PART 5

THE IMPACT OF BIOENERGETICS ON CELLULAR, PHYSIOLOGICAL AND BIOTECHNOLOGICAL PROCESSES

How could we express in terms of the statistical theory the marvellous faculty of a living organism, by which it delays the decay into thermodynamical equilibrium (death)? We said before: 'It feeds upon negative entropy', attracting, as it were, a stream of negative entropy upon itself, to compensate the entropy increase it produces by living and thus to maintain itself on a stationary and fairly low entropy level.

Erwin Schrödinger: What is Life?

Erwin Schrödinger around 1927

CHEMICAL FORCES IN THE CELL: CALCULATION FOR THE ATP SYSTEM

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INTRODUCTION

While ATP is widely accepted to be pivotal for life phenomena, the significance of phosphorylcreatine (PCr) and of the creatine kinase (CK) reaction for energy metabolism of cells and tissues with high and fluctuating energy demands has often been neglected. However, a detailed knowledge of the thermodynamics of ATP and PCr hydrolysis is crucial for a deeper understanding of cellular and tissue energetics.

In the past, attempts to describe the thermodynamics of ATP hydrolysis or of the CK reaction have been hampered by the lack of a complete set of thermodynamic constants or by their inappropriate application. While Mg^{2+} and H^+ binding to the adenine nucleotides and to PCr have been incorporated in most thermodynamic approaches, K^+ binding has been considered in only very few studies (*e.g.* [1]). In addition, temperature and ionic strength effects on dissociation and equilibrium constants have frequently been neglected.

In ^{31}P-NMR experiments, the intracellular free [ADP] in most instances is calculated from [ATP], [PCr] and [creatine] on the assumption of near-equilibrium conditions for the CK reaction. To provide a sound basis for calculation of the apparent equilibrium constant *K'* of the CK reaction for any experimental condition, we developed a simple algorithm for solving multiple equilibria of any complexity (see also [2]). For illustration, we applied this algorithm to ATP hydrolysis under a wide range of cellular conditions. The available thermodynamic information on the adenine nucleotide and PCr/creatine systems is compiled in Table 1.

MULTIPLE EQUILIBRIA COLLAPSED INTO A SIMPLE REFERENCE REACTION

A vast complexity of equilibrium reactions has to be taken into account when describing the thermodynamics of ATP hydrolysis or of the CK reaction. For simplicity, it is desirable to reduce a complex equilibrium system to a simple *reference reaction* with clearly defined thermodynamic species (each in a specified ionic form) and characterized by charge balance. For instance, if ATP hydrolysis is written as

$$ATP + H_2O \leftrightarrow ADP + P_i \qquad (R1)$$

ATP actually represents the sum of all ionic species of ATP, ΣATP (Fig. 1). The corresponding apparent equilibrium constant, *K'*, therefore, depends on pH and metal ion activities. The respective reference reaction is defined such that an 'absolute' equilibrium constant can be obtained, *e.g.* for the reference reaction,

$$ATP^{4-} + H_2O \leftrightarrow ADP^{3-} + HPO_4^{2-} + H^+ \qquad (R2)$$

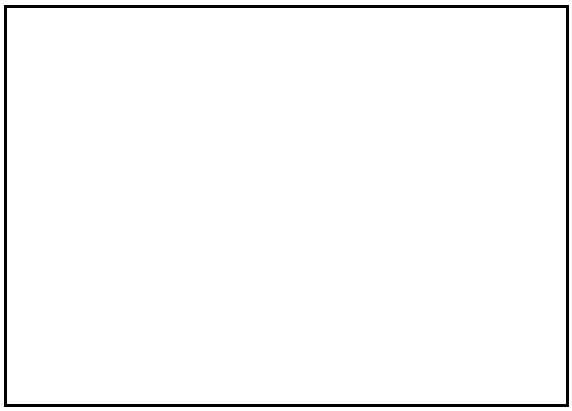

Fig. 1: The concept of a reference compound. Under equilibrium conditions, the fraction of each ionic species is strictly defined by the equilibrium constants K_1-K_8 and by the activities of H^+, K^+, Mg^{2+} and Ca^{2+}. Therefore, knowledge of the sum concentration of all ATP species, of pH, pK, pCa, pMg and K_1-K_8 allows calculation of the concentration of any one ionic species (e.g. Mg_2ATP) and, in particular, of the reference compound, ATP^{4-}.

In contrast to the reactants in (R1), the concentrations of ATP^{4-}, ADP^{3-} and HPO_4^{2-} are not measurable by analytical methods, but must be calculated with the aid of dissociation constants listed in Table 1 (pK=-log K). The chemical species defined in (R2) are related to the sum concentrations in (R1) by the fractions, *e.g.* for ATP^{4-} ,

$$f_{ATP^{4-}} = \frac{[ATP^{4-}]}{[\sum ATP]} \tag{1}$$

The simplification in (R2) is based on the fact that at any given pH, $[K^+]$, $[Mg^{2+}]$ and $[Ca^{2+}]$, the fractions are defined by the equilibrium constants of the relevant dissociation reactions (K_1 to K_8 in Fig. 1). Subsequent multiplication by the measured sum concentration of a reactant and insertion into the reference reaction (R2) fully defines the system and allows calculation of the molar Gibbs energy change (*i.e.* Gibbs force, Δ_pG_B). In addition, proton and magnesium stoichiometries can be calculated for the specified conditions of pH, $[K^+]$, $[Mg^{2+}]$ and $[Ca^{2+}]$.

STANDARD REACTION QUANTITIES (T = 298.15 K, I = 0 M) FOR COMPUTER SIMULATIONS

Table 1 represents a compilation of the available thermodynamic data for adenine nucleotides, inorganic phosphate and phosphorylcreatine. As far as possible, data were extracted from compilations by Alberty and Goldberg and corrected, where necessary, to I = 0 M.

The Gibbs force of ATP hydrolysis (R1), Δ_pG_{ATP}, under actual reaction conditions is given by,

$$\Delta_pG_{ATP} = \Delta_pG_{ATP}^{o'} + RT \ln ([ADP][P_i]/[ATP]) \tag{2a}$$
$$= \Delta_pG_{ATP}^{o'} + RT \ln M' \tag{2b}$$
$$= RT \ln (M'/K') \tag{2c}$$

M' = $[ADP][P_i]/[ATP]$ represents the apparent mass action ratio of the reaction, not incorporating protons and other ions. The influence of the latter on the Gibbs force is lumped into the apparent equilibrium constant, K'. Eq.(2) may also be written as,

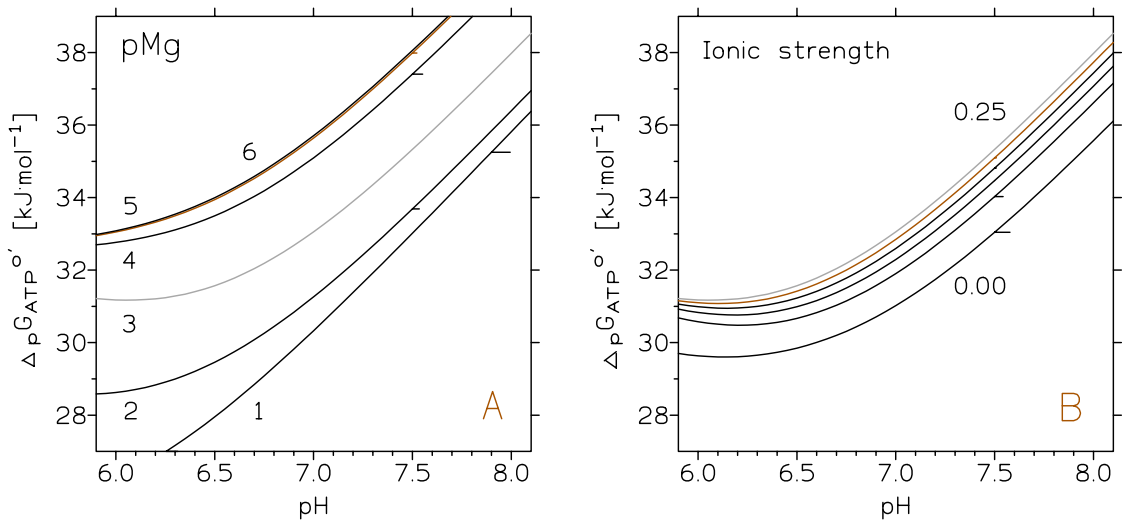

Fig. 2: Dependence of $\Delta_p G_{ATP}^{o'}$ on pMg (A) and on the ionic strength (B). (A) pMg was varied from 1 to 6, while temperature, ionic strength, pCa, pK and pH were held constant at 25 °C, 0.25 M, 8, 1 and 7, respectively. (B) The ionic strength was set at 0.00, 0.05, 0.10, 0.15, 0.20 and 0.25 M (curves from bottom to top), while temperature, pMg, pCa, pK and pH were held constant at 25 °C, 3, 8, 1 and 7, respectively. Note the rather large increment in $\Delta_p G_{ATP}^{o'}$ associated with an increase in *I* from 0.00 to 0.05 M, while around *I* = 0.25 M, $\Delta_p G_{ATP}^{o'}$ is fairly independent of variation in ionic strength.

$$\Delta_p G_{ATP} = \Delta_p G_{ATP}^{o'} + \Delta_p G_{ATP}^{\oplus'} \quad (3)$$

emphasizing the additive effect of the concentration-independent term, $\Delta_p G_{ATP}^{o'}$, and the concentration-dependent term, $\Delta_p G_{ATP}^{\oplus'}$, on the Gibbs force. For a quantitative assessment of the relative contribution of the substrate-dependent term to $\Delta_p G_{ATP}$, let us assume two extreme energetic situations. In resting skeletal muscle, [ATP], [ADP] and [P_i] can be considered to be 4 mM, 20 µM, and 0.5 mM, yielding at 25 °C a $\Delta_p G_{ATP}^{\oplus'}$ of -32 kJ·mol^{-1}. After heavy stress, the respective substrate concentrations may change to 2 mM, 0.5 mM and 20 mM, yielding a $\Delta_p G_{ATP}^{\oplus'}$ of only -12.4 kJ·mol^{-1}. This is a remarkable entropic, concentration-dependent effect, considering that $\Delta_p G_{ATP}^{o'}$ approximates -33 kJ·mol^{-1} (Table 2). The concentration-dependent term $\Delta_p G_{ATP}^{\oplus'}$ contributes 27-49% to $\Delta_p G_{ATP}$ under physiological conditions.

Effects of Ionic Strength, [K^+], [Mg^{2+}], [Ca^{2+}] and Temperature on the Thermodynamic Properties of the ATP System

For appropriate calculation of $\Delta_p G_{ATP}^{o'}$ as well as of the proton and magnesium stoichiometries in ATP hydrolysis, a variety of parameters have to be considered. pH, [Mg^{2+}], [Ca^{2+}] and [K^+] (indicating the free concentrations) are incorporated into the calculation as mentioned above, using either experimental values or an estimated range. The temperature dependence of $\Delta_p G_{ATP}^{o'}$ and of

TABLE 1
Standard thermodynamic quantities for the ATP and CK systems
T = 298.15 K, I = 0 M. Values in parentheses are semi-empirical or estimated rather than experimental. ΔC^{o}_{p} values in parentheses apply to I = 0.2 M [5].

Reaction	pK	$\Delta_r H^o$ kJ mol^{-1}	ΔC^{o}_{p} J mol^{-1} K^{-1}	Ref.
$HATP^{3-} \leftrightarrow ATP^{4-} + H^+$	7.60	-6.3	(-126)	4
$H_2ATP^{2-} \leftrightarrow HATP^{3-} + H^+$	4.68	15.0	(-63)	4
$HADP^{2-} \leftrightarrow ADP^{3-} + H^+$	7.18	-5.6	(-126)	4
$H_2ADP^{-} \leftrightarrow HADP^{2-} + H^+$	4.36	17.6	(-63)	4
$H_2PO_4^{-} \leftrightarrow HPO_4^{2-} + H^+$	7.22	3.6	(-226)	4,6
$H_3PO_4 \leftrightarrow H_2PO_4^{-} + H^+$	2.12	-10.4	(-125)	
$HPCr^{-} \leftrightarrow PCr^{2-} + H^+$	5.06	2.66		7
$HAMP^{-} \leftrightarrow AMP^{2-} + H^+$	6.73	-5.4		4
$H_2AMP \leftrightarrow HAMP^{-} + H^+$	3.99	18.1		4
$MgATP^{2-} \leftrightarrow ATP^{4-} + Mg^{2+}$	6.18	-22.9	(-126)	4
$MgHATP^{-} \leftrightarrow HATP^{3-} + Mg^{2+}$	3.63	-16.9	(-188)	4
$Mg_2ATP \leftrightarrow MgATP^{2-} + Mg^{2+}$	2.69	-10.8	(-63)	4
$MgADP^{-} \leftrightarrow ADP^{3-} + Mg^{2+}$	4.65	-19.0	(-188)	4
$MgHADP \leftrightarrow HADP^{2-} + Mg^{2+}$	2.50	-12.5	(-251)	4
$MgHPO_4 \leftrightarrow HPO_4^{2-} + Mg^{2+}$	2.71	-12.2	(-251)	4
$MgPCr \leftrightarrow PCr^{2-} + Mg^{2+}$	2.73	-8.18		8
$MgAMP \leftrightarrow AMP^{2-} + Mg^{2+}$	2.79	-11.3		4
$CaATP^{2-} \leftrightarrow ATP^{4-} + Ca^{2+}$	5.68	-1.16	(-126)	6
$CaHATP^{-} \leftrightarrow HATP^{3-} + Ca^{2+}$	3.42	-2.46	(-188)	6
$CaADP^{-} \leftrightarrow ADP^{3-} + Ca^{2+}$	4.15	1.28	(-188)	6
$CaHADP \leftrightarrow HADP^{2-} + Ca^{2+}$	2.44	0.02	(-251)	6
$CaHPO_4 \leftrightarrow HPO_4^{2-} + Ca^{2+}$	2.40	-10.0	(-300)	6
$KATP^{3-} \leftrightarrow ATP^{4-} + K^+$	2.13	(-6.3)	(-126)	9
$KADP^{2-} \leftrightarrow ADP^{3-} + K^+$	1.38	(-5.6)	(-126)	9
$KHPO_4^{-} \leftrightarrow HPO_4^{2-} + K^+$	1.02	(3.6)	(-226)	9
$KAMP^{-} \leftrightarrow AMP^{2-} + K^+$	0.74			9
$ATP^{4-} + H_2O \leftrightarrow ADP^{3-} + HPO_4^{2-} + H^+$	0.531	-20.5	-237 ± 30	4,6
$PCr^{2-} + ADP^{3-} + H^+ \leftrightarrow ATP^{4-} + Cr$	-8.70[a]	-17.6		7

[a] for I=0.25 M.

TABLE 2.
Gibbs force and stoichiometric numbers of H^+ and Mg^{2+} for ATP hydrolysis, and fractions of ATP, ADP and P_i species calculated for various experimental conditions
$\Delta_p G_{ATP}^{o'}$, v_{H}^{+}, v_{Mg}^{2+}, and the fractions of the ATP, ADP and P_i species were calculated using a computer program (BASIC). Values $<10^{-5}$ are represented by hyphens

T	***310.15***	298.15	298.15	298.15	298.15	298.15
pH	7	7	7	7	7	7
I	0.25	0.25	***0***	0.25	0.25	0.25
pMg	3	3	3	3	3	***14***
pCa	8	8	8	***5***	8	8
pK	1	1	1	1	***14***	1
$\Delta_p G_{ATP}^{o'}$	-33.15	-33.05	-31.02	-33.05	-32.49	-35.71
v_{H^+}	0.697	0.686	0.576	0.686	0.627	0.731
$v_{Mg^{2+}}$	0.424	0.428	0.292	0.427	0.449	0
$f_{ATP^{4-}}$	0.0751	0.0932	0.0004	0.0929	0.103	0.438
$f_{HATP^{3-}}$	0.0233	0.0272	0.0017	0.0271	0.0300	0.128
$f_{H_2ATP^{2-}}$	0.00001	0.00001	-	0.00001	0.00002	0.00008
$f_{MgATP^{2-}}$	0.799	0.758	0.661	0.756	0.835	0
$f_{HMgATP^{-}}$	0.0024	0.0023	0.0074	0.0023	0.0025	0
$f_{CaATP^{2-}}$	-	-	-	0.0024	-	0.00001
$f_{HCaATP^{-}}$	-	-	-	0.00001	-300	-
$f_{KATP^{3-}}$	0.0682	0.0921	0.0059	0.0919		0.434
f_{Mg_2ATP}	0.0324	0.0272	0.324	0.0272	0.475	0
$f_{ADP^{3-}}$	0.369	0.409	0.0200	0.409	0.101 0.00006	0.645
$f_{HADP^{2-}}$	0.0836	0.0873	0.0302	0.0872	0.421	0.137
$f_{H_2ADP^{-}}$	0.00003	0.00005	0.00006	0.00005	0.0023	0.00008
$f_{MgADP^{-}}$	0.414	0.363	0.892	0.363	-	0
f_{HMgADP}	0.0023	0.0020	0.0096	0.0020	-	0
$f_{CaADP^{-}}$	-	-	-	0.0011	0	-
f_{HCaADP}	-	-	-	0.00001		-
$f_{KADP^{2-}}$	0.131	0.138	0.0479	0.138	0.673	0.218
$f_{P^{2-}}$	0.574	0.565	0.237	0.565	0.302 0.0253	0.577
$f_{HP^{-}}$	0.243	0.254	0.393	0.254	-	0.259
f_{MgP}	0.0254	0.0212	0.122	0.0212	0	0
f_{CaP}	-	-	-	0.0001		-
$f_{KP^{-}}$	0.159	0.160	0.248	0.160		0.164

the p*K* values was calculated under the assumption of constant molar heat capacities (i.e. $\partial\Delta_r H_B/\partial T$ is constant) over the temperature range under consideration (4-37 ^{o}C). Ionic strength effects on the thermodynamic properties of the ATP system were calculated according to the extended Debye-Hückel theory.

At 25 °C, the following equations describe the influence of ionic strength, I, on the molar enthalpy of reaction, $\Delta_r H_B^\circ$, and on the equilibrium constant K_j (see [3]):

$$\Delta_r H_B^\circ(I) = \Delta_r H_B^\circ(I=0) + [1.4775\ \sqrt{I}\ /(1 + 1.6\ \sqrt{I}\ )] \times \Sigma \nu_i z_i^2 \quad (4)$$

$$pK(I) = pK(I=0) - [0.51065\ \sqrt{I}\ /(1 + 1.6\ \sqrt{I}\ )] \times \Sigma \nu_i z_i^2 \quad (5)$$

where ν_i and z_i are the stoichiometric numbers and electric charges of reactants i, respectively.

As can be seen in Table 2, a rise in $[Ca^{2+}]$ from 10^{-8} M to 10^{-5} M has virtually no effect on $\Delta_p G_{ATP}^{\circ\prime}$ or ν_{H}^{+} and ν_{Mg}^{2+} and only slightly influences the fractions of the different ionic species. Likewise, minimal differences are observed for $\Delta_p G_{ATP}^{\circ\prime}$ or ν_{H}^{+} and ν_{Mg}^{2+} between 25 °C and 37 °C. In contrast, $[Mg^{2+}]$, $[K^+]$ and ionic strength considerably affect most of the parameters listed, as is also shown for $\Delta_p G_{ATP}^{\circ\prime}$ in Fig. 2.

CONCLUSIONS

The algorithm applied for reducing a complex system of multiple equilibrium reactions to a simple reference reaction with well-defined thermodynamic properties proved to be a valuable tool for the calculation of $\Delta_p G_{ATP}$ as well as of the H^+ and Mg^{2+} stoichiometries of ATP hydrolysis under varying conditions of temperature, pH, ionic strength, $[Mg^{2+}]$, $[Ca^{2+}]$ and $[K^+]$. As a control for our approach, we obtained the same $\Delta_p G_{ATP}^{\circ\prime}$ for ATP hydrolysis at 25 °C, pH 7, pMg 3, pK 14 and I = 0.25 M (-32.49 kJ mol^{-1}) as published previously [4] for the same conditions. In addition, it is shown that the effects of $[K^+]$ (see also [1]) and of ionic strength on $\Delta_p G_{ATP}^{\circ\prime}$ are far from negligible, especially when intracellular conditions are to be simulated.

The algorithm in our computer program can be adapted easily for the binding of additional metal ions and, even more importantly, to any other reaction for which the thermodynamic properties of a reference reaction are known. We will apply this approach to the creatine kinase reaction. In spite of 65 years of research, the controversy on the physiological function(s) of the CK system is still ongoing. Hopefully, mathematical modelling will aid in understanding CK function and in designing future experiments.

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