2	6.26×10^2	5.23×10^2	4.60×10^2	4.18×10^2	3.88×10^2	3.65×10^2	3.48×10^2	3.34×10^2	3.23×10^2	3.14×10^2	3.06×10^2	3.00×10^2	2.95×10^2	2.90×10^2

TABLE 6: Calculated Free [Mg^{2+}] in Vivo for Brain, Liver, and Perfused Heart

tissue	[citrate], μmol/g	[isocitrate], μmol/g	[citrate]/[isocitrate]	free [Mg^{2+}], mM
brain,[a] fed	0.288	0.019	15.2	0.52
brain,[b] 48 h starved	0.336	0.0149	22.6	1.27
liver,[c] fed	0.300	0.026	11.5	0.21
liver,[c] 48 h starved	0.308	0.017	18.1	0.80
perfused heart,[d] glucose + insulin	0.883	0.051	17.31	0.72
perfused heart,[d] glucose	0.432	0.018	24.00	1.44

[a] Reference 53. [b] Reference 17. [c] Reference 54. [d] Reference 55.

conditions will depend on the tissue of interest. For this reason, Table 4 can be utilized to find the appropriate value of the combined equilibrium constant for the conditions of interest. It is important to note that this system of calculations is only valid within physiological pH (pH 6.8−7.4). The reason for this restriction concerns the acid and magnesium constants used in the *f* functions. The variation of $K'_{GG\text{-}(TPI+LDH)}$ with changing pH (6−8) and free [Mg^{2+}] (pMg^{2+} 0−6) is depicted in Figure 1.

By analyzing Table 4, it becomes apparent that free [Mg^{2+}] has a larger effect on the constant than pH. Free [Mg^{2+}] varies the constant from 1.68×10^5 at 0 mM to 9.46×10^5 at 1.5 mM. This evidence supports the view that free [Mg^{2+}] plays a critical role in the quantification of the equilibrium constant for this combined enzyme system.

Effect of Free [Mg^{2+}] and pH on K'_{GS}. As with $K'_{GG\text{-}(TPI+LDH)}$, an apparent equilibrium constant for the glutamine synthetase, K'_{GS}, can be calculated for physiological conditions as described by eq 24 using the calculated value for $K_{ionicGS}$ as 1.160×10^3. As with all of these calculations, it should be noted that these calculations are only relevant at physiological pH. The variation of K'_{GS} with changing pH and free [Mg^{2+}] is plotted from pH 6−8 and pMg^{2+} 0−6 in Figure 2.

It should be noted (Table 5) that free [Mg^{2+}] affects the magnitude of K'_{GS} as it affected $K'_{GG\text{-}(TPI+LDH)}$. The apparent equilibrium constant changes from 1.91×10^3 at 0 mM free [Mg^{2+}] to 4.53×10^2 at 1.5 mM free [Mg^{2+}]. The change is opposite in magnitude because of the differences in the *f* function term in eqs 20 and 24.

Free [Mg^{2+}] in Vivo. Since free [Mg^{2+}] has been demonstrated to greatly affect equilibrium constants, Table 6 has been included to demonstrate the variations in free [Mg^{2+}] for in vivo conditions. While analyzing Table 6, it becomes clear that free [Mg^{2+}] was increased in starved tissue. It can be concluded that free [Mg^{2+}] was increased by a factor of 2 in the starved brain, by a factor of 4 in the starved liver, and by a factor of 2 in the starved heart tissue as compared to tissue from fed sources. In the perfused heart model, the glucose only perfusate is considered a "starved" condition, since there is no insulin to assist in glucose uptake. The reason for the increase in free [Mg^{2+}] could be due to a decrease in substrates binding magnesium, or it could be a response to some other cell energy-regulating process.

Calculation of $\Delta G'$ of ATP Hydrolysis in Various Tissues. It can be hypothesized that starvation should affect the Gibbs free energy of ATP hydrolysis in different tissues because of the decrease in energy status of the animal. Table 7 supports this view by presenting values for the $\Delta G'$ value of ATP hydrolysis in various tissues under starved or fed conditions. As hypothesized, the energy is decreased in starved tissues from both liver and perfused heart. It was not possible to find reliable data for starved brain tissue. In the liver, the $\Delta G'$ value of ATP hydrolysis changes from −59 to −53.5 kJ/mol after starvation. In the perfused heart model, the $\Delta G'$ value of ATP hydrolysis changes from −61.7 to −59.5 kJ/mol. This information coupled with the fact that free magnesium is increased during periods of starvation (Table 6) provides interesting insight into the thermodynamics of starvation.

A Comparison between $\Delta G'$ of ATP Hydrolysis and $\Delta G'$ of Mitochondrial Complex I−II. The most abundant source of energy, i.e., ATP, in a living cell is from the electron transport chain of the mitochondria. In this chain, mitochondrial reducing agents, NADH or flavoproteins, donate their electrons to the chain where, after a series of steps, they combine with oxygen to form water at the irreversible cytochrome oxidase reaction. While these electrons are traveling down the chain, protons are being pumped out of the mitochondrial matrix and into the cytosol. This provides a powerful proton gradient between mitochondrial and cytoplasmic phases, that is used to drive ATP synthesis through F1−F0ATPase. There are three complexes that pump protons across the wall in the mitochondrion with excess nonstochiometric cofactors:[39,40] NADH dehydrogenase, ubiquinol-cytochrome C reductase, and cytochrome oxidase. NADH dehydrogenase is proposed to pump four protons across the wall.[41−43] ATP synthase operates with near-100% efficiency using four protons to reversibly convert ADP to ATP.[44,45] Because of the efficiency of the F1−F0ATPase, the energy of the proton gradient between mitochondrial and cytosolic phases, as indicated by the difference between the redox states of the free mitochondrial NAD couple and the free coenzyme Q couple can equal the energy of ATP hydrolysis in cytosol under a number of in vivo conditions.

Table 8 contains a comparison, using previously measured values, between the Gibbs free energy of ATP hydrolysis, $\Delta G'_{ATP\ hydrolysis}$, and the Gibbs free energy of the difference between the redox states of the free mitochondrial NAD couple and the free coenzyme Q couple, $\Delta G'_{mito\ compI-II}$. Interestingly, the values agree quite well in fed liver, −57.5 kJ/mol vs −56.8 kJ/mol. However, in fed rat brain samples, the value for the

TABLE 7: Gibbs Free Energy of ATP Hydrolysis in Various Tissues under Starved or Fed Conditions

tissue	[DHAP], μmol/g	[3PG], μmol/g	[pyruvate], μmol/g	[lactate], μmol/g	$\Delta G'°$, kJ/mol	$\Delta G'$, kJ/mol, free [Mg^{2+}] = 0.5 mM	$\Delta G'$, kJ/mol, free [Mg^{2+}] = 1 mM
brain,[a] fed	0.019	0.018	0.102	1.35	−33.29 at free [Mg^{2+}] = 0.5 mM, −32.61 at free [Mg^{2+}] = 1 mM	−61.0	−61.2
liver,[a] fed	0.043	0.387	0.258	1.36		−57.6	−57.8
liver,[b] starved	0.011	0.156	0.010	0.171		−53.4	−53.6
muscle,[a] fed	0.017	0.038	0.095	0.924		−59.6	−59.8
erythrocytes,[a] fed	0.017	0.073	0.068	0.921		−57.1	−57.2
perfused heart,[c] glucose + insulin	0.055	0.064	0.085	0.737		−61.6	−61.8
perfused heart,[c] glucose	0.036	0.071	0.055	0.683		−59.3	−59.5

[a] Reference 21. [b] Reference 54. [c] Reference 55.

TABLE 8: A Comparison of $\Delta G'_{\text{ATP hydrolysis}}$ and $\Delta G'_{\text{mito compI−II}}$ in Fed Brain and Liver

	brain, fed[a]	liver, fed
Measured Values, μmol/g of Tissue		
[citrate]	0.205	0.300[b]
[isocitrate]	0.0109	0.026[b]
[3PG]	0.034	0.387[c]
[DHAP]	0.017	0.043[c]
[pyruvate]	0.086	0.258[c]
[L-lactate]	1.69	1.36[c]
[fumarate]	0.074	0.075[d]
[succinate]	0.077	0.74[d]
[α-ketoglutarate]	0.138	0.140[e]
[L-glutamate]	13.4	2.41[e]
$[NH_4^+]$, M	3.71×10^{-4}	4.70×10^{-4} [e]
Calculated Values		
free $[Mg^{2+}]$, mM	0.87	0.21
$K'_{\text{ATP hydrolysis}}$, M	3.13×10^5	5.68×10^5
$\Delta G'^{\circ}_{\text{ATP hydrolysis}}$, kJ/mol	−32.7	−34.3
phosphorylation potential, M^{-1}	18913	7825
$\Delta G'_{\text{ATP hydrolysis}}$, kJ/mol	−58.2	−57.5
$[NAD^+]/[NADH]_{\text{mito}}$	0.623	4.451
$\Delta G'_{\text{mito compI−II}}$, kJ/mol	−67.7	−56.8

[a] Reference 56. [b] Reference 54. [c] Reference 21. [d] Reference 57. [e] Reference 11.

mitochondrial energy, $\Delta G'_{\text{mito compI−II}}$, is significantly higher, −67.7 kJ/mol vs −58.2 kJ/mol. The proposed explanation for this discrepancy could be that brain contains uncoupling proteins[46−49] that may use the proton gradient to produce heat by transferring protons across the wall without the use of ATP synthase. Therefore, the free energy of the difference between the redox states of the free mitochondrial NAD couple and the free coenzyme Q couple, $\Delta G'_{\text{mito compI−II}}$, is hypothesized to be higher than the Gibbs free energy of ATP hydrolysis.

Conclusion

Significant errors can be made in determining the value of equilibrium constants where reactants have unequal Mg^{2+} binding constants. The method of calculating the value of an equilibrium constant using the most ionized forms of the reactants, called K_{ionic}, and then mathematically adjusting the value depending upon the acid dissociation constants and Mg^{2+} binding constants developed by Robert Alberty overcomes this source of error. This has allowed for the use of equilibrium constants in living tissues under different conditions of pH and free $[Mg^{2+}]$ bringing significant advances not only in physiology but also in medicine, particularly in the new field of NMR spectroscopy. As such, this very rigorous mathematical approach has furthered the application of classical thermodynamics to living systems. In addition, the described changes in free $[Mg^{2+}]$ and Gibbs free energy provide additional insight into biochemical thermodynamics with a specific focus on the electron transport chain and ATP hydrolysis.

Supporting Information Available: A detailed Mathematica (Mathematica 7.01) calculation script for the determination of free $[Mg^{2+}]$ in the glutamine synthetase reaction. Also included are the acid dissociation constants and magnesium binding constants adjusted for $T = 38$ °C and $I = 0.2$ as well as an explanation on how the ionic strength and temperature corrections were performed. This material is available free of charge via the Internet at http://pubs.acs.org.

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